Applied Sciences, Biology, and Territory

Achieved findings and new research frontiers at the University of Molise

Department of Bioscience and Territory

Termoli, December 20th, 2022

DIPARTIMENTO
DI BIOSCIENZE
E TERRITORIO

Organizing Committee

Piera Di Marzio – Rocco Oliveto – Marco Petrella – Marco Segatto

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DIBT, Università degli studi del Molise, August 2023
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Detailed program

09:30 – 10:00 Welcome

10:00 – 11:00 Established lines of research and achievements

- *Biology*
- *Nature, Environment, and Forests*

11:00 – 11:30 Coffee break

11:30 – 13:00 Established lines of research and achievements

- *Landscape, Economy, and Planning*
- *Engineering*
- *Computer science and Mathematics*

13:00 – 14:00 Light lunch

14:00 – 16:30 Poster session and working sessions

16:30 – 18:00 Department meeting and wrap-up

18:00 – 18:30 Closing and best poster award ceremony

Welcome

For the sixth year, the Department of Biosciences and Territory is proposing the Scientific Research Days event, the periodic gathering of the department's researchers to discuss research, dialogue and exchange views on the analyses, lines of action and activities carried out in the laboratories of the department's three facilities (Pesche, Campobasso and Termoli).
In addition, young researchers, Ph.D. students, postdoctoral fellows, and students present their research projects in the form of posters: from single cells to natural ecosystems, from new technologies in engineering and computer science to new approaches in mathematics and molecular biology.

This year the event was held, for the first time at the headquarters of the Biosciences and Territory Department in Termoli.

Sponsors to thank for their support: the Publisher EdiSES Università, which will publish the proceedings of the event, and the company Datasound srl, a spin-off of the University of Molise, which funded the prizes for the three best research posters.

The Organizing Committee

Research

BIOLOGY UNIT

Coordinator: Prof. Eleonora Sgambati
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MISSION

The Biology unit comprises research fields that study the basis and evolution of biological processes in a plethora of living organisms: viruses, bacteria, lower and higher eukaryotes, plants, humans. The research has a functional and application profile at different levels of biological organization, from molecules to cells to tissues, organs and complex organisms as well as in populations and ecological communities in an evolutionary context. The Biology unit, thanks to the coexistence of highly specific and differentiated skills, offers a valuable opportunity to integrate the heterogeneity of knowledge into interdisciplinary projects.

The main research lines are structured in four thematic clusters: 1) Biochemistry, Cell and Molecular Biology, Human Anatomy and General Physiology; 2) Microbiology; 3) Plant Biology and Physiology; 4) Chemistry.

Cluster of Biochemistry, Cell and Molecular Biology, Human Anatomy and General Physiology

The cluster of Biochemistry, Cell and Molecular Biology, Human Anatomy and General Physiology deals with studies at molecular, structural, metabolic and physiological level on cells and tissues of human and animal models, in both physiological and pathological conditions such as metabolic alterations, inflammation, sepsis, tumors, neurodegeneration and other morphofunctional alterations.

Principal subjects (1-9):

1) Structural and functional analysis of human ferroportin and ceruloplasmin produced in the yeast *Pichia pastoris* - Head: Prof. Giovanni Musci (SSD BIO/11);
2) Effect of lactoferrin on oxidative stress and iron disbalance induced by HIV-Tat and SARS-CoV-2 Spike viral proteins- Head: Dr. Antimo Cutone (SSD BIO/10);
3a) Autophagy role in cancer biology: the autophagy master regulator ULK1 in glioblastoma pathogenesis- Head: Sabrina Di Bartolomeo (SSD BIO/06);
3b) Autophagy role in cancer biology: epigenetic reprogramming of GBM cells - Head: Prof. Sabrina Di Bartolomeo (SSD BIO/06);
4) Sialylation status in cells and tissues of human organs and of animal models- Head: Prof. Eleonora Sgambati (SSD BIO/16);
5) Telocytes in human tissues and organs- Head: Prof. Eleonora Sgambati (BIO/16);
6) VEGF and its receptors in human bone tissue. Head: Prof. Eleonora Sgambati (SSD BIO/16);
7) Regulation of cholesterol metabolism in brain physiopathology- Head: Prof. Marco Segatto (SSD BIO/09);
8) Elucidation of molecular mechanisms and identification of novel biomarkers in neurodegenerative diseases - Head: Prof. Marco Segatto (SSD BIO/09);
9) Study of the cell processes regulating muscle mass- Head: Prof. Marco Segatto (SSD BIO/09).

Cluster of Microbiology

The cluster of Microbiology studies the distribution and role of microorganisms in nature and microbial interactions.

Principal subjects (10-11):

10) Analysis of microbial communities in different habitats: Isolation and characterization of microorganisms degrading pollutants- Head: Prof. Gino Naclerio (SSD BIO/19);
11) Multilevel characterization of truffle populations (Tuber spp.)- Head: Dr. Antonio Bucci (SSD BIO/19).

Cluster of Plant Biology and Physiology

The cluster of Plant Biology and Physiology studies various functional aspects of plant organisms in terms of development and response to biotic and abiotic stress. The research ranges from molecular interactions to the

whole organism, combining multiple experimental approaches that involve the integrated study (morphological, biochemical, physiological and molecular) of model plants and species of agronomic interest grown under controlled conditions and/or in the field.

Principal subjects (12-14):

12) Conservation and characterization of plant biodiversity- Head: Prof. Gabriella Stefania Scippa (SSD BIO/01);
13) Plant-environment interaction: the mechanisms involved in roots response to environmental stress conditions, i.e. drought, heavy metals and mechanical stresses- Head: Prof. Dalila Trupiano (SSD BIO/01);
14) Host-pathogen interaction: molecular cross-talk between plants and phytopathogenic fungi- Head: Prof. Claudio Caprari (SSD BIO/04).

Cluster of Chemistry

The cluster of Chemistry uses NMR spectroscopy, mass spectrometry and computational methods for the structural characterization of new "hit compounds" deriving from sources of natural origin with potential antimicrobial, anti-inflammatory, antioxidant and anticancer activity and for the design and synthesis of new platforms potentially bioactive molecules.

Principal subjects (15-18):

15) Isolation, characterization and antioxidant activity of bioactive natural products from plant kingdom combined with a heavy metals analysis of natural sources- Head: Prof. Maria Iorizzi (SSD CHIM/06);
16) Stereochemical assignment and target identification by Direct (VS) and Inverse Virtual Screening (IVS) of secondary metabolites extracted from natural sources and design of novel bioactive molecular platforms- Head: Prof. Maria Giovanna Chini (SSD CHIM/06);
17) Metabolomic and multivariate data analysis- Head: Prof. Gabriella Saviano (SSD CHIM/03);
18) Characterization and chemical variability of Essential Oils (EOs) extracted from aromatic plants and their correlation with pharmacological properties- Head: Prof. Gabriella Saviano (SSD CHIM/03).

RESEARCH IMPACT

The research activity of Biology unit, both basic and applied, has resulted in a high level scientific production (247 products published on peer review journals in the last five years), and in obtaining patents. The unit goal is to continue and extend the research interests, in order to increase the knowledge in the different fields and to obtain results that can be translated to human and environmental being.

The components of the Biology unit carry out research activities in various laboratories of the Department of Biosciences and Territory such as Plant Biology, Chemistry and Instrumental Chemical Analysis, Cell and Molecular Biology, Microbiology, Plant Physiology and Advanced Microscopy. In addition, several research lines are performed in collaborations with other Universities and National and International Research Institutions/Centers.

NATURE, ENVIRONMENT, AND FORESTS UNIT

Coordinator: Prof. Maria Laura Carranza
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MISSION

NEF has sound experience in the field of Natural Sciences, Environmental Biology, Spatial and Landscape Analysis, Physical Environment and Forestry. NEF integrates the scientific research on a wide range of biotic and abiotic processes at multiple scales with academic teaching and third mission activities, along with the promotion of the sustainable development of culture, society and economy.

NEF is composed of 10 researchers organized in five laboratories that collaborate sharing high level disciplinary competences and knowledge, also joining a large network of national and international collaborations. Laboratories are: a) MicroLab, b) PLAN.TA Lab, c) EnvixLab, d) ForestryLab and e) GeoGisLab.

MicroLab: Environmnetal Microbiology and biorestoration – Prof. Giacarlo Ranalli: Agricultural Microbiology (AGR/16).

The MicroLab deals with environmental microbiology studies on different environments such as air, water, soil and works of art. Main topics: 1) Microbiological and enzymatic analyses to study the state of bio-preservation of internal and external cultural heritage; 2) Physiology of methanogenic bacteria, isolation, selection and evaluation of anaerobic microorganisms in different habitats. Microbial biodiversity as a resource for innovation; 3) Selection and use of microorganisms in bio-restoration, and bio-removal of undesirable alterations on works of art.

PLAN.TA Lab: Plant taxonomy and Systematics – Prof Paola Fortini: Plant Taxonomy (BIO/02); Prof. Piera Di Marzio: Environmental and Applied Botany (BIO/03).

PLAN.TA Lab, in cooperation with "Museo delle Scienze Naturali del Molise" (MuSNaM) and "Giardino di Flora Appenninica" (GFA), is focused on research, services, third mission and teaching within bio-ecological and botanical disciplines. In particular, the research focuses on: 1) Floristic surveys and proposal of new records for the regional and national Flora; 2) Classification of critical species of the Italian flora and assessment of their ecological and biogeographic features; 3) Identification and Monitoring of plants included in 92/43/EEC Directive; 4) Models of genetic diversity, phylogenetic classification and phylogeographic frameworks, through the acquisition, synthesis and comparative analysis of chorological, biological-reproductive, populationistic, macro and micro-morpho-anatomical, phytochemical, molecular information on herbarium collections; 5) Identification of possible phenotypic response and plant functional traits (PFTs) variations from stands characterized by different environmental conditions to establish ecological and evolutionary trends for plant species and communities.

EnvixLab- Environmetrics Lab – Prof Anna Loy and Mirko Di Febbraro: Zoology (BIO/05); Prof Angela Stanisci: Applied Botany (BIO/03); Prof Maria Laura Carranza and Michele Innangi: Ecology (BIO/07).

http://envixlab.unimol.it/

EnvixLab is an interdisciplinary laboratory of research, services, third mission and teaching combining ecological, zoological and botanical disciplines. Implemented in 2008, EnvixLab has coordinated several national and international projects and promoted many educational and dissemination activities in ecological monitoring and modelling, biodiversity conservation and environmental sustainability.

Main topics include: a) Ecosystems and species monitoring adopting standard field protocols (IUCN, Habitats directive, etc.) and collecting data in situ and supported by remotely sensed data, b) Innovative metagenomics (eDNA, non-invasive genetic sampling, landscape genetics), c) Assessment and modelling of biodiversity threats at different scales (local, regional, global) including climate change, invasive alien plants, fire, road kills, pollution, urban expansion, d) Ecosystems and protected areas management (SAC, National and Regional Parks), integrated coastal management, e) Implementation and management of biodiversity databases: fauna, flora

and ecosystems, f) Ecology of populations, g) Integrative taxonomy and geographic variation of wide ranging species investigated through geometric morpho-metrics applied to explore geographic variability of endangered species, h) Environmental DNA for studying animal species of conservation concern (e.g. endangered species, problematic species as alien taxa), i) Spatially-explicit modelling of the impacts of anthropogenic pressures (i.e., climate and land-use change, invasion risk by alien species, human overpopulation) on animal biodiversity, j) Analysis of the effects of past climate change on mammal communities as experimental set to improve future prediction capabilities: effects of Late Pleistocene climate modifications on mammal megafauna, k) Soil Ecology: plant tissues decomposition, soil Carbon and Nitrogen cycle on different ecosystems (forests, and agro ecosystems), soil quality assessment, l) Applications of remote sensing to ecological issues: multitemporal landscape analysis and landscape processes, passive and active sensors for assessing landscape integrity, remote sensed data as variables for building ecological models, multispectral data for mapping vegetation phenology, use of drones for ecosystem monitoring and satellite upscaling, m) Ecosystem functioning and services.

Forestry Lab – Prof. Vittorio Garfì: Forest management and silviculture (AGR/05) http://www.ecogeofor.unimol.it/

The Forestry Labs develop research activities referred to ecology, monitoring and management of forest ecosystems and urban forests. Forestry lab quantifies and monitors tree response and forest resilience to changes in disturbance regimes, stand dynamics and land use. This knowledge is applied to the development of sustainable forest management practices and the provision of vital forest ecosystem services and to establish a framework for the formulation of optimal forest planning. Main topics: Sustainable management of forest systems, forest fire and arboriculture for wood production in Mediterranean areas and in particular: 1) Forest management: i) Silvicultural interventions for Mitigation and Adaptation to Climate Change; ii) Definitions of forest and pre-forest types; iii) Treatments; iv) Assessment of reforestation plant effects, 2) Ecology of forestry systems: i) Adaptation to climate change; ii) Climate change and growth; iii) Intra and interspecific competition of forest species; 3) Forest fire: i) Forest fire-fighting plans; ii) Fire behavior; iii) Fuel models; iv) Fire risk assessment; v) Economic damage assessment); 4) Forest planning: i) Sustainable planning of ecosystem services; ii) Forest management plan; iii) Urban forest planning; iv) Forest biomass assessment; v) Biomass for energy purposes.

GeoGisLab – Prof. Carmen Maria Rosskopf, Vincenzo Amato: Physical Geography and Geomorphology (GEO/04)

The mission of the GeoGisLab is the investigation and characterization of the environment/territory by means of geological + geomorphological research based on field, laboratory and GIS analyses (geological-geomorphological and topographic/bathymetric surveys, stratigraphic and sedimentological analyses, remote sensing, geophysical investigations, geothematic mapping etc.).

Major Research Themes and Goals: a) Landscape characterization + short to long-term landscape evolution, b) Understanding natural and anthropic controls of recent landscape evolution and present-day dynamics, with particular attention to the role of ongoing climate changes. c) Understanding the past to present-day relationships between man and its environment/territory, d) Preservation + valorization of geodiversity/geological heritage + archaeological sites, e) Geological and geomorphological hazard + risk assessment and zonation (landslides, flooding, seismic susceptibility). Support of and collaboration with other environmental researches: Bioconservation + Biodiversity, Forest Management, Archaeology/Tourism/History, Engineering. Among the Most Important Projects: National Inventory of Italian Landslides (IFFI) Seismic Microzonation (Campobasso + Isernia provinces); CARG – Production of the new Geological Map of Italy, Geological sheets Trivento, Campobasso, Isernia, Castel di Sangro; Inventory of Geosites in Molise Region, 20 year-long monitoring of Molise coast.

RESEARCH IMPACT

The research activity of NEF section is evidenced by the relevant number (>320 papers) of highly-cited (>5200 citations) scientific products published on peer-reviewed journals in the last ten years, as well as several international research projects coordinated by NEF members financed in the last 10 years for approximately 3.5 million €.

LANDSCAPE, ECONOMY, AND PLANNING UNIT

Coordinator: Prof. Monica Meini
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MISSION

The Section deals primarily with the territory considered as a living space, thus in constant elaboration by man in co-evolution with the environment. The research carried out is characterized by synergic cultural and scientific aspects, has a multidisciplinary and multifunctional profile, both basic and applied, and presents various levels of scientific development and technological maturity: very high in some of the more consolidated areas of study, lower in others where recent experiments have been launched, as a result of a constant attempt to align with the problems of today's societies, to which we try to provide answers. The coexistence of differentiated competences, which find their fields of application in numerous areas of the European Research Council – mainly in the Social Sciences and Humanities area and the SH7 sector – allows the Section to offer a valuable opportunity to integrate the heterogeneity of knowledge in interdisciplinary projects.

ORGANIZATION

The main lines of research are traced back to five thematic clusters, which correspond to the Research Units in which the Section is structured: 1) Geographical Sciences; 2) Historical Sciences; 3) Forestry Sciences; 4) Economic-Agricultural Sciences; 5) Spatial Planning Sciences. Various research themes converge on these clusters, carried out by specific groups of researchers within the following laboratories:

1. MoRGaNA Lab (Mobility, Regions, Geo-economics and Network Analysis) - Head: Prof. Monica Meini (SSD M-GGR/01);
2. SAP Lab (History, Environment and Heritage) - Head: Prof. Rossano Pazzagli (SSD M-STO/02);
3. Forestry LABs- Head: Prof. Marco Marchetti (SSD AGR/05);
4. Lab_LATE (Land, Agriculture, Territory, Environment) - Head: Prof. Davide Marino (SSD M- AGR/01);
5. LISP (Laboratory for Interactive Spatial Planning) - Head: Prof. Luciano De Bonis (SSD ICAR/20).

BASIC AND APPLIED RESEARCH OBJECTIVES

The main objects of study in basic research are:
- the forms of inhabiting the world, which are realized through the relationships between biotic and abiotic factors, activate feedback chains and modify resources and development paths;
- the relationships that innervate territorial systems of material and immaterial flows and the construction of regional networks of variable geometry;
- the processes of territorialization, deterritorialization and reterritorialization;
- the anthropogenic causes of ecological-environmental changes and their local and global impact;
- the configurations of territoriality, agency and resilience dynamics.

Word-cloud based on the word frequency of the current research themes in the Section (in Italian, PEP stands for "Paesaggio Economia Pianificazione").

The applications, conducted at various scales of analysis, use a place-based approach in the assessment of territorial dynamics and planning of sustainable co-evolution pathways, an approach that, if supported by the theoretical lines and conceptual models elaborated by basic research, is substantially fuelled by the knowledge processes activated through empirical research based on the direct relationship with places and their communities.

THIRD MISSION ACTIVITIES

The Section coordinates scientific research with respect to a significant set of different but related strategic objectives:
1) Geo-historical, anthropological and economic analysis of the territory, with

particular attention to social and economic structures and the landscape, with planning, management and reconstruction purposes of the interaction between territorial components (city-countryside, mountain-plains, coast-inland, etc.).
2) Conservation, protection, management, valorization and regeneration of the 'heritage', understood in its broadest meanings of environmental, historical-cultural, landscape, urban and built environment.
3) Policy, governance and intervention models on specific critical issues aimed at better management of the environment and the territory, through the study of the interactions between the social, institutional and economic systems and the environment, so as to highlight the dynamic and evolutionary phenomena that involve these systems. In particular, there are three areas of major interest in the study of these relationships:
(a) the economic assessment of environmental quality, understood both in a negative sense - environmental damage - and in a positive sense - the economic benefits of the environment or ecosystem services;
b) the evaluation of interventions aimed at restoring, conserving and enhancing environmental functionality;
c) land planning methodologies aimed at organizing the non-dissipative use of the territory by man through regulatory prescriptions and design visions that relate environmental, social and economic aspects.

RESEARCH IMPACT

The research activities, both basic and applied, of the various members of the Section have resulted in a high level of scientific production and a highly developed third mission activity. The intention is to continue and expand the current lines of research as well as add new ones, increasing both scientific knowledge and the ways of making it available to society, through doctoral and post-doctoral projects, participating in competitive calls, providing technical advice, raising public awareness, and supporting decision-making processes at national, regional and local levels. Some research topics include the possibility of activating spin-offs and obtaining patents, thanks also to the possible contamination with other research areas in the Department of Biosciences and Territory that are more oriented towards technology transfer.
The Section intends to characterize itself as an environment of transdisciplinary meeting and confrontation within the Department, with the aim of channeling the scientific knowledge of individual researchers towards reflection on the critical issues of the human-environment relationship expressed through social demand. The impact in terms of the results obtained and expected is therefore on the one hand traceable in the publications produced by each disciplinary scientific sector, aimed at the advancement of the theoretical-methodological apparatus and epistemologies of reference, and on the other hand it follows experimental paths and finds forms of concretization in action-research projects, capacity building, international cooperation and territorial restitution.

ENGINEERING UNIT
Analysis and perspectives of the research in safety and sustainability: the civil engineers of the Next Generation EU

Coordinator: Filippo Santucci de Magistris
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MISSION

To call "Engineering" a research unit of the Department of Biology and Territory is extremely ambitious and partly misleading. Because, as it is well known, this term includes a large group of disciplines oriented towards the creation of works and artifacts of an extremely variable and rapidly evolving nature, always analyzed and designed starting from the knowledge that develops in the scientific and technological fields. Our Engineering unit is constituted of researchers working in the area of Civil Engineering and Architecture, and Physics and Chemistry.

More specifically, the research interests fall on the one hand in the field of engineering and territory, in structural and geotechnical engineering, in architectural design, and, on the other, in the physics of fundamental interactions and inorganic and technological chemical sciences. However, the heterogeneity of these themes can be summarized through some groups of keywords that can help to identify the mission of the section: *planning, analysis, verification, and recovery of structures and infrastructures on a punctual and territorial scale*- individual buildings and building aggregates, villages, minor towns, and historic structures, dams, bridges, tunnels, pipelines, retaining works and water treatment plants – *analyses of Earthquake Engineering problems*- advanced numerical modeling using FEM codes, mechanics of porous media, localization problems, liquefaction problems, structural engineering problems in seismic areas, reinforced earth- *structural and environmental monitoring problems*- monitoring of structures and the environment- ending with *elementary particle physics*. The researchers work towards the solution of cases on an extremely variable scale, passing from the analysis of territorial problems through structural and environmental monitoring and the analysis of distributed infrastructures, through the analysis or renovation of specific structures or infrastructures, ending up with the analysis of single particles. The common aspect is the analytical-experimental research method which also requires skills in the physical-mathematical modeling of the problems under examination.

However, almost all of the research topics carried on in the units can be also understood if read into two keywords that are also going to characterize a new Civil Engineering class that hopefully will be proposed at the University of Molise, starting from next academic year: *safety* and *sustainability*.

While "safety" in Civil and Environmental Engineering is a well-known and technically sound concept, few words need to be spent about "sustainability" borrowing some sentences and definitions reported by the International Organization for Standardization (i.e., ISO 21931-1:2022).

"Buildings constitute both a living and working environment and affect the safety, comfort and performance of the user, as well as the quality of coexistence/life in a community. Aspects of urban integration and architectural design of buildings are important for the overall quality of the built environment: they can represent a cultural value. Buildings and constructed assets represent a high economic value both from a private, micro-economic and macro-economic point of view. Their construction and maintenance contribute to the preservation and creation of jobs, whereas the building-related negative effects on the environment contribute to external costs. The construction, use and maintenance of buildings are associated with significant energy and material flows, as well as adverse effects on the local and global environment. This includes health risks and risks to the environment. The type of design, construction and operation of buildings, together with the future construction of building stocks, have a great effect on sustainable development". In the same document it is also reported that "Against the background of climate change and the related SDG Goal 13 on Climate Action, and the importance of energy-saving, resource-saving, and healthy and cost-effective design, construction and management of buildings, there is a need for the creation of a basis for the assessment of environmental, social and economic performance".

Worth remembering too- again we partially copy ISO 21931-1:2022 that "Life-cycle-based approaches play an increasingly significant role for setting performance criteria within methods of assessment of environmental, social and economic performance of buildings. However, methods of assessment of the environmental, economic and social performance of buildings need to refer to limited criteria and seek a balance between rigour and practicality. Target conflicts can occur when attempting to plan environmentally- and health-friendly buildings, which are characterized by a high user acceptance and are at the same time economically advantageous. These target conflicts can be identified through the combined analysis of ecological, social and economic aspects. Already in the planning phase, the consequences of decisions on the energy and material flows with resulting environmental impacts, on the life cycle costs as well as on the social performance can be identified and influenced. The effects of decisions on the size and shape, the construction method, the choice of materials or the energy sources, among others, can be analysed."
The reader should have in mind all the above concepts, to interpret the specific research that going to develop in the Engineering unit of the Department.

RESEARCH IMPACT
The research activity is mainly of an applied type, even if some topics fall in the area of basic research. The overall scientific production of the group is, on average, very good. Most researchers belong to so-called 'bibliometric' sectors, some of them having a track record of scientific papers well above the reference medians of that of their scientific area.
In various circumstances, the research activities have a significant impact not only in terms of scientific publications already produced or which will be written shortly- which constitute the key element with which one evaluates what is the advantage of the researcher of a public institution returns to his community which bears their costs- but also because they are in line with the directions towards which European research is moving in the context of Horizon 2020, or because they have a clear applicative influence. Applicative because they mainly contribute to the design, analysis, evaluation, and recovery of construction engineering works. Or because they are linked to issues that can then be poured into the drafting or updating of technical standards or guidelines.
Basic research on particle physics also has a significant impact because it is linked to important experiments carried out using cutting-edge experimental equipment and in collaboration with the main research groups in this specific area.

COMPUTER SCIENCE AND MATHEMATICS UNIT

Coordinator: Prof. Fausto Fasano
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MISSION

The Computer Science and Mathematics Section is characterized by a strong multidisciplinary connotation, thanks to the collaboration between researchers in quantitative areas, especially mathematical and statistical disciplines, and applied sciences like computer science. This mix of skills has the goal of building, on top of a scientific theoretical background, functional and applied solutions. Examples of topics that benefit from such an interaction are the area of machine learning, empirical software engineering and formal verification in the field of security and parallel computing.

The main mission is therefore to study and advance the state of the art of the "exact sciences" to adapt and apply them to concrete problems characterized by an IT component.

The application areas most explored so far can be summarized as follows:

Software engineering: in this area, the main contributions are in the field of development methodologies and processes, with the aim of providing increasingly effective tools to support the development team during the various phases of the software development cycle, and the software quality, where the focus was on the proposal of approaches aimed at verifying and guaranteeing a high quality of software

Artificial intelligence: in this area, the members of the section intend to explore the potential of machine learning as a tool for recommending solutions to complex problems, automatically verifying software models and the quality of data and procedures for processing them to create models starting from the data.

Virtual and augmented reality: in this area the members of the section explore applications of the most modern technologies in the field of virtual environments to the problem of remote access and maintenance in distant or dangerous environments. In the same field there are studies aimed at improving the quality of life, such as, for example, systems to support individuals with disabilities or limited sensory capacities who can take advantage of computer vision systems. Finally, virtual reality has been applied to archaeological and cultural settings, to allow users enjoying art and archaeological sites.

Cryptographic methods for access control: this area covers both theoretical and practical aspects of information security with particular attention to the modeling, analysis, and implementation of access control systems. Examples of active researches include selecting the most effective descriptors for some biometric characteristics, objectively comparing their performance according to consolidated metrics and modeling and implementing multilevel access control policies using cryptographic methods and formal methods for the analysis of the security of administrative access control policies.

Mobile security: in this area the members of the section address the security of applications for mobile devices such as smartphones, tablets, and wearable devices. A working group has recently been created to tackle the problem of the ever-increasing diffusion of malicious software within devices which, by their nature, are used with high frequency and collect a considerable amount of potentially sensitive data. The working group, in addition to the study of techniques and tools for identifying anomalous behavior in applications running on these devices, also has the objective of proposing technological solutions aimed at increasing awareness in the use of sensitive information and the perception of risk by the end.

Distributed Ledger: in this area, the section aims to study new application areas, with reference to the field of industrial applications, blockchains and smart contracts. Examples of areas in which the advantages of similar technologies have been studied are those of supply chain management, agri-food, finance, and the biomedical.

Parallel computing: in the context of parallel computing, the section aims to propose a solution to problems related to the creation and verification of concurrent programs running on distributed systems or in the cloud. In particular, the problem to be addressed in this area is that of the complexity in the development and verification of correct, scalable, and efficient concurrent programs, a complexity linked to the high number of possible concurrent executions that must be considered.

Computational mathematics: in this area, the members of the section aim to apply mathematical models to different fields, such as digital image processing and cryptanalysis.
Collaborative environments for learning: this line of research involves the design, implementation, and testing of a digital collaborative environment useful for studying and understanding the arguments, both at process and product level, of students in solving a mathematical problem within a story. The objective of the activity is to study the dynamics, reported through collaborative scripts by the students, useful for producing and justifying the solution of a mathematical problem.
Computational statistics: in this area, the section aims at proposing statistical models and applying them to phenomena of a different nature, in the biological, epidemiological and environmental fields.
Legal informatics: this area includes the study and in-depth analysis of some legal-social implications connected to the process of desubstantialization of law entailed by the legal-positivist and functionalistic-systemic theories prevalent in today's technical age. The main objective is to thematize a "law by principles", which can contribute to the understanding and refinement of the "ratio" underlying the current "law by rules", according to a substantial and not just formalistic legal logic, more suited to the needs semantic values emerging from the growing technological development.

RESEARCH IMPACT
The research activity of the Computer Science and Mathematics sectio, both basic and applied, has resulted in a high-level scientific production and a highly developed third mission activity. The intent is to continue and expand current research lines as well as add new ones, increasing both scientific knowledge and technology transfer, participating in competitive calls, providing technical consultancy, raising awareness in public opinion, supporting decision-making processes at national, regional, and local levels.
In terms of the third mission, the section contributed to the creation of two academic spin-offs, Datasound and BB-Smile, participated in the creation of the Stat Group-19 and the first Center of excellence on data Science and Machine Learning, applied to Medicine and Finance. Among the projects with the greatest impact on society we can mention the ATTICUS project, which aims to create a hardware/software system based on artificial intelligence capable of constantly monitoring an individual and warn in case of anomalies concerning both his state of health and his behavior, and the Nova Tellus project which aims at developing an innovative IT solution, integrated with new technologies and processes, for precision farming and sustainable agriculture capable of being applied in different contexts.

Posters:

Proof-of-concept or early-stage studies with preliminary results

Isolation and characterization of hydrocarbonoclastic bacteria for bioremediation

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Petroleum hydrocarbons include several hundred hydrocarbon compounds originating from crude oil and consisting mainly of hydrogen (H) and carbon (C). They are divided into volatile petroleum hydrocarbons and extractable petroleum hydrocarbons. While the former group includes small-chain hydrocarbons such as benzene, toluene, ethylbenzene and xylene (BTEX), the latter includes long-chain hydrocarbons and polycyclic aromatic hydrocarbons (PAHs). On a structural level, aromatic hydrocarbons are considered mainly recalcitrant in nature and classified as priority contaminants [1], causing carcinogenic, mutagenic, neurotoxic and immunotoxic effects on living beings [2, 3].

Nowadays, industrialization and urbanization have determined an increase of the demand for petroleum hydrocarbons and, accordingly, of the risk for air, soil and water pollution, because of oil extraction and fuel transport accidents. Therefore, due to the increasing number of hydrocarbon-contaminated sites, it has become essential and a priority to develop effective strategies to restore environmental quality.

To restore contaminated sites, biological technologies, such as *bioremediation*, can be used, since they are more cost-effective, environmentally friendly, and socioeconomically acceptable to communities [4] than traditional physico-chemical techniques.

The main aim of this research was to isolate and characterize hydrocarbonoclastic bacteria from two natural hydrocarbon outcrops located in Val d'Agri, Basilicata region, whose existence is known since the late 19th century [5]. These outcrops have always aroused curiosity in the scientific community, interested to study the adaptive strategies of microorganisms, plant and animal species living in an environment "chronically contaminated" by hydrocarbons.

The isolation and characterization of hydrocarbonoclastic bacteria is a key strategy for the bioremediation of hydrocarbon-contaminated sites.

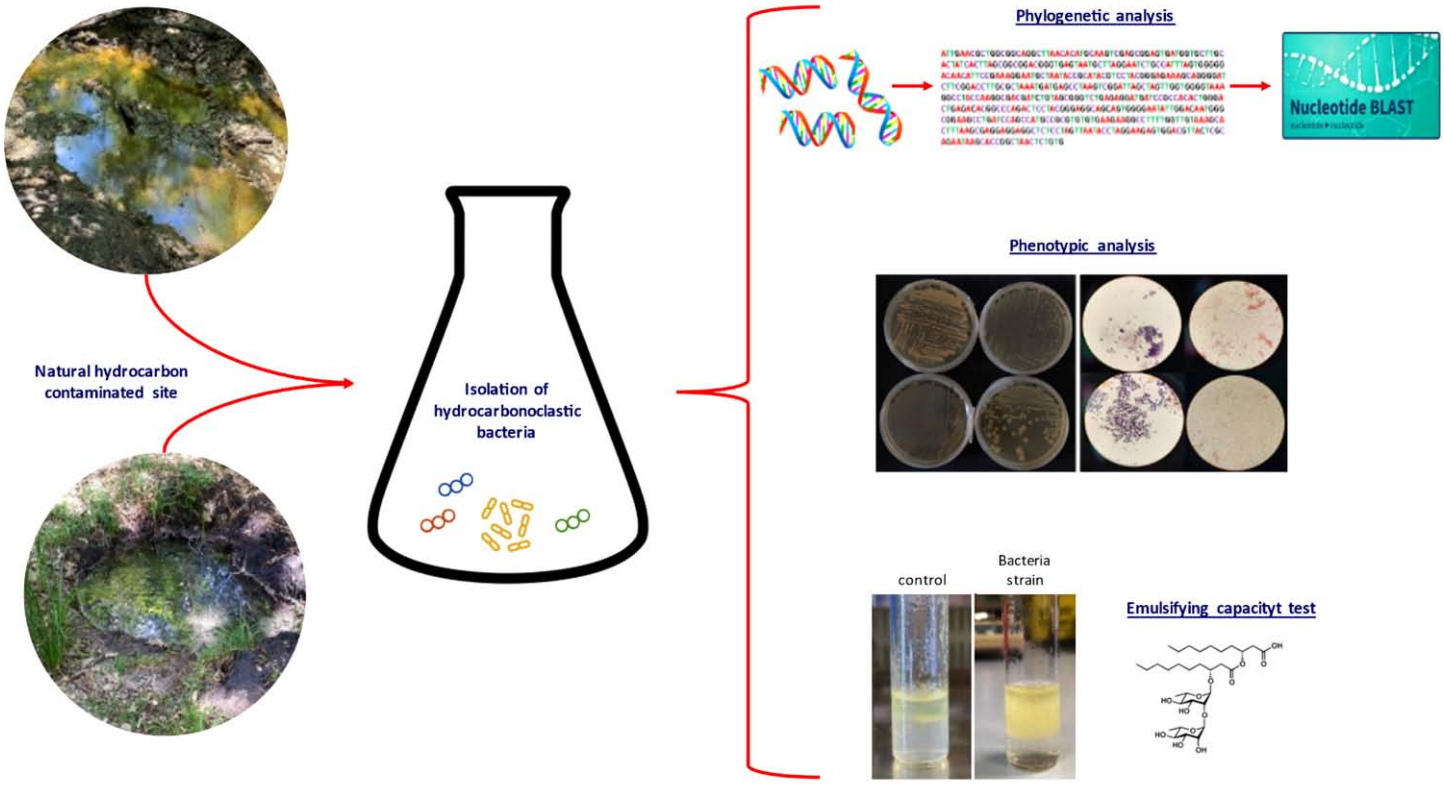
Six different strains of aerobic hydrocarbonoclastic bacteria, belonging to the genera *Stutzerimonas*, *Acinetobacter*, *Rhodococcus*, *Gordonia* and *Comamonas* and capable of growing in minimal medium with diesel oil as the sole carbon source, were isolated. These strains are currently being characterized from a morphological, physiological, biochemical and genetic perspective.

Tests to verify their capacity to produce biosurfactants have been conducted.

Biosurfactants are amphiphilic secondary metabolites with both hydrophobic and hydrophilic groups that possess significant surface and interfacial activity. Due to these properties, biosurfactants tend to solubilize complex hydrophobic compounds and other contaminants in aqueous system. Surfactants derived from microorganisms are an effective choice because of their structural diversity, low toxicity and higher biodegradability compared with their synthetic counterparts [6,7].

The results obtained from emulsifying capacity (EC) and emulsion index (EI) assays showed that bacterial strains were able to produce emulsions with diesel fuel with high stability over time.

These preliminary results pave the way for further investigations aimed at defining in more depth the degradation capacity of the isolated bacterial strains, and/or microbial consortia, for the remediation of hydrocarbon-contaminated sites and their ability to produce molecules with a promoter effect for the removal of petroleum hydrocarbons.



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Addressing the Target Identification and Accelerating the Repositioning of Anti-Inflammatory/Anti-Cancer Organic Compounds by Computational Approaches

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In silico methods play a key role in the early stages of the drug discovery development processes [1, 2]. In this field, a high interest has been ever devoted to organic natural products (NPs) and their derivatives, in particular for the development of multi-target drugs. On the other hand, NPs usually show important limitations, such as the low yields, unknown biological targets, and difficulties in the structural modifications and in the total synthesis. Accordingly, the re-investigation of already reported natural products ("old" NPs) with lacking structural/biological information represents a valid and solid strategy to obtain new applications of known compounds and for accelerating the discovery of new pharmacological agents. In a hypothetical flowchart aimed at extensive investigation of such agents (Figure 1), further key steps to be accounted concern the elucidation of structural chemical features. For instance, data regarding the conformational and configurational behaviors of organic compounds can be obtained through the combination of experimental methods (e.g., NMR spectroscopy and electronic circular dichroism (ECD)) and computational techniques, usually at the quantum mechanical (QM) level (QM/NMR/ECD integrated approach). The correct elucidation of the structural features of "old" and "new" NPs represents the early key step for accelerating their complete biochemical and pharmacological characterization, with the final goal to use this information for the development of semi-synthetic/synthetic derivatives with optimized potency and selectivity towards specific macromolecules involved in pathological events in the inflammatory/cancer field (Figure 1) [3].

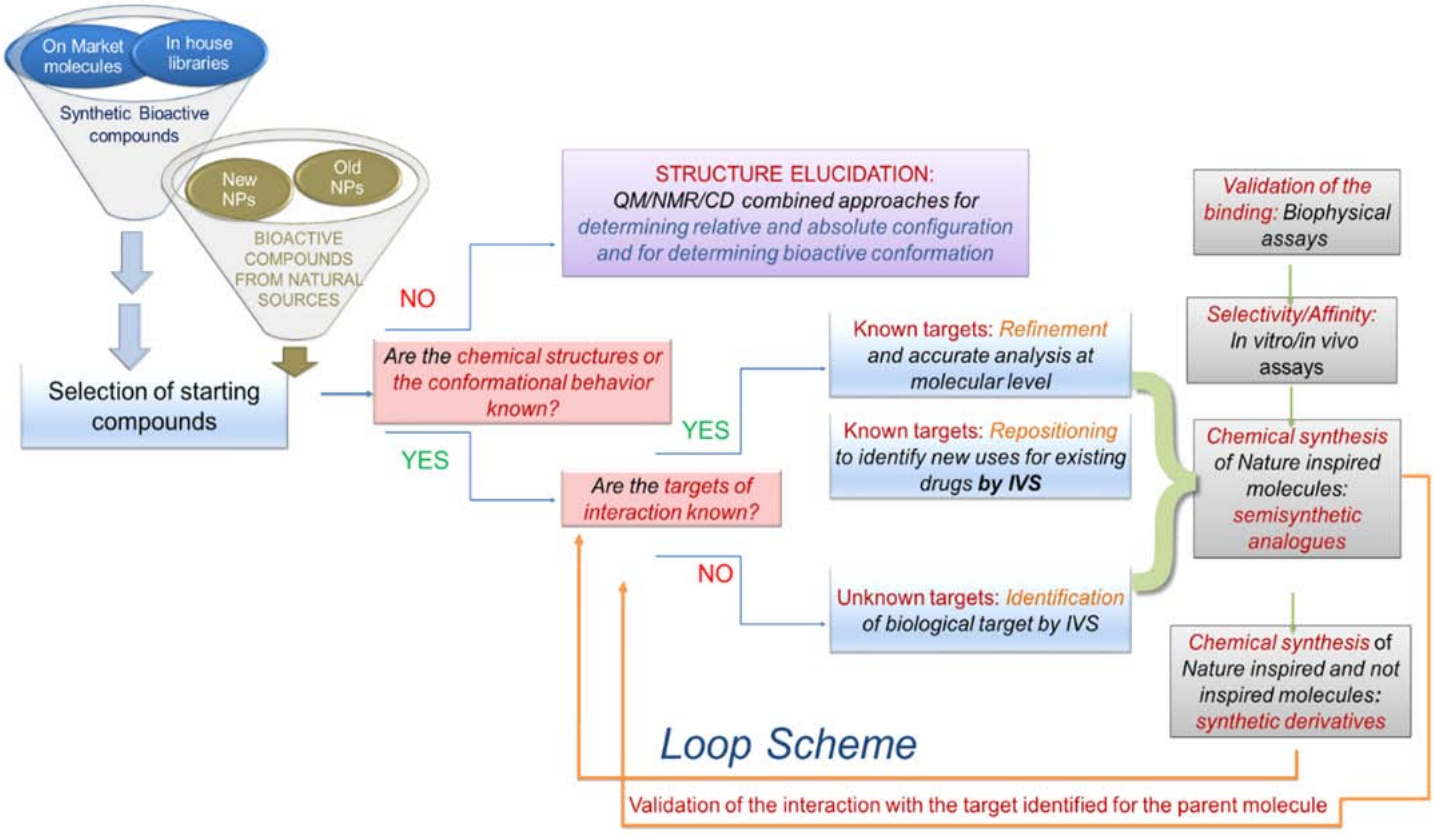

Figure 1. Schematic workflow for the design, development, and repositioning of organic compounds from both natural and synthetic sources.

Prompted by the interest in discovering the potential interacting targets of the investigated compounds, also useful for achieving the drug repurposing task [3, 4], we introduced and implemented the Inverse Virtual Screening (IVS) approach [5, 6]. This tool emerged as the *in silico* prototypical technique to disclose the most likely target(s) of a known small molecule, which is crucial for understanding the mechanisms of action and for developing new drug candidates. In this way, IVS can quickly orient the subsequent biological investigations, such as the specific binding assays of the investigated compounds with the identified putative interacting proteins as well as subsequent in vitro and in vivo specific pharmacological tests. It is worthy of noting that such computational tools are becoming even more interesting in the drug repositioning, namely the identification of new uses for approved or investigational drugs that are outside the scope of the original medical indication or selected research field [7]. Considering the low number of approved drugs, substantial costs, and slow pace of new drug discovery and development, the repurposing of "old" drugs to treat both common and rare diseases is becoming an attractive strategy, because it involves the use of de-risked compounds featuring feasible development costs. Also, computational methodologies represent key approaches not only for clarifying how a given compound exerts its activity, e.g., elucidating the molecular basis behind its interaction with a precise target at a molecular level, but also for shedding light about its ability to bind more than a single target (polypharmacology).

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AUTOMATING PLANT STRESS DETECTION USING IMAGE ANALYSIS

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Plants live in constantly changing environments that are often unfavorable or stressful for their growth and development. The study of plant responses to these conditions is traditionally based on destructive methods, that predominantly focus on biochemical and molecular approaches [1-3]. The investigation based on visible symptoms is a low-invasive method but heavily relies on the experience of plant biologists, which may incur errors due to the occurrence of confounding symptoms [4]. Moreover, manual processes are tedious, time-consuming, and suffer from inter and intra-operator variability [5]. The present study aims to address these problems using image analysis combined with explainable machine learning approaches, in order to automate the process of plant stress identification, classification, quantification, and prediction. Actually, regarding abiotic stresses, only a few examples of machine learning models exist in the literature, that can recognize plants from RGB images, extract relevant morphological traits (geometrical/colorimetrical), and use these data to predict plant health status [6, 7]. Thus, it is critical to implement and improve these models. To reach these goals, we propose the use of digital phenotyping based on image analysis and its comparison to standard laboratory analytical techniques. The first goal of this study is to obtain a robust pipeline for the model species *Arabidopsis thaliana*. The same protocol will be subsequently implemented to study other species (e.g. plant species with agro-industrial or officinal relevance). Generalizing the model will allow us to: (i) identify and ensure optimal growth conditions; (ii) increase yield and productivity; (iii) improve the efficiency and sustainability of management procedures; and (iv) predict plant responses to extreme disturbance levels.

To achieve the first goal, a pot experiment was conducted using *A. thaliana* plants exposed to salt stress. Two different growth media (soil and perlite) and two different treatments ("medium" and "high" stress, 50 mM and 150 mM NaCl concentrations, respectively) were tested. As summarized in Figure 1, plants were subjected to specific analyses for the measurement of biochemical and morphological parameters. The data obtained were then analyzed using Student's t-test and Principal Component Analysis. Our results showed that response patterns can vary depending on the growing condition and the influence of the substrate type on the development of plants. Overall, *A. thaliana* plants subjected to stress conditions were generally smaller (in terms of area, perimeter, major axis, and minor axis of leaves and rosettes) and presented higher chlorosis levels than the untreated plants. With regard to biochemical parameters, in response to the highest NaCl concentrations, Relative Water Content (RWC) decreased with Dry Weight (DW) values, while Electrolyte Leakage (EL) increased. Differences were mostly significant ($p \leq 0.05$) between the untreated and high-stressed plants. On the contrary, the results obtained for the medium-stressed plants were not always significantly different from any of the other conditions. The PCA (PAST, v.4.03) performed on this data confirmed the observations: the scatter plot built from PC1 and PC2 (consisting of features derived from automated image analysis) showed a strong separation between the two different substrates (soil and perlite) and between the untreated and salt stress-treated plants, but not between the two intensity levels of stress. These assumptions were further confirmed by training and testing machine learning models (Weka, v.3.8.6) for classifying the stress presence (2-classes/binary) and level (3-classes). The highest levels of accuracy were obtained, for the 2-class model, using the *Decision Stump* tree (90%), and, for the 3-class model, using the *Random Forest* classifier (73%). We might assume that EL is probably one of the key features since the principal decision nodes are based on this parameter. Nevertheless, the 3-class model still shows some confusion in identifying the medium-stress class.

Preliminary results suggest that the expansion of the dataset is necessary to aim to completely avoid destructive methods, prevent overfitting and concurrently improve the accuracy, robustness, and reliability of the predictive model. Upcoming perspectives will thus include larger-scale experimentations, identifying and selecting relevant

morphological traits with higher predictive power, and the model's generalization to various species and abiotic stress conditions.

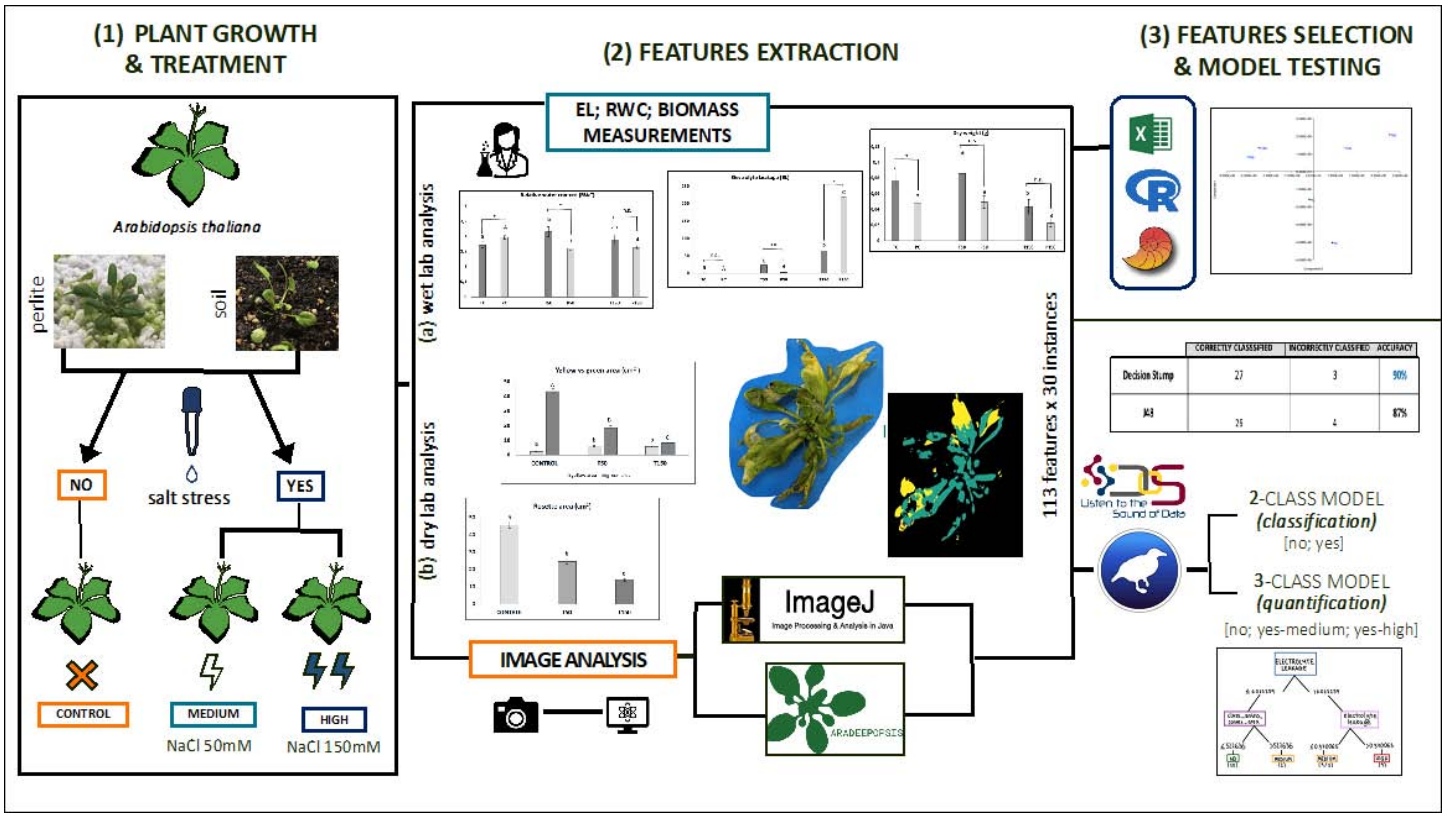

Fig. 1. The experiment's three steps: (1) A. thaliana plants grown in different conditions (optimal/adaptogen/stressful); (2) measurement of (a) biochemical - Electrolyte Leakage (EL), Relative Water Content (RWC), biomass - and (b) morphological parameters (using ImageJ v.1.53 and Aradeepopsis v.2.0 on RGB pictures); (3) analysis of extracted features and their use in constructing a model of classification and quantification for stress detection.

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A COMPARATIVE STUDY ON CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OIL AND METHANOL EXTRACTS FROM SEEDS OF DAUCUS CAROTA L. SUBSP. CAROTA GROWING IN THE CENTRAL APENNINES (ITALY)

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For several decades there has been a growing interest in natural products from medicinal plants as an alternative to synthetic drugs. This explains why, a large number of plant species are being studied with the aim of finding both natural bioactive compounds and green, sustainable alternatives that reduce or eliminate the use of dangerous substances in daily life.

The purpose of this study is to determine the phytochemical composition and biological activity of essential oil and methanol extracts of *Daucus carota* L. seeds (subsp. *Carota* Apiaceae family) (Fig. 1).

Essential oils are a complex mixture of chemical components with great interest, both for scientific aspects and for applications in pharmacology. The determination of the chemical composition of EOs isolated from carrot seeds by GC-MS will be performed and the determination of mineral contents (Al, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Se, Sr, V and Zn) by ICP-AES is also planned.

Although this plant has been studied [2-5], some scientific reports do not refer to subspecies, a crucial aspect of this polymorphic species, which is very common in Europe where at least 19 subspecies have been described [6]. Moreover, from the literature search of the last two decades concerning the ethnobotany of the Apennines, little data have been found [7] and the use of wild carrot in "folk medicine" over time seems to be completely neglected.

Fig. 1. Daucus carota L. subsp. carota - Scheda IPFI, Acta Plantarum.

At present, the in vitro antioxidant activity (DPPH method) of essential oils and methanol extracts of wild *Daucus carota* L. subsp. *carota* seeds (Fig. 2), collected in the central Apennines (Italy), Capracotta (Is) and the total phenolic content of the methanol extract of the seeds (Tab. 1) has been analyzed.

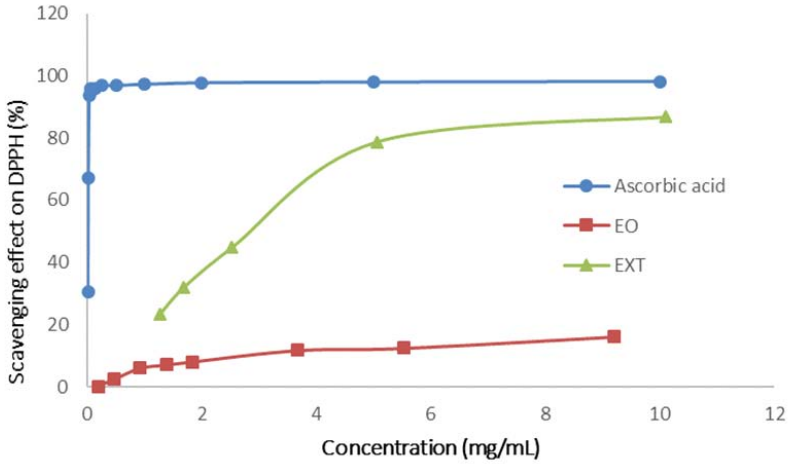

Fig. 2. DPPH radical-scavenging activities of essential oil (EO) and methanol extracts (EXT) of wild Daucus carota L. seeds and standard (Ascorbic acid) measured at different concentration.

Tab. 1 Antioxidant activities and total phenolic content of methanol extracts of wild Daucus carota L. seeds. IC_{50}: 50% inhibition concentration.

	DPPH IC_{50} (mg/mL)	*Total phenolic content (mg GAE/g)*
Daucus carota L. seeds	2.92	1.44

These data will be compared with those obtained from *Daucus carota* seeds from other geographical areas.

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Role of the Autophagy Regulator ULK1 on Glioblastoma Biology

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Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumor in human characterized by a poor prognosis despite the existence of multimodal therapy [1]. This could be explained by GBM chemoresistance, heterogeneity, and infiltrative patterns that make it challenging to complete tumor resection [2, 3]. In 30 years the only significant improvement in overall survival occurred with the introduction of Temozolomide (TMZ) in addition to surgery and radiotherapy [4,5]. As the response of GBM tumors are not quite evident towards these standard of cares, there is an immense need for novel treatment methods [6]. The Cancer Genome Atlas (TGCA) research network recently analyzed whole-genome sequencing of GBM tumors highlighting epigenetic mechanisms and main pathways deregulations [7]. Understand in detail the molecular mechanisms involved in the onset and progression of this aggressive form of brain malignancies may therefore reveal new approaches for development of more effective therapies [8]. Particularly, transcriptomic analyses from TGCA and REMBRANDT (Repository of Molecular Brain Neoplasia Data) have shown lower expression of some autophagy related protein with an intriguing down-regulation of the autophagy initiator ULK1 and an accumulation of the autophagy substrate p62. Consistently, a turning off of ULK1 expression has been observed between GBM cells grown as monolayers and those cultured as tumorspheres, thus suggesting a progressive decrease in autophagy proficiency during glioma progression. Otherwise, there are also opposing evidences of an increased autophagic rate associated with an increased level of autophagic protein in high-grade gliomas rather than low-grade glioma and normal tissue [9]. Therefore, clarify the pro-tumorigenic or the tumor-suppressive role of autophagy in Glioblastoma onset and progression is still an open challenge.

Experimental plan

In order to assign an established role to autophagy in glioma biology, it will be performed a monitoring of main tumorigenic properties of some *in vitro* GBM models, modulating autophagic flux proficiency through an ULK1 dosage expression. GBM primary cells, not expressing ULK1, will be transduced with cDNA encoding for wild type ULK1 by lentiviral infection to obtain an efficient and stable protein expression and their capability to proliferate and to migrate, in presence and in absence of TMZ, will be evaluated in comparison with non-infected cells. In detail, proliferation, colony-formation and migration/chemotaxis assays will be performed, by means of tools and protocols well consolidated in our lab [10-13]. Apoptosis occurrence will be also evaluated by nuclear fragmentation/condensation and by western blotting analysis of Caspase 3 and PARP cleavage. Proliferation, migration and apoptosis analyses will be also performed on ULK1-silenced GBM cell lines (e.g. U87MG and GL15) in order to evaluate the response to TMZ in different ULK1 genetic settings. If an effect of ULK1 dosage on cellular response to TMZ will be confirmed, experiments will be also performed upon ectopic expression of the ULK1 kinase-dead construct, which is unable to induce autophagy, in order to discriminate between an autophagy-dependent or-independent role of ULK1 in GBM drug response. Because of ULK1 fine modulation in colture [9], it will be investigated molecular mechanisms by means ULK1 down-regulation occurs during gliomagenesis. It will be assessed the hypothesis of a decreased transcription of ULK1 gene due to a promoter hypermethylation via measuring ULK1 transcript and protein level in presence and in absence of a methyl-transferase inhibitor on GBM cell line (U87MG), GBM primary colture (GH2) and their tumorspheres. In a second moment it will be also evaluated ULK1 stability treating GBM cells colture and tumorspheres with cycloheximide and measuring ULK1 protein levels by western blotting at different time point after the treatment with protein synthesis inhibitor.

Expected results

The expected results from this project are: a) to identify a tumor suppressing role for autophagy in glioma onset and progression by a negative influence on main oncogenic properties; b) to clarify the mechanism of ULK1 down-regulation during gliomagenesis.

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Investigating the woody roots' early response to mechanical stress

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The mechanical stresses that impact plants' development and stability take various forms including wind, herbivory, gravity alteration, compacted soils, etc. When roots encounter some of these mechanical constraints, the stability of the plant and its potential to ensure water and nutrient uptake are threatened. Thus, as a response to these challenging environments, plants have developed complex stress perception mechanisms that occur through three subsequent phases; (i) an early sensory period of stress perception and gene expression (seconds to hours), (ii) a period of phytohormone signaling and metabolic feedback; and (iii) a phenotypic plasticity phase that facilitate s the stress acclimation [1]. Previous studies have shown that prolonged bending stress (2-14 months) induces sector-specific responses in woody poplar taproots [2, 3]. This asymmetrical response includes lateral root emission on the convex and reaction wood formation on the concave side, both temporally and spatially modulated by a complex interplay between different signal transduction pathways involving reactive oxygen species (ROS), hormones, and specific molecular factors regulating lignin deposition, cell wall integrity, and lateral root formation [4-6].
However, substantial research gaps regarding woody roots' response to mechanical stresses remain, particularly regarding how these roots respond to short-term stress bending.

Hypothesis
We hypothesize that short-term root bending is perceived by specific factors named "mechanosensors" triggering a complex cascade of secondary messengers, channelled through a network of calcium channels and finally resulting in stress-specific transcriptional changes. The present study aims to (i) identify the specific stress perception mechanisms and signalling pathways, and (ii) reveal of the early transcriptomic response to short-term bending stress. By using both a model herbaceous plant species (*Arabidopsis thaliana*) and a woody tree model specie (*Populus nigra*) (Fig. 1), we also (iii) aspire to generate a comparative transcriptional network analysis, to identify any conserved plant strategy to sense and respond to root mechanical stress.

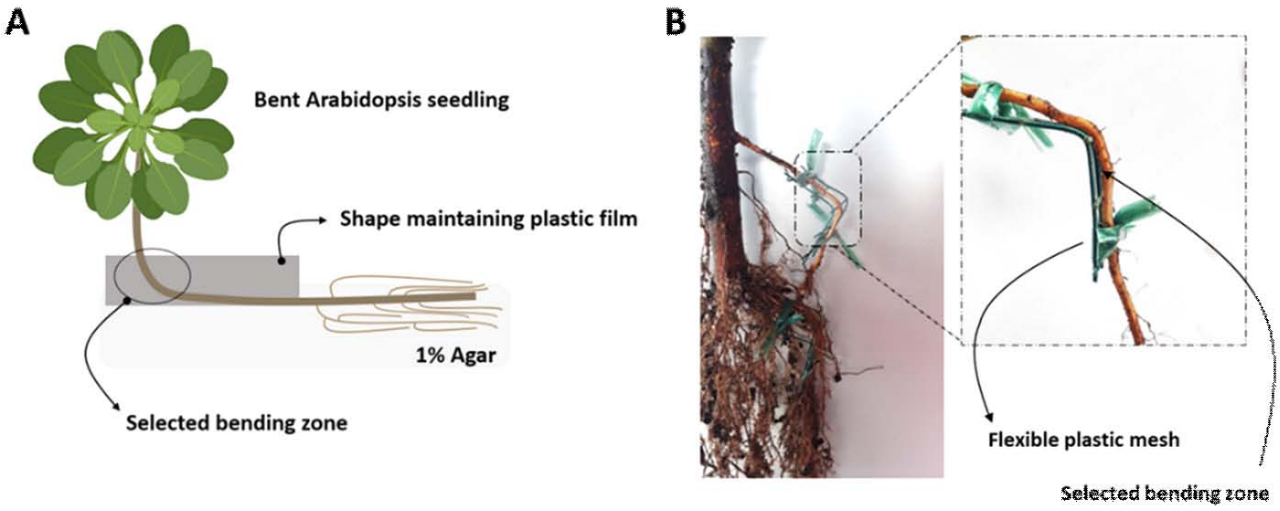

Fig. 1. Bending experimental setup for both model species (A: Arabidopsis, B: Poplar)

Preliminary results
In *A. thaliana*, the RT-PCR analysis of relative gene expression levels of calcium and mechanosensitive ion channels (Cyclic nucleotide-gated channels (CNGCs) CNGC6, 10, and 17, and the mechanosensitive Mid1-Complementing Activity (MCAs) – (MCA1, 2) revealed a potential upregulation in the relative gene expression levels, following a

short-term bending. This was especially for the case of CNGC6, where a significantly higher expression was observed during the analysed time points (Figure 2). This preliminary observation suggests an early potential activation of the calcium channel CNGC 6 in response to mechanical stress.

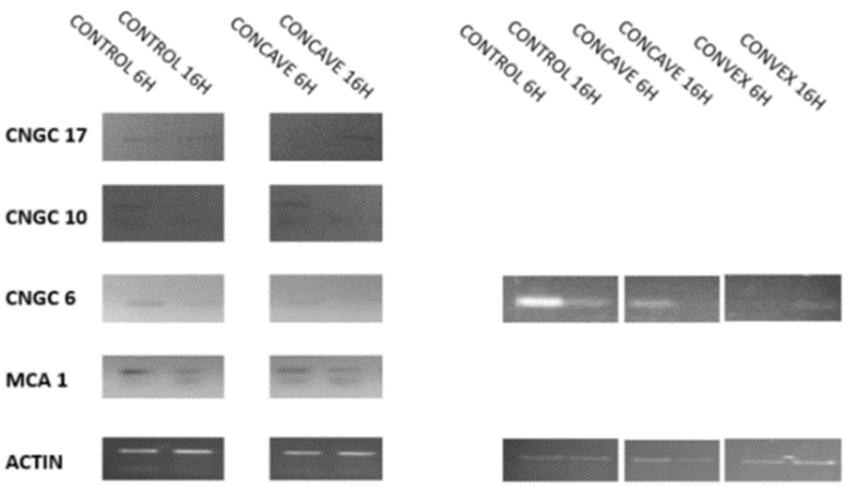

Fig. 2. RT-PCR analysis of calcium-related channels (CNGC 17, 10, 6, MCA 1, 2) in Arabidopsis thaliana.

Future perspectives

RNA sequencing of bent poplar and *Arabidopsis* woody root tissues will identify the differentially expressed genes in early response to bending constraint. Besides, drawing a comparative network between the herbaceous and woody plant models will allow for the identification of differences and similarities between the two model species, in terms of their response to mechanical stress. Furthermore, using a genetically encoded calcium indicator named GCAMP3 [a synthetic fusion of green fluorescent protein, calmodulin and a peptide sequence from myosin light-chain kinase], we will be able to track *in vivo* the calcium flow in the area of interest (different woody root sectors and sides) in response to the short-term bending treatment. Therefore, we will be able to determine the appropriate time interval on which to focus our research. Similarly, tracking another secondary messenger, such as reactive oxygen species (ROS), will help us better understand signatures related to the root's response to mechanical stress, and correlate these observations with RNA sequencing data.

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Lavender essential oil modulates cholesterol metabolism in the hepatic cell line HepG2

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The proper homeostatic maintenance of lipid metabolism is essential for several key cellular processes involved in numerous biological functions. In particular, cholesterol is known for its important structural and functional roles, being the precursor of steroid hormones, vitamin D, and bile acids and regulating the assembly of specialized membrane microdomains. Therefore, it is not surprising that the body employs an intricate homeostatic control to assure the correct cholesterol supply for cells and tissues. This control primarily occurs in the liver, where most lipid metabolism takes place [1]. In fact, hepatic cells are specialized to produce the largest amount of cholesterol and fatty acids in the body.

Cells regulates intracellular cholesterol levels by relying on a delicate balance between *de novo* biosynthesis, uptake, efflux, and storage systems, which guarantee the homeostatic maintenance of this lipid [2]. One of the main proteins that ensures homeostatic balance of cholesterol is the sterol regulatory element binding proteins 2 (SREBP2): when intracellular cholesterol levels decrease, the nuclear fragment (nSREBP) generated by proteolysis promotes the expression of key genes involved in cholesterol biosynthesis and uptake.

Increasing evidence reports that defects in cholesterol metabolism are involved in different pathological conditions such as cardiovascular disease, several types of cancer and neurodegenerative diseases [3]. Therefore, the identification of natural compounds capable of modulating cholesterol metabolism has long attracted biomedical research. Several molecules found in essential oils (EO) have demonstrated cholesterol-lowering properties [4], furthermore Rabiei and colleagues have also observed that the dietary extract of *Lavandula angustifolia* reduces serum cholesterol in rats [5]. However, the molecular mechanisms elucidating these properties are still poorly characterized. Therefore, the aim of this work is to evaluate whether lavender EO (LEO) modulates cholesterol metabolism in a liver cell line. To achieve this goal, we used the HepG2 cells as experimental model, since this cell line strongly retains hepatic metabolic functions.

First, we analyzed the putative effects of LEO on intracellular cholesterol. HepG2 cells were treated with LEO at a concentration of 0.005% (v/v) for 24 hours and then subjected to filipin staining, a gold standard method for assessing intracellular cholesterol. The analysis showed that LEO treatment promoted an accumulation of intracellular free cholesterol (*Fig 1A*). In addition, the data was confirmed by the colorimetric enzymatic test (*Fig 1B*), which showed that intracellular total cholesterol was higher in LEO-treated HepG2 cells.

Next, we evaluated whether LEO could affect SREBP-2, the major transcription factor of cholesterol homeostasis. Immunofluorescence and confocal analysis showed that overall SREBP-2 immunoreactivity significantly increased in LEO-treated cells, particularly at nuclear level (*Fig 1C*).

Our results demonstrate that LEO promotes cholesterol accumulation in hepatic cells. This effect is accompanied by an increased activation of SREBP-2. Even though these data provide new indications on the biological activities of LEO, it would be interesting to better characterize the molecular mechanisms participating in the observed modulations.

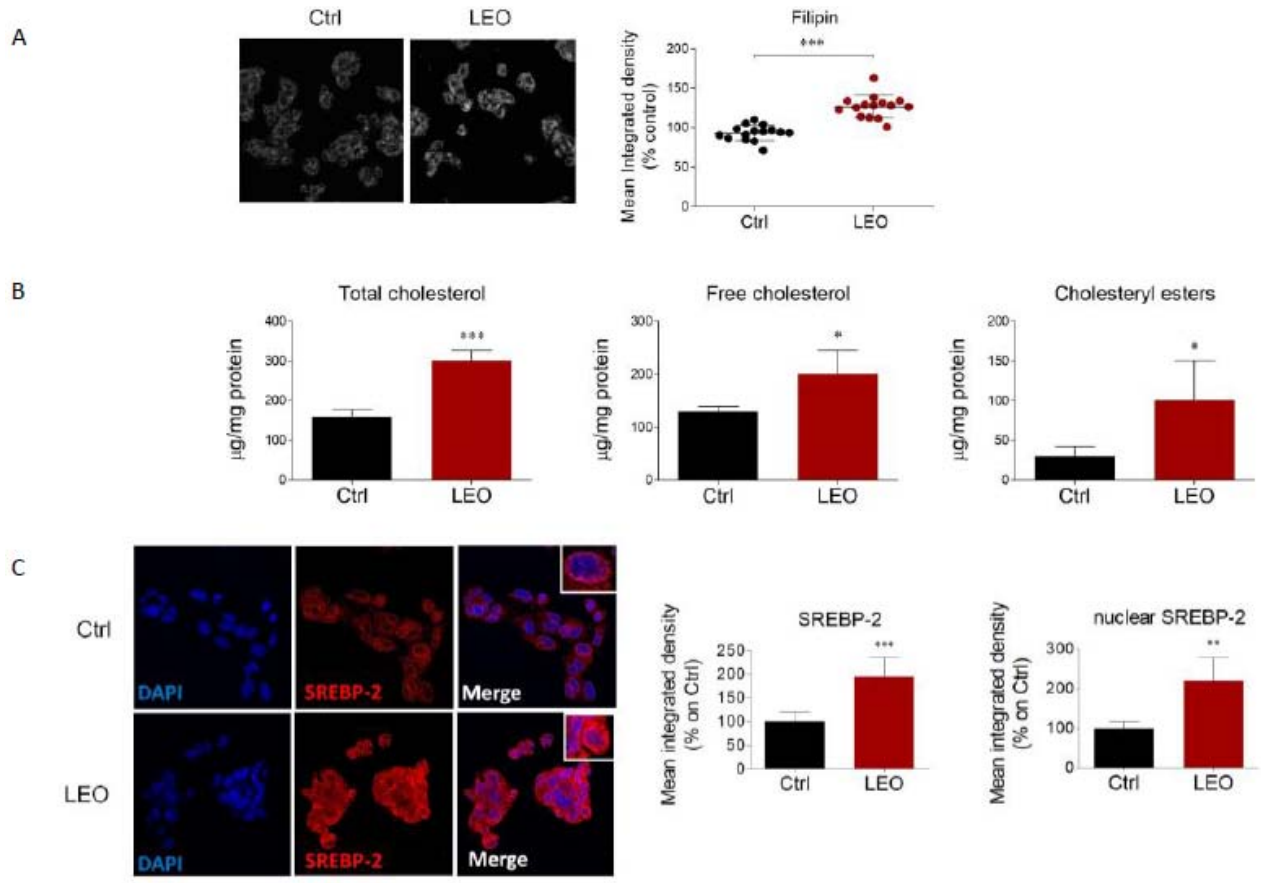

*Fig. 1. LEO accumulates intracellular cholesterol and enhances the expression of SREBP-2 in HepG2 cells. (A) Representative images and signal quantification of filipin staining performed on HepG2 cells after treatments with vehicle (Ctrl) and LEO (0.005%) for 24 hours. n = 15 experimental replicates. (B) Quantification of total cholesterol, free cholesterol, and cholesteryl esters in vehicle- and LEO-treated HepG2 cells. n = 5 different experiments. (C) Representative immunofluorescence images and quantitative evaluation of the total and nuclear fluorescence intensity of SREBP-2 in HepG2 cells stimulated with vehicles (Ctrl) and LEO (0.005%) for 24 h. n = 7 different experiments. Data represent means ± SD. Statistical analysis was assessed by using unpaired Student's t test. * p < 0.05; ** p < 0.01; *** p < 0.001.*

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Development of an artificial poly(HEMA) lenticular capsule to contain the artificial lens by 3D bioprinting

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The lens is a biconvex lens located in the eyeball between the iris and vitreous body. It is one of the main components of the dioptric system of the eye, together with the ciliary muscle responsible for accommodation. The lens presents a problem, the so-called opacification, due to oxidative phenomena associated with aging (senile cataract), diabetes, prolonged use of cortisone drugs, trauma, exposure to UV rays and glaucoma (an increase in intraocular pressure) [1]. To date, there are no pharmacological therapies capable of healing cataracts to restore transparency to the opaque lens, only microsurgery can heal this pathology, by implanting an artificial lens. The problem arises when the patient is aphakic, i.e., completely devoid of the capsular sac, due to trauma or surgical necessity, and in this case the lens can be implanted either by fixing it to the sclera (scleral suspension) or to the iris (iris suspension). In the very rare case in which the patient also lacks the iris (anirid), the surgical solution is further complicated, with the use of photo-usable materials.
For this reason, the goal of this research is to develop a lenticular device that acts as a capsular bag in all subjects who do not have the natural one in which to deposit the artificial lens during the cataract operation.
In this study, different materials were used for the development of the artificial lenticular capsule. Among all, poly(2-hydroxyethyl methacrylate) was found to be the best, being a biomaterial with excellent biocompatibility and cytocompatibility, eliciting a minimal immunological response from host tissue making it desirable for several biomedical applications. Il poly(HEMA), obtained by polymerization of monomer HEMA, is a biocompatible, optically transparent, hydrophilic and non-degradable polymer, used in the manufacture of soft contact lenses [2]. Figure 1 shows the device design and the finale poly(HEMA) device obtained by 3D bioprinting. The chemical-physical and mechanical properties of this device were analyzed, and the manufacture of this artificial lenticular capsule was the subject of a patent in Italy [3].
In this way, it has been possible to develop an artificial lenticular capsule to contain the artificial lens in all patients who, for various reasons, lack the capsular bag or the iris.

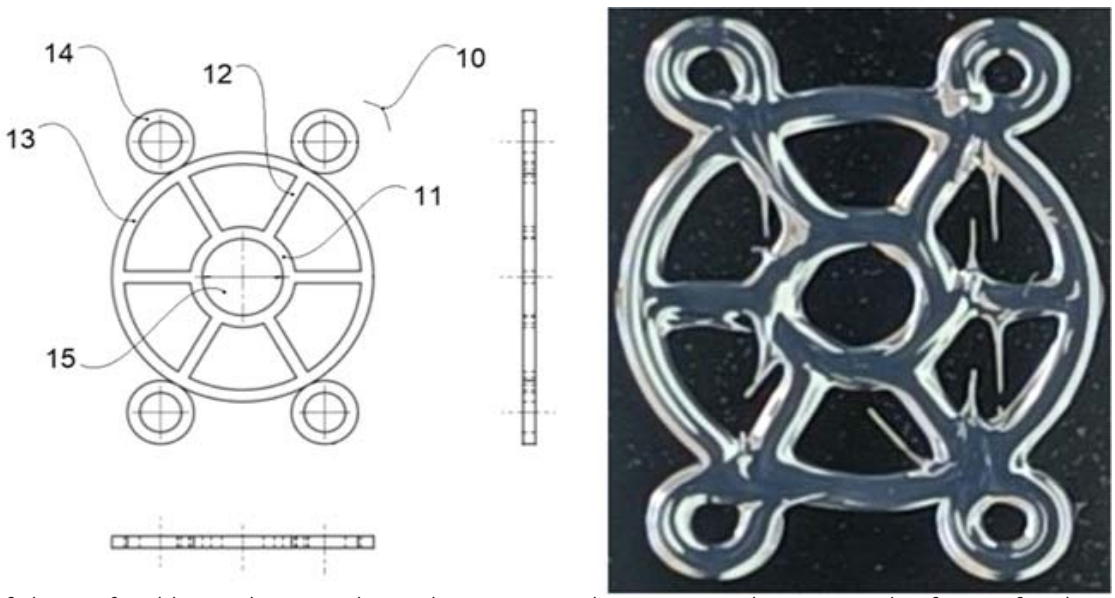

Fig. 1. Design of the artificial lenticular capsule made using a 3D bioprinter with integrated software for the generation of CAD (Computer Aided Design) sections.

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The role of sialic acid in the biological activity of lactoferrin

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Introduction

Lactoferrin (Lf) is a 689 amino acid cationic glycoprotein synthesized at infection and inflammation sites by exocrine glands and by neutrophils. In terms of structure, Lf folds into N- and C-lobes, each of which bears a high affinity iron-binding site (K_d ~10^{-20} M). Iron binding induces large conformational changes which define the open (iron unsaturated, apo-Lf) or closed (iron saturated, holo-Lf) state (1). Since human (hLf) and bovine (bLf) lactoferrins show high sequence homology and share identical multifunctionality, most studies have been carried out using commercial bLf. Notably, bLf is a substance generally recognized as safe (GRAS) by the Food and Drug Administration (2) and approved as a dietary supplement by the European Food Safety Authority (3).

Lf exerts a number of functions including antibacterial, antiviral, anti-inflammatory, antioxidant, and immunomodulatory activities. Its biological function is influenced by glycosylation, the most common post-translational modification that affects protein folding, immunogenicity, protein solubility, and resistance to proteolysis. The glycosylation status can change depending on species, cell type expressing the glycoprotein, amino acid sequence, physiological conditions, and purification strategy. In the case of bovine milk, the main source of commercial bLf, the distribution of glycans differs depending on the breed of cow, the lactation period, the animal's diet, and the glycoprotein purification strategy (4). Five N-linked glycosylation sites are present on bLf (Asn233, 281, 368, 476, and 545) but only four sites are invariably glycated (Asn233, Asn368, Asn476, and Asn545) (5) whereas Asn281 can selectively undergo glycosylation, giving rise to a bLf with higher molecular mass (84kDa vs. 81kDa) (6). BLf possesses two oligomannosidic type (linked to Asn233 and Asn545) and three biantennary N-acetyllactosamine type glycans, partially fucosylated and sialylated (linked to Asn281, Asn368 and Asn476). Sialic acid, usually present in a ratio of about three residues per molecule, could affect the functionality and bioavailability of Lf, however, little effort has yet been made to unravel its influence on the various functions of the glycoprotein (4).

Hence, this project aims to investigate the role of sialic acid on the biological activity of Lactoferrin. For this purpose, a desialylated version of bLf will be produced and tested for anti-oxidant, anti-inflammatory, anti-viral and anti-bacterial activity.

Preliminary results

In order to evaluate the role of sialic acid, a desialylated bLf was produced by treatment with neuroaminidase, an enzyme able to specifically cleave and release sialic acid from glycan chains. The enzymatic digestion was performed at 37°C for 20 h. BLf was purified by a cation exchange chromatography, which allows the selective binding of the glycoprotein and the release of neuroaminidase (pI around 6). The integrity of desialylated protein was examined through SDS-PAGE and Coomassie Blue staining (Fig. 1A). As a control for the experiment, a bLf sample undergoing the same treatment, excluding the enzymatic digestion, was loaded on SDS-PAGE as well.

As shown in figure 1A, each lane presents an 80-kDa band, corresponding to the molecular mass of an intact bLf, suggesting that neither the treatment at 37°C nor the enzymatic digestion altered the integrity of the glycoprotein. To analyze the efficiency of desialylation, a lectin-based Western blot was carried out. It can be seen in Figure 1B that desialylation occurred for the bLf sample treated with neauroaminidase. In particular, the densitometric analysis evidenced the production of a 90% desialylated bLf.

These preliminary experiments show that a good quality desialylated protein can be obtained, which will be employed to investigate the role of sialic acid on the biological activity of Lf.

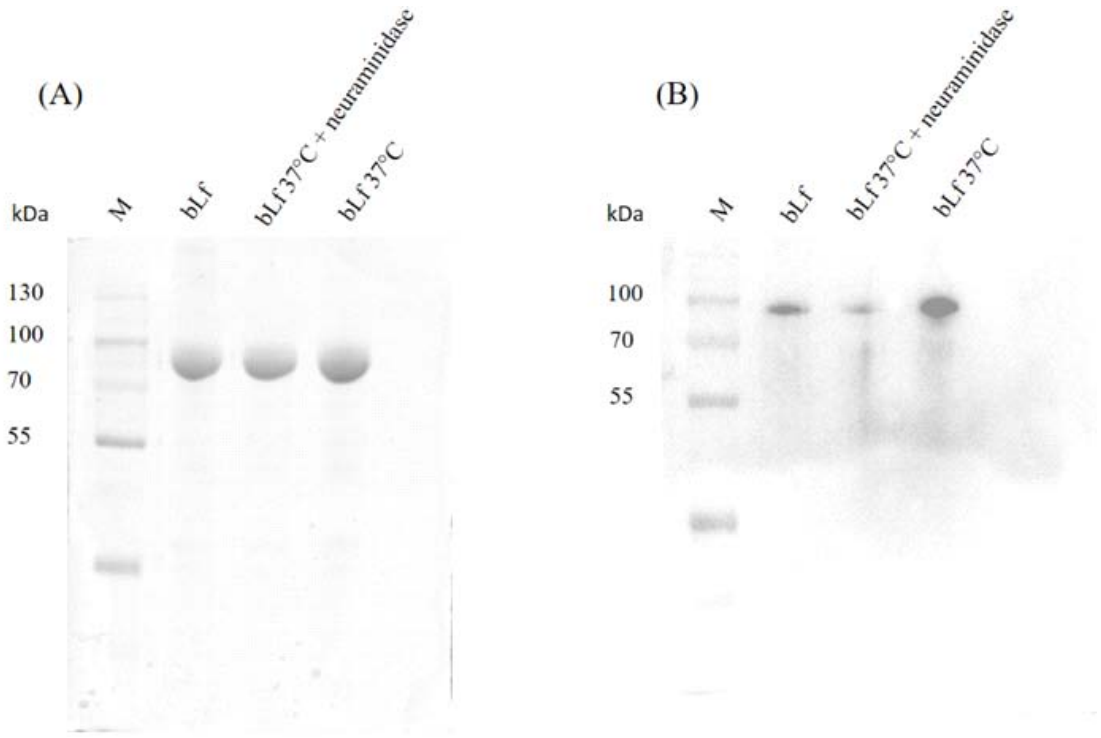

Fig. 1. SDS-PAGE with Coomassie blue stain (A) and lectin-based Western blot (B). Lane 1: native bLf; lane 2: bLf treated at 37°C with neuraminidase; lane 3: bLf treated at 37°C.

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Biodiversity conservation of autochthonous lentil (*Lens culinaris* Medik.) landraces of Molise region

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Agro-biodiversity is a kind of biodiversity, which provide genetic resources relevant to food and agriculture and ecological services that are important for human well-being [1]. However, agro-biodiversity and in particular local varieties are gravely threatened by extinction due to the improper human activities on the environment over the past century, and their replacement by modern genetically uniform varieties [2]. Generally, local varieties are characterized by high genetic variability, specific adaptation to the environmental conditions of the cultivation area (i.e., tolerance to biotic and abiotic stresses) [3], and the presence of secondary metabolites and health-promoting compounds [4]. For example, many legume landraces, such as lentils and common beans, are reported to present higher contents of minerals and oligo-elements than commercial varieties [5, 6]. Thus, their diversity is relevant for food security, not only in nutritional terms, but also due to the content of phytochemicals or secondary metabolites [7]. To preserve autochthonous landraces, their genetic variability, and their metabolite diversity, as well as to make them an ideal alternative for commercial varieties and to draw the interest of local farmers and consumers involved in their conservation, it is very important to identify their unique characteristics [8]. However, local varieties are often totally neglected and still understudied with a consequent lack of scientific knowledge about their characteristic [9].

In this context, the present study aimed to characterize three autochthonous lentil landraces from different villages of the Molise Region - Capracotta, Rionero Sannitico, and Agnone – analyzing them with a multi-integrating approach (morpho-physiological, genetic, and metabolomic analysis), in comparison to three other populations- one from Umbria (Castelluccio di Norcia, IGP), one from Lazio (Rascino) and one commercial variety (Turca Rossa) - (Fig. 1A).

In the first phase, the morpho-physiological analysis was performed through the germination seed test and 9 morphological descriptors of *the International Board for Plant Genetic Resources* (IBPGR) to evaluate the quality of autochthonous germplasm and diversity among populations. The genetic analysis was carried out through 8 Inter Simple Sequence Repeat (ISSR) molecular markers, to define genetic variability and phylogenetic relationships among different populations.

The germination tests results showed that each population was viable and had a good germination capacity, reaching a germination percentage (%G) above 80%, except for the Castelluccio di Norcia lentil (%G < 66.67%; T50 = 6.60 days) (Fig. 1B). Instead, the morphological descriptors showed high similarity among populations, except for the Rascino lentil, that is smaller than the other ones, also confirmed by the Principal Component Analysis (PCA) and clustering analysis (Fig. 1C). The PCA and clustering analysis of the genetic profiles divided all populations into two main groups: one made up of the three autochthonous Molise Region populations and one formed by the other three (Fig. 1D).

Further genetic investigation coupled by metabolomic analysis and *in vivo* testing of bioactive compounds (in collaboration of the Chemistry and Physiology research groups of the University of Molise) will identify relationships among autochthonous populations and explore their characteristics, such as the possible presence of specific phytochemical compounds or secondary metabolites that can be enhanced from a nutritional, nutraceutical, and health point of view, supporting their conservation, and promoting diversity preservation.

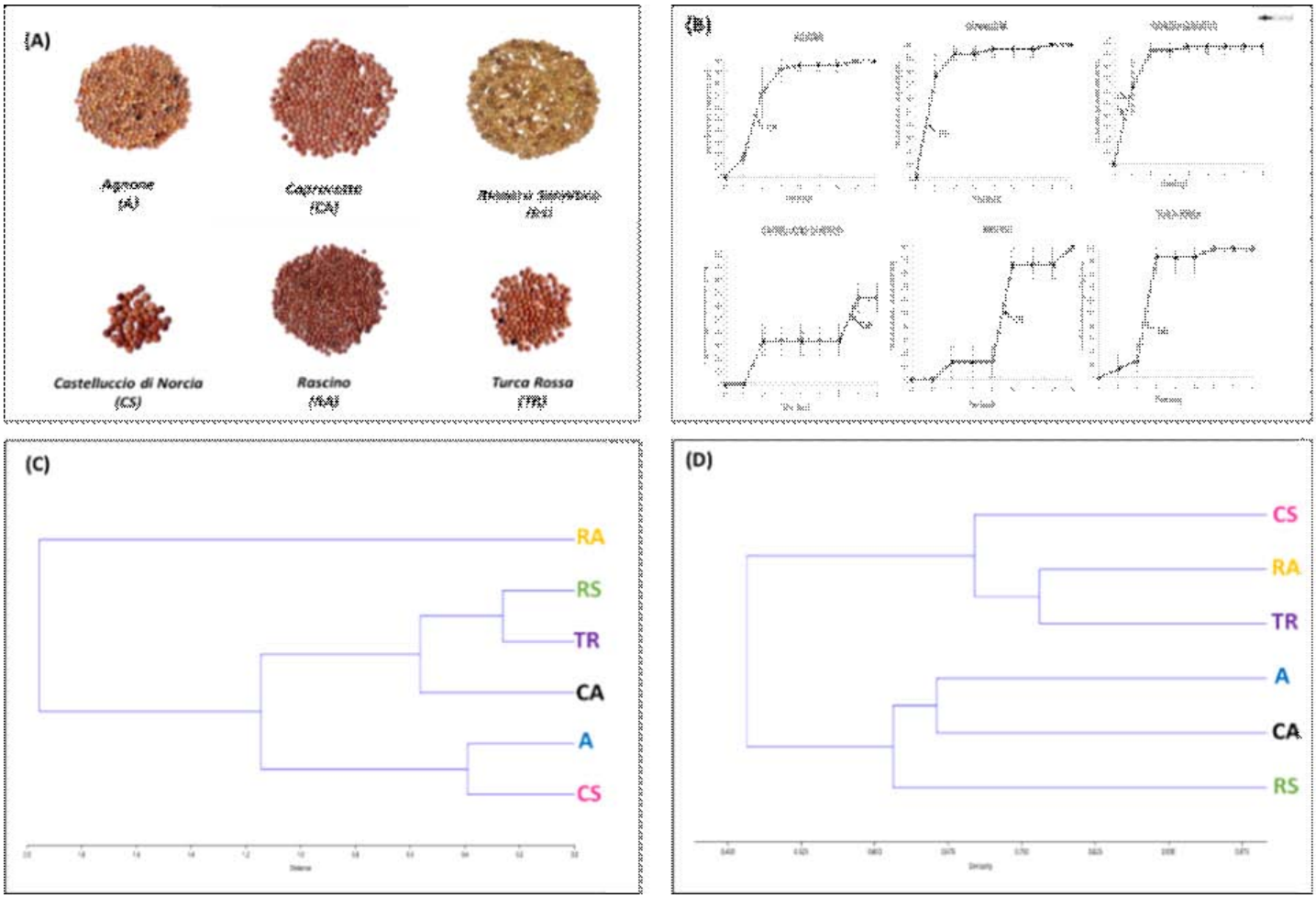

Fig. 1. Results of morpho-physiological and genetic analysis of autochthonous lentil (Lens culinaris Medik.) landraces. A – Autochthonous populations characterized; B - Results of germination tests under control conditions; C - Clustering of morphological parameters; D - Clustering of genetic analysis data.

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The BET protein inhibitor JQ1 modulates autophagy and induces differentiation in glioblastoma cells

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Autophagy is an evolutionarily conserved degradative process used by cells to keep proper homeostasis, and its de-regulation has been associated with different human diseases, including glioblastomas (GBM). We previously demonstrated that autophagy modulation triggers a molecular switch from a mesenchymal to an epithelial-like phenotype in GBM cellular models, likely due to the inactivation of signal transduction pathways, such as the Wnt/beta-catenin and the MAPK/ERK pathway that are both crucial for tumour proliferation, invasiveness and stemness maintenance [1-3]. The involvement of autophagy in tumorigenesis is showed in the literature, highlighting its dual role depending on tumour stage and specific microenvironmental conditions. The regulation of autophagy as a mechanism of death in cancer cells has led to the use of autophagy inhibitors and inducers to block cancer progression. Inhibition of the autophagy process can significantly increase the sensitivity of glioma cells to cytotoxic therapies and enhance the effect of treatments in clinical trials [4]. Due to the mutated genome and hyperactivation of tyrosine kinase receptors, glioma is resistant to various therapies. Autophagy stimulation, in fact, impairs cell proliferation and sensitizes cells to Temozolomide (TMZ) in vitro. In addition, growing evidence supports the proposition that analogous epigenetic alterations can contribute to the acquisition of hallmark capabilities during tumour development and malignant progression; in fact, preclinical studies suggest a role for epigenetic readers like BET proteins as conducting tumorigenesis in a wide range of human neoplasms [5]. Specifically, BET proteins are involved in a wide range of biological processes and regulate cell plasticity and differentiation process that occurs during development in which there is a progressive loss of phenotypic plasticity and the acquisition of increasingly specialized cell fates. The result of cell differentiation is in most cases antiproliferative and a clear barrier to the continued proliferation needed for neoplasia. Increasing evidence shows that unlocking the normally limited capacity for phenotypic plasticity to evade or escape the terminal differentiation state is a critical part of cancer pathogenesis [6]. The discovery of small molecules capable of inhibiting BET bromodomains has enabled the use of these epigenetic readers as pharmacological targets in cancer treatment. One of the first molecules to be found was JQ1 that can competitively bind the binding pocket for acetylated lysine residues. Thus, JQ1 can prevent the interaction between BET readers and chromatin and prevent their pro-tumorigenic activity. Clinical and preclinical studies highlighting the involvement of JQ1, and next generation BET inhibitors for the treatment of hematopoietic malignancies and solid tumours. Preliminary in vitro and in vivo studies have shown that Glioblastoma (GBM) models treated with JQ1 or I-BET151 show promising antitumor activity. Specifically, GBM is the most common and aggressive form of tumour in the Central Nervous System (CNS). The high degree of GBM malignancy is defined by characteristic hallmarks such as rapid cell growth, extensive tumour heterogeneity, sustained local invasiveness, and high resistance to therapy due to several factors. For instance, the presence of the blood-brain barrier is one of the main obstacles to the ineffectiveness of most drug therapies directed toward glioblastoma that still make it an incurable disease and ensure that the standards of treatment still in use remain surgical removal of the tumour mass combined with radiotherapy and chemotherapy with TMZ as an adjuvant agent. Considering all these reasons, the curiosity to further investigate the role of BET proteins in glioblastoma biology and the need to identify more suitable therapeutic strategies is what prompted our research group to employ the pan-BET inhibitor JQ1 as a drug treatment on an immortalized human glioblastoma line (U87MG) and on a primary culture (GH2). Stimulation of GBM cells with the BET inhibitor JQ1 resulted in de-regulation of PI3K/Akt/mTOR pathway. As mTOR is a known autophagy regulator, we analyzed autophagy occurrence by western blotting and immunostaining analyses. Significant changes in the expression of the main autophagy regulators and autophagy induction were observed in our GBM models. Intriguingly, we

observed that JQ1 induced a morphological change; a relevant increase in the number of cells with cytoplasmic projections was observed and the length of projections increased upon JQ1 stimulation (Figure 1).
In order to test a possible cell fate reprogramming upon JQ1 stimulation we are analyzing the expression levels of

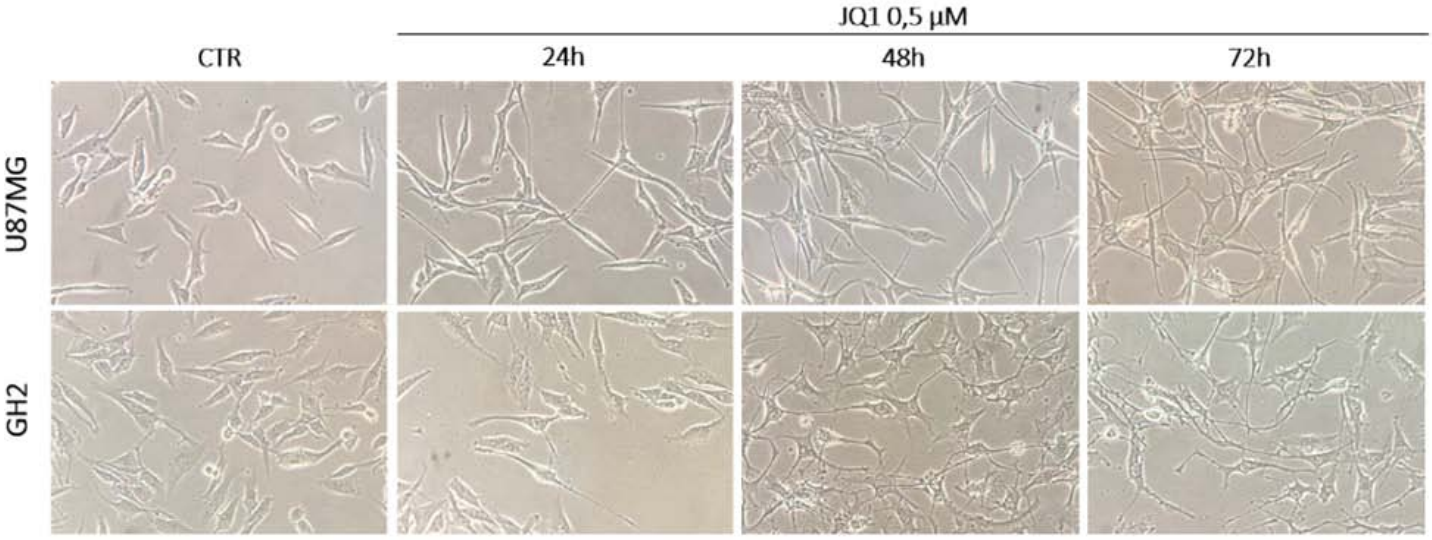

Fig. 1. Brightfield images of U87MG and GH2 treated with 0,5 µM JQ1 for 24, 48 and 72h. The images were obtained using on optical microscope Nikon Eclipse 7S100 al 20X magnification.

stemness and neural and glial markers [7]. We are also studying the effect of JQ1 alone or in combination with the chemotherapeutic drug Temozolomide on GBM cells proliferation and apoptosis. Finally, the functional role of autophagy activation, if any, on cell fate reprogramming induced by JQ1 will be investigated.The results obtained suggest that BET protein regulation may be involved in the molecular alterations that occur during gliomagenesis and support the hypothesis that these epigenetic regulators may represent a novel therapeutic target in neuro-oncology field.

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Identification and characterization of constituents of Malus domestica and Pyrus communis of Molise region, evaluation of bioactivity of secondary metabolites and development of nutraceuticals, cosmeceuticals, food supplements and cosmetics

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Natural sources such as plants are an important resource for both food field and drug discovery field. This because they offer not only primary metabolites that satisfy our food requirement but also a large library of secondary metabolites which can show different bioactivities and can find a use in different areas such as nutraceuticals, cosmeceuticals and for the drug discovery.

The proposed project is based on the enhancement of the Molise region, exploiting autochthonous cultivars belonging to *Malus domestica* (Suckow) Borkh. and *Pyrus communis* L. through chemical profiling and quali-quantitative determination of the constituents of each selected species disclosing active secondary metabolites (SAMs) and functional ingredients for nutraceuticals, cosmeceuticals, food supplements and cosmetics. *Malus domestica* (Suckow) Borkh, commonly known as apple, represents an important source of polyphenols which are responsible for their well-known antioxidant and hepatoprotective activity. In addition, polyphenols have been shown to possess a role in the prevention of degenerative diseases [1]. Pears (*Pyrus communis* L.) represent a good source of phytochemicals, especially phenolics, that are important bioactive compounds known for their health benefits such as antioxidant and anti-inflammatory properties, furthermore anticancer properties are due to the content of triterpenoids [2]. Among the autochthonous cultivars of *Malus domestica* and *Pyrus communis*, different varieties will be evaluated.

The chemical profile of the selected species will be determined through extraction methods and identification of the constituents by UPLC-MS/MS and NMR analysis of the extracts obtained. All components of the extracts will be characterized unequivocally by isolation protocols with chromatographic techniques and the structure of pure compounds will be determined by spectrometric (MS) and spectroscopic (NMR) methods. Furthermore, bidimensional homonuclear and heteronuclear NMR experiments (e.g., COSY, HMBC, HSQC, etc.) will be performed to confirm the structure of the compounds and to allow the stereo-assignments. Moreover, high-resolution magic angle spinning (HR-MAS) NMR analysis will be performed on plant samples to obtain a chemical profile in the complete lack of any chemical manipulation of the sample. The qualitative analysis will be followed by a quantitative analysis by HPLC-UV, LC-MS and qNMR (Quantitative NMR). All NMR experiments will be performed by collaborations with University of Salerno.

Once the chemical profile of the species has been determined, DARTS (Drug Affinity Responsive Target Stability) will be performed to explore the molecular binding assessment of interesting SAMs disclosing their biological partners suitable as potential therapeutic targets of the SAMs. DARTS is a new approach based on the thermodynamic stabilization of the target proteins upon small molecule binding and, consequently an increase in the resistance to proteolysis that can be detected using a proteomic workflow [3].

In the context of the possible application of the SAMs, their potential antioxidant, anti-ageing, and anti-inflammatory activity will be validated in vitro with a particular interest devoted to SAMs interfering with multiple pathways associated with inflammatory-based and inflammatory-related diseases.

The extracts and/or SAMs isolated from the fruits as well as the waste and by-products of Molise cultivars will find application also for the formulation and development of nutraceuticals, cosmeceuticals, food supplements and cosmetics in collaboration with some industry companies that already study, realize, and propose a new concept of application of natural products in fields reported above with high performance and a high level of technological innovation. This phase represents the final goal of the entire project, accounting for the exploitation of the typical products from the Molise region.

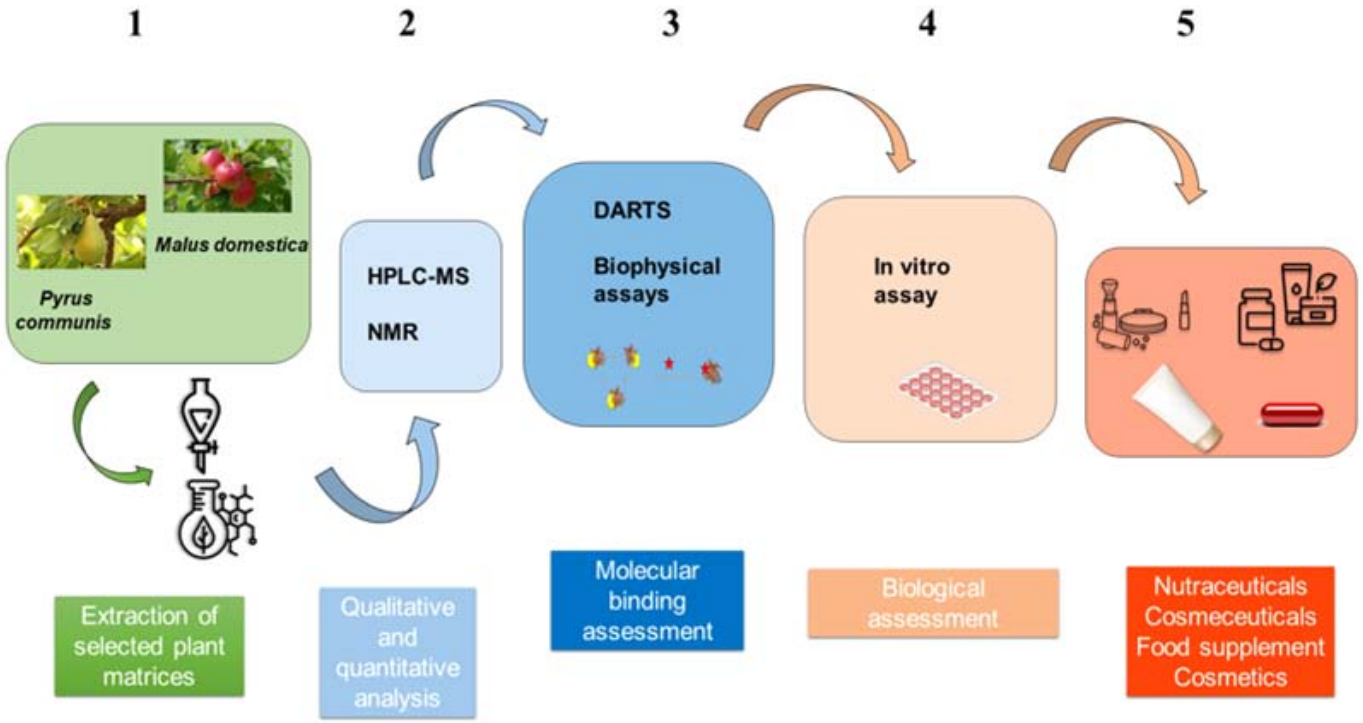

Fig. 1. Workflow of the project.

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Studying the role of autophagy in neurogenic muscle atrophy

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Denervation, as well as nerve damage, is a condition that leads to the activation of catabolic pathways, resulting in loss of muscle mass and contractile properties of skeletal muscle. Muscle atrophy is generally characterized by an imbalance of the processes controlling protein anabolism/catabolism. For instance, it is well-documented that anabolic pathways responsible for the increase in muscle mass, such as Akt/mTOR axis, are altered in denervated muscles, leading to decreased protein synthesis [1]. Conversely, AMPK results to be hyperactivated in neurogenic muscle atrophy. AMPK operates an activating phosphorylation on FoxO3, a crucial transcription factor which regulates the transcription on proteins and enzymes involved in protein degradation through the ubiquitin-proteasome system, such as Atrogin-1 and MuRF-1 [2]. Thus, it has been speculated that loss of innervation leads to muscle atrophy by decreasing protein anabolism through the activation of Akt/mTOR pathway and, concurrently, by inducing protein catabolism through AMPK activation. It is interesting to note that Akt and AMPK represent the two main upstream kinases controlling the activation of autophagy [3]. From these premises, several research groups investigated whether Akt and AMPK could regulate muscle loss during denervation by influencing autophagy. The role of autophagy in neurogenic muscle atrophy is controversial, and different hypothesis have been proposed. Some studies sustain that autophagy is hyperactivated during muscle atrophy: this event could be detrimental as it may contribute, together with the ubiquitin-proteasomal system, to the degradation of muscle proteins. Conversely, other research groups suggest that autophagy is suppressed upon muscle denervation: this condition could worsen the quality of muscle tissue since basal autophagy is required for the clearance of damaged proteins and organelles [4]. Despite the efforts, the putative involvement of autophagy in neurogenic muscle atrophy still remains elusive. Thus, the objective of this proof-of-concept study is to perform an extensive characterization of autophagy in the atrophic muscle following denervation. To reach this aim, Balb/c mice will be subjected to sciatic nerve crush in order to induce nerve damage and subsequent muscle atrophy. 14 days after sciatic nerve damage, mice will be sacrificed, and hindlimb muscles will be collected to study the expression of autophagic proteins. For instance, mRNA and protein expression of Ulk1, Beclin1, Atg9, p62, LC3 will be analyzed by qRT-PCR and Western blot, respectively. LC3 will be also evaluated by immunofluorescence analysis on muscle sections, to better identify autophagosomal structures. Since autophagy is a catabolic process associated to lysosomal degradation, the expression of lysosomal proteins will be also assessed, such as Cathepsin D, Cathepsin L, Lamp1 and Lamp2. The results obtained from this study will be useful to define the role of autophagy in neurogenic muscle atrophy: the increase of knowledge may help to identify novel molecular targets to counteract muscle mass loss occurring following nerve damage.

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Terpinen-4-ol affects cholesterol metabolism in the hepatic cell line HepG2

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Cholesterol is the most abundant sterol present in animal tissues. It is an essential component of eukaryotic cells, where it is mainly located in cell membranes. In this context, it interacts with adjacent lipids regulating the rigidity, fluidity and permeability of the membrane. In addition to its role in membrane structure and function, cholesterol is the precursor of all steroid hormones, bile acids and vitamin D. Given these crucial functions in different physiological contexts, alterations in cholesterol metabolism can cause several congenital diseases in humans. In addition, increasing experimental evidence defines the close relationship between cholesterol metabolism and acquired diseases such as cardiovascular disorders, Alzheimer's disease and many types of cancer. Although almost all human cells can synthesize cholesterol, about 50% of total synthesis takes place in the liver. The crucial role of this lipid and the energy expenditure attributed to its biosynthesis lead to a strict regulation of its metabolism, exerted by an intricate protein network that can be conditioned by different exogenous compounds, such as essential oils (EOs). Notably, EOs are rich in monoterpenoids and sesquiterpenoids which, as evidenced by recent data, are characterized by hypocholesterolemic properties [1]. In addition, dietary extract of *Lavandula angustifolia* has been shown to modulate serum cholesterol levels in rats [2].
Lavender essential oil (LEO) consists of several compounds with potential ability to modulate lipid metabolism, however the biological activity of the single components has not yet been evaluated. Monoterpenoid alcohols are the main constituents of EOs, and among these, *Terpinen-4-ol* is one of the most abundant compounds in LEO [3]. Therefore, the aim of this work is to evaluate the possible modulatory activity of *Terpinen-4-ol* on the protein network involved in the maintenance of cholesterol homeostasis. To this objective, the hepatic cell line HepG2 was used as experimental model. First, we evaluated the effects of *Terpinen-4-ol* on intracellular free cholesterol and neutral lipids in HepG2. For this purpose, HepG2 were treated with *Terpinen-4-ol* (Ter-4-ol) for 24 h at a concentration of 0.00034%. Filipin test, a gold standard method for assessing intracellular cholesterol, showed significant cholesterol accumulation in HepG2 treated with *Terpinen-4-ol* (*Fig 1B*). Oil Red O staining reported that neutral lipid content increased in Terpinen-4-ol-treated HepG2. In addition, lipid droplet sizes increased following treatment with *Terpinen-4-ol*, although this trend does not reach statistical significance (*Fig 1A*).
Subsequently, the expression levels of the main enzymes involved in biosynthesis, uptake and intracellular cholesterol transport were evaluated. Western blot analysis showed that *Terpinen-4-ol* had no effect on the expression levels of 3β-hydroxy-3β-methylglutaryl CoA reductase (HMGCR), the rate limiting enzyme of cholesterol biosynthesis, and Niemann-Pick type C1 protein (NPC1), involved in intracellular lipid distribution (*Fig 1C-D*). In contrast, the expression of LDL receptor (LDLr), primarily involved in cholesterol intake, was elevated in HepG2 treated with *Terpinen-4-ol*, as demonstrated by immunofluorescence and signal quantification (*Fig 1E*).
Our results show that *Terpinen-4-ol* promotes the accumulation of cholesterol and neutral lipids in HepG2, enhancing LDLr expression. This evidence sustains the importance of seeking new natural compounds from EOs for their possible modulatory activity on lipid metabolism.

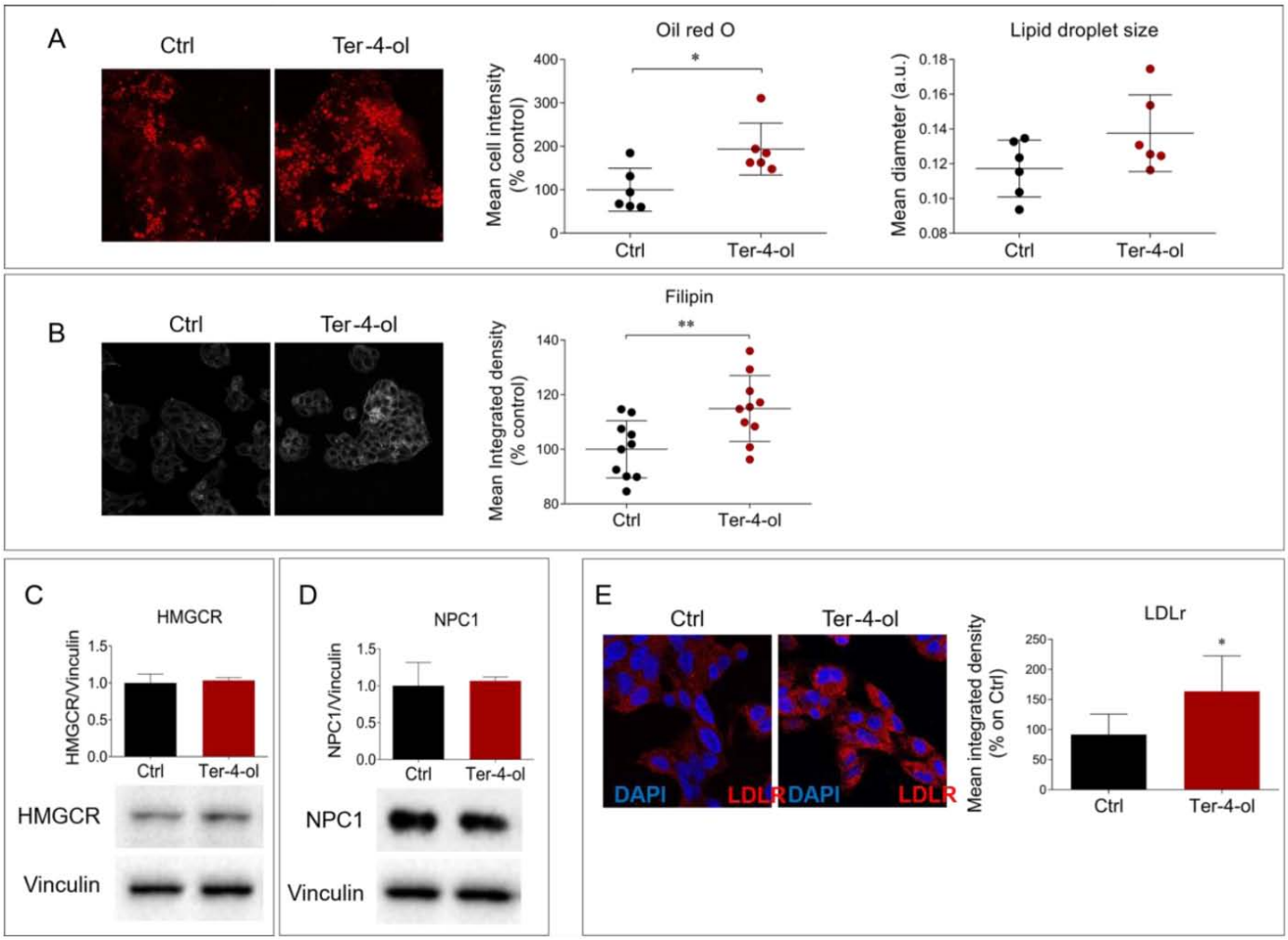

*Fig. 1. Terpinen-4-ol induces the accumulation of cholesterol and neutral lipids in HepG2. (A) On the left images and signal quantification of the Oil Red O staining, on the right analysis of lipid droplets size in HepG2 treated with Terpinen-4-ol (0.00034%) for 24 h. n = 6 independent experiments. (B) Images and quantitative analysis of the filipin signal in HepG2 under the same experimental conditions defined in (A). n = 10 different experiments. (C-D) Western blot analysis of HMGCR and NPC1 protein expression levels in HepG2 treated as in (A). Vinculin was used as loading control. n = 3 independent experiments. (E) On the left, immunofluorescence and confocal analysis for LDLr (red). DAPI (blue) was used to counterstain nuclei. On the right quantification of the main integrated density. n = 6 different experiments. Data represents mean ± SD. Statistical analysis was assessed by using unpaired Student's t test. * p < 0.05; ** p < 0.01.*

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Assessment of Climate-Smart Forestry indicators in forests with different management intensity. the case study of the "Collemeluccio Forest"

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Climate-Smart forestry (CSF) is a new sustainable forest management approach to forefront climate crisis aimed to identify adaptation and mitigation management strategies valorising forest conservation, productivity and the provision of ecosystem services [1, 4]. Despite there is a general increment of interest about CSF worldwide, studies focused to assess CSF are rather limited [1, 5, 6]. Recently, the Technical University of Munich used a network of long-term experimental plots in the Bavarian Alps to test a practical method to assess CSF at the stand level [2], while no studies on Mediterranean forests are carried out.

This study aims to test the assessment method of CSF in Mediterranean forests, assessing and comparing the CSF between two forest management intensities. The study was carried out belonging to the Collemeluccio forest (Isernia, Molise), within two forest stands, one untouched since post-war period (State forest), and one regularly managed for commercial purposes (private forest). The research questions of the study were: are there any Climate-Smart difference between the two forest management intensities? Is the regularly forest management systems Climate-Smart?

The methodological approach includes: (i) selection of CSF indicators form the current set of sustainable forest management indicators; (ii) collecting field data for each indicator; (iii) asses the CSF for each forest stand; and (iv) verify if the differences among forest management intensities are similar through ANOVA test.

The CSF assessment method compares the values measured in field (actual value) with reference values from national forest inventory or literature (index value). Moreover, a constant, which is useful for making smartness value comparable, is determined by assessing a score for each indicator according to Bowditch et al. [1] classification.

The results show a higher smartness value in the unmanaged area (3.6) than in the managed area (2.2). Except for "*stability*" and "*diameter distribution*", all other indicators show higher values for the unmanaged area, particularly for "*carbon stock*" and "*deadwood*" (Table 1).

Table 1: Values of CSF indicators in two areas of Collemeluccio forest.

	Growing stock	*Diameter distribution*	*Carbon stock*	*Stability*	*Tree species composition*	*Regeneration*	*Deadwood*	***Total***
Unmanaged area	0.49	0.09	1.19	0.14	0.35	0.24	1.09	**3.6**
Managed area	0.23	0.15	0.42	0.17	0.31	0.11	0.78	**2.2**

However, the ANOVA test confirm that the two management intensities have no significant statistical difference. For this reason, it is possible to state that correct forest management can be considered Climate-Smart contributing to promote forest productivity reducing the impacts of climate change. In addition, it should be considered that the short period since the last silvicultural intervention of managed forest stand has an effect on the evaluation of CSF (e.g., on "*regeneration*", "*growing stock*" and "*carbon stock*"). In a long-term perspective, these indicators could increase with positive effect on CSF evaluation. The selected set of indicators used also significantly impact on the results. So, the methodology tested for the assessment of indicators is an excellent starting point to assess CSF, resulting easy, timesaving and very practical to implement. However, the approach needs to be improved in some critical points, such as enlarging the set of indicators for a more uniform analysis of the three CSF pillars (ecological, economical, societal); testing the method in other forest types and other forest management systems to obtain a more robust method for CSF evaluation.

The study highlight that the method can be considered suitable, but it is strongly recommended that more efforts are necessary to improve its applicability and transferability through practicable forest management guidelines that forest managers can be applied in field. The idea is to develop a digital application for smartphone that allows to measure the indicators in a user-friendly way.

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Incorporating fossil data into climate change vulnerability assessments

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Climate change represents one of the most concerning anthropogenic threats to biodiversity and ecosystems. Among the several approaches proposed to quantify the vulnerability of the species to climate change effects, the "Climate Change Vulnerability Assessment" (CCVA) [1]; emerged as one of the most promising. In particular, recent studies proposed to incorporate Species Distribution Models (SDM) and environmental niche quantification (via ecological niche factor analysis) [2]; into CCVAs, as to take this conservation tool to a next level [3]. The rationale behind such an inclusion is that SDMs are able to predict the distribution of a species across geographic space and time, using species occurrences and environmental data; thus offering a spatially–explicit context to CCVAs.
Some of the most widely used methods to predict distribution changes – correlative distribution models [4] – assume that the current distribution of a species reflects its ecological and physiological limits [5], thus treating current conditions as conservation baselines for vulnerability assessments. This assumption is often violated, since the habitat preferences expressed geographically by a species in the current time may well be a subset of their physiological tolerances, with several factors (both biogeographical and anthropogenic) preventing a species to occupy the entire spectrum of its environmental preferences. This issue, called "niche truncation", is known to hamper SDMs ability to predict the effects of climate change on species distribution accurately [6], and thus, to quantify species vulnerability.
Fossil and phylogenic information can help to reduce niche truncation by feeding SDMs with information on species–climate relationships coming from the past, where e.g. the effects of anthropogenic pressures were absent, thus capturing the portions of the physiological tolerances that are not available in the current time (Figure 1). In light of that, the inclusion of fossil data to calibrate SDMs and CCVAs on extant species will likely offer new perspectives on the real vulnerability of these species to climate change.
In this scenario, we propose a study aimed at assessing vulnerability to climate change of a set of megafauna (>44kg) extant species in Eurasia, comparing the CCVA scores obtained from current occurrences only and current–plus–fossil data, as to investigate if and how much the inclusion of past climatic preferences might influence future vulnerability values. Among the studies that investigated a similar topic on vertebrate species, most focused only on a single species [7] or relied on simple reconstructions of past geographic distributions in lieu of fossil data [8]. In our case, we will be able to rely on a vast database of fossil occurrences, going back as far as 200 kya, including species as e.g.: *Alces alces, Canis lupus, Capra ibex, Capra pyrenaica, Capreolus capreolus, Cervus elaphus, Equus ferus, Equus hemionus, Lynx pardinus, Rangifer tarandus, Rupicapra rupicapra, Sus scrofa, Vulpes lagopus* and *Vulpes vulpes*.

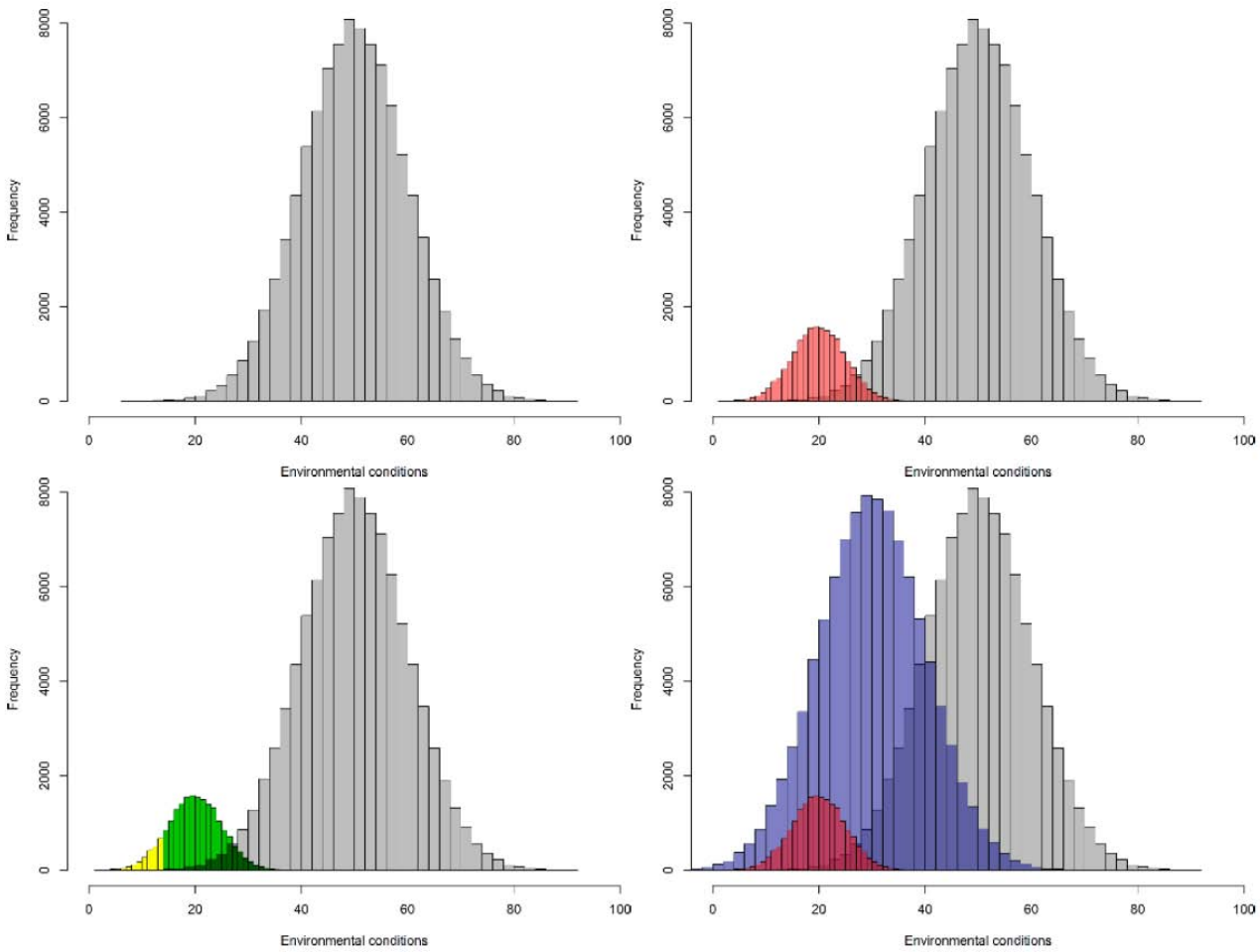

Fig. 1. The panel illustrates an example of how fossil occurrences and paleoclimate data can help distribution models to get closer to the species fundamental niche. The histogram in gray rappresents the distribution of the current thermal range of Eurasia, while red bars depict the species full thermal preferences in the fundamental niche. These preferences are only partly available in the current geographic space of Eurasia (green bars; i.e., the realized species niche), whereas the colder values (in yellow) are excluded. Including fossil data and paleoclimate reconstructions of Last Glacial Maximum (in purple) into species distribution models allow to cover the full thermal preferences of the species, thus better capturing its fundamental niche.

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Phenological monitoring in beech stands: a preliminary study for adaptive silviculture

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The increase in weather and climate extremes due to the climate change has led to the deterioration of ecosystems structure and function, resilience, and their natural adaptive capacity; in particular, it's esteemed that projected climate change, combined with non-climatic drivers, will cause loss and degradation of much of the world's forests [1]. For this reason, it's crucial to improve forest adaptation to climate change due to reduce the risk and the impact on forest, decreasing vulnerability- which is the degree of system susceptibility to cope with climate change- increasing their resilience and resistance [2].
AForClimate Project aim is to adapt the forest planning of beech forests to climate variability. The phenology is an adaptive trait able to shape itself and the length of the growing season in response to local environmental conditions [3].
The study site is in the Italian Southern Apennines (Matese mountains), in Molise region located in the municipality of Roccamandolfi (IS) and, to cover climatic extremes of local beech distribution range, four sectors have been selected: high altitude and northern exposure (HN), low altitude and northern exposure (LN), high altitude and southern exposure (HS) and low altitude and southern exposure (LS). In each sector, 15 permanent circular sample plots were established [3] and in 3 different sectors, a different approach to cutting was adopted for each site: in two sectors, thinning was applied between 15% and 25% of the basal area and one wasn't cut. This approach aims to find out how trees respond to different thinning intensities in order to understand which forest management is better for their adaptation to climate change.
Cambium activity have been monitored at different spatial and temporal scale to define a method that improves the efficiency in forest management. The objective of this study is to investigate cambium phenology during the vegetation period in 2021 thought monitoring cambium activity collecting wood microcores biweekly at the breast height with Trephor tool [4]. The microcores were stored in Eppendorf microtubes in a fixative solution containing 50% ethanol and 50% distilled water. Sample preparation was then performed according to the protocol of Prislan et al. (2022) in the Wood Anatomy Laboratory of the Department of Wood Technologies, Biotechnical Faculty, University of Ljubljana, Slovenia. Firstly, the microcores containing inner bark, phloem, cambium and at least two youngest xylem growth rings, where dried and embedded in paraffine [5]. The block of paraffine were produced and then the samples were cut with rotatory microtome [5, 6]. Cross-section of 9 µm of thickness were prepared and for better adhesion of the section, slides were pre-treated with albumin and after that they were treated in different mixture to be colored with safranine and astra-blue and finally embedded with Euparal and covered with coverslips. To assess the beech's cambial activity, we considered the following forming tissues: cambial cell (CC), postcambial cells (PC), secondary wall (SW) and mature cells (MT) [5]. In this study, the data were collected biweekly during the spring of 2021 between doy 120 and 197 (30th April-16th July) and only the CC phase were considered and measured (CCm). The values reported are the medium valued for each site. The first results show that comparing the two sites at the higher height but different expositions (north and south), HN site starts with more CC and CCm rather than HS, but HN reach the maximum on DOY 170, whereas HS has a faster and speedy growing cell reaching the maximum on DOY 141. Afterwards, from DOY 170, in HN and HS sites cambial production gradually decrease. Comparing the lower sites at different expositions, LN and LS start in the same DOY 120 with a similar amount of Cambial cells numbers and their measurements reaching the maximum in the same DOY 148 but LN has the higher value. On the other hand, considering the same exposition (South) but different altitude, LS has a higher number of cells compared to HS at the beginning and at the end they reach the maximum with the same number of cells but in different DOY (LS in 148 and HS in 141 DOY). Overall, the first hypothesis of these differences

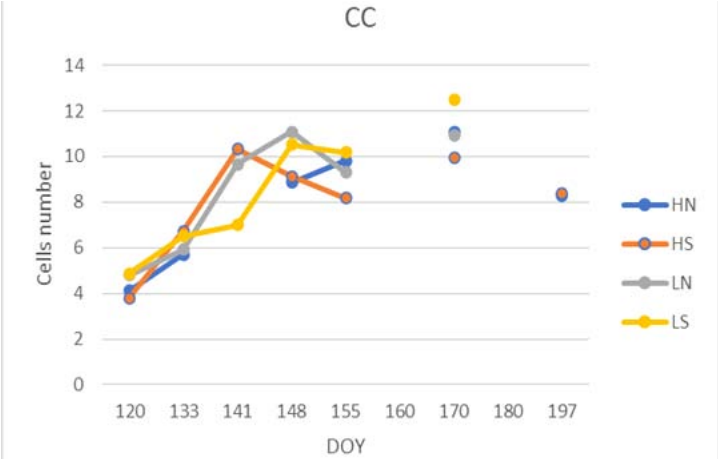

Fig. 1. Cambial Cells related to DOY.

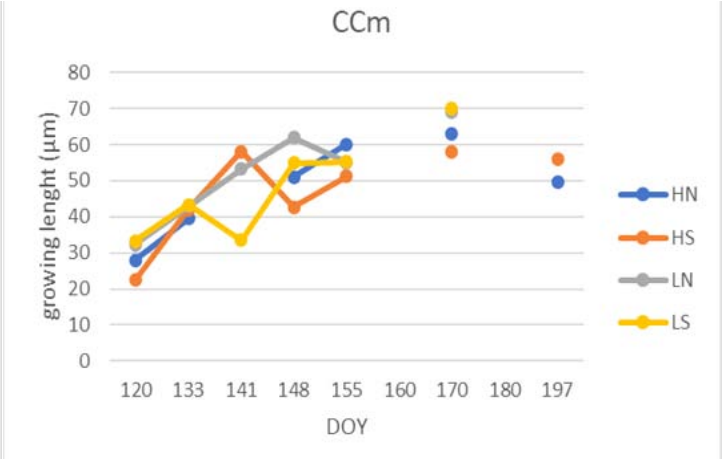

Fig. 2. Growing length (µm) related to DOY.

between the site and the time of starting cambial cells growing is related to climate difference. In the southern sites, which are warmer and more exposed to the sunlight much longer rather than the northern sites, cells differentiate earlier. Furthermore, LS show an absolute maximum in cells number and growing length on DOY 170 that can be associated to the warmer and positive climate condition trough the time, with an extension of the growing season. These hypotheses should be validated by the analysis of climate parameters for each site to better understand how beech react to climate change. Only with this analysis it's possible to improve forest management plan based on adaptive silviculture in order to promote Mediterranean beech forest adaptation to climate change.

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Exploring the potential of "Carta della Natura" for the development of new ecological indicators for urban ecosystems

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Nature Map (Carta della Natura) information system was envisaged by the Italian Law 394/1991, with the aim to identify the status of the natural environment in Italy with a particular focus to assess natural values and territorial vulnerability [1]. It is a national project based on two main products: the maps and the assessment system. The maps describe the type and the distribution of Italian terrestrial ecosystems on a national, regional and local scale; the assessment system assigns values to each polygon mapped, using a set of indicators and indexes, giving information on the state of ecosystems and highlighting the areas of greatest natural value and those most at risk of degradation. The whole system is structured at different scales of analysis and is organized as an informative system which allows to access all the maps and associated database resources and to maintain a constant upgrade of its structure and contents [2].
Current final indexes used to represent the state of ecosystems in the assessment system are: Ecological Value, Ecological Sensitivity, Anthropogenic Pressure and Environmental Fragility [3].

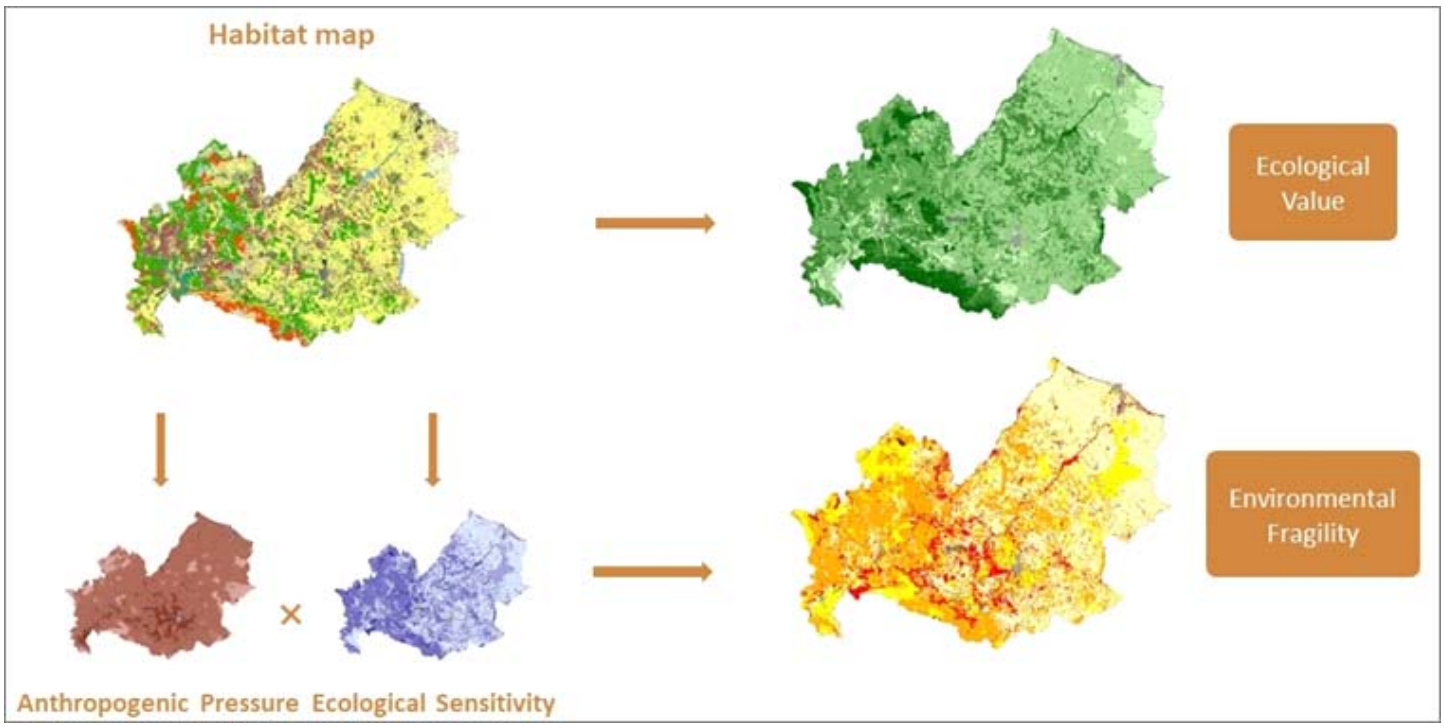

Fig. 1. Carta della Natura: assessment system and derived maps [4].

Due of the new available IT tools, with greater calculation capacity, cartographic data even more numerous and detailed and the development of new research experiences at a national and international level, it is timely, and scientifically interesting, to build a new solid and innovative assessment model, which is functional to the uses of Carta della Natura required by national law 394, such as decision support in the environmental field.
To do this, the first step is to develop a new system of indicators to assess the ecological value (EV) of each habitat mapped [5].
The aim of the research is to develop an innovative methodology for evaluating EV taking the urban functional area of Campobasso as a case study, considering the habitats mapped in Carta della Natura and data collected by

vegetation sampling and by literature review [4, 6].
Here, we present a conceptual frame that will be organized in the following steps: a) Development of a new set of indicators for the Ecological Value; b) Development of a new experimental methodology for the aggregation of indicators into a new index of Ecological Value; c) Development of an automated procedure for the calculation and aggregation of indicators into a synthetic EV index.
Each indicator will respect three essential properties: to be available and homogeneous throughout the study area, to be significant at the scale of analysis and to be quantifiable [4].
EV indicators will be specific for each habitat type. For example, in our case, the parameters considered for the assessment natural habitats may differ from those needed for the assessment of anthropic habitats.
To explore the effectiveness of the proposed indicators, a dedicated geographic database of the Campobasso FUA and surroundings will be implemented [7]. A detailed habitat map, classified according to the new Carta della Natura scheme, will be used to develop the new assessment methodology on urban ecosystems.
The research will provide a contribution to the implementation of an assessment model of urban biodiversity exportable to other small cities of the Italian inner areas.

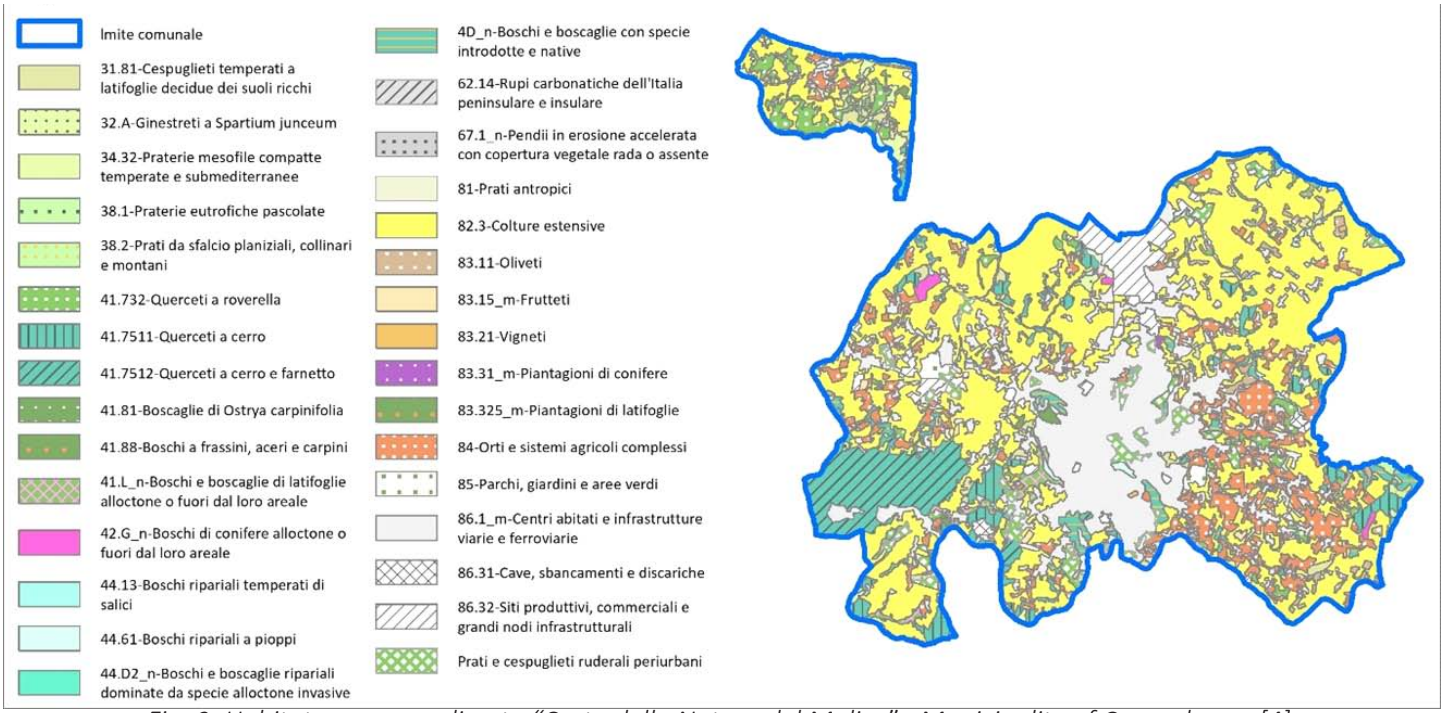

Fig. 2. Habitat map according to "Carta della Natura del Molise" - Municipality of Campobasso [4].

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A MULTISPECIFIC PROTOCOL FOR ENVIRONMENTAL DNA MONITORING OF ENDANGERED AND INVASIVE FRESHWATER SPECIES

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Every organism release DNA in the environment where it lives (shredding of epithelial cells, egg, sperm, etc.). That DNA is called Environmental DNA (eDNA). The analysis of eDNA is a novel technique to detect species occurrences from environmental samples (water, soil and air), particularly useful with species that are difficult to detect through traditional methods. Freshwater is one of the most endangered ecosystems at the whole global scale, downgraded mainly by human pollution, habitat loss and invasive alien species. Detecting several species of different taxa is time-consuming and requires many experts and logistical costs. The eDNA single species approach is a very quick sampling method but it is rarely used to monitor contemporaneously different species of the animal community, especially if species belong to different systematic groups [1]. With this work we are testing an eDNA protocol to simultaneously detect 14 endangered species listed in the Habitat Directive 92/43/EC and 9 invasive alien species representing a threat to the first ones living in freshwater bodies of Central Italy.
We collected water samples from 52 sampling localities in the Latium region, covering different freshwater habitats, like rivers, lakes, creeks, marshes, and ponds. We performed 2 different sampling sessions (year 2021, year 2022) according to the phenology of the target species, collecting 3 liters of water per site and measuring 9 environmental parameters (pH, nitrites, nitrates, air temperature, water temperature, water speed, river width, river depth, water turbidity) to better understand the influence of environmental variables on eDNA persistence. Water samples were filtered employing filters of cellulose mixed esters with a pore size of 0.22 µm and prefilters with a pore size of 8 µm. Sterile water samples were used as negative controls to keep track of possible contamination.
Developing genetic probes to detect the species of interest is an essential first step. Genetic probes have species-specific bounding to the animal's DNA scattered in the environmental matrix and is required a three-step probe validation process to ensure a proper accuracy in species detectability: i) in silico validation, checking genetic databases for possible matches with congeneric or sympatric species; ii) in vitro validation on target species tissues; and iii) in situ validation, on environmental samples from both ascertained presence and absence sites. After filtration, DNA was extracted from the filters by using a commercial extraction kit (DNeasy Power Soil Pro Kit, Qiagen), following the manufacturer's instructions with some modifications.
To detect the presence of the target species in the water samples, the DNA previously extracted was amplified by using the genetic probes properly designed for the target species. Species distribution modelling was based on species occurrences and connectivity analyses were implemented to understand the potential distribution and dispersal routes of each target species using appropriate software (R, CIRCUITSCAPE).
The study is still ongoing, and definitive results have not yet been obtained. The use of eDNA techniques will be essential in monitoring wildlife on a large geographical scale. Their impact is on understanding species distribution and modeling their presence in space to infer their habitat requirements, comprehending connectivity among habitats, revealing the efficacy of protected areas on these endangered habitats and driving where to implement eradication plans for invasive species.

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Monitoring Alien Plant invasions with drones: an insight into coastal dunes invaded by Carpobrotus sp. pl. (Aizoaceae)

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Coastal ecosystems, due to their transitional nature between terrestrial and marine environments, are among the most productive ecosystems and provide a large number of ecosystem services. [1]. At the same time, human activities have had a considerable number of direct and indirect impacts on coastal ecosystems, including habitat reduction, overexploitation, eutrophication and pollution. [2]. These threats are made worse by the inherent nature of coastal ecosystems, which are usually narrow strips in a landscape very often fragmented by human activities [3]. Among the coastal ecosystems most impacted by human activities, especially those related to tourism, are beaches and coastal dune systems [3-5].

Among the various threats to dune ecosystems, invasive alien species (IAPs) are among the most serious [6]. In fact, IAPs are considered the second biggest threat to biodiversity globally, as they affect species composition and threaten the conservation status of invaded habitats [7]. Early identification and good mapping of the presence of IAPs is one of the fundamental prerequisites for any containment or eradication plan for these plants [8, 9] and for modelling future invasion risks [10]. Traditional detection and identification of invasive plants usually involves intensive field research, which can be time-consuming and costly [11]. Thanks to the availability of numerous satellites, whose data are acquired almost daily and possess information, remote sensing technologies have expanded the possibilities and range of IAP detection [12]. However, an even more innovative frontier is offered by the use of drones, which can fly at low altitude and acquire RGB, multispectral and hyperspectral images at higher resolution [13]. However, despite their potential, the use of drones for IAP invasion management is still largely surpassed by satellite and aerial imagery or even field measurements [14].

An important example of IAP that heavily affects coastal dunes is the genus *Carpobrotus* N.E.Br. (Aizoaceae). *Carpobrotus* are succulent, trailing perennial grasses, native to South Africa. They have been introduced to Europe for ornamental and soil stabilisation purposes since the early 17th century and are now widely naturalised in the coastal habitats of southern and western Europe [15].

Carpobrotus can invade both sandy coastal ecosystems- from embryonic dunes to juniper groves- and rocky ones with equal success [15]. *Carpobrotus* represents one of the most important plant invaders in the Mediterranean [15, 16], where its spread in coastal ecosystems is seriously threatening the conservation of biodiversity both on a local and global scale, and numerous eradication initiatives have been implemented.

In this study we will address several technical issues concerning the application of drones for the identification and monitoring of *Carpobrotus* invasions on coastal dunes. In particular, our approach will allow us to evaluate: a) the set of variables best suited to predict the presence of *Carpobrotus* among those derived from RGB and multispectral sensors; b) the best approach to predict the whole plant or only the green parts or flowers; c) establish the minimum training area in which to perform a manual interpretation of the presence of the species for optimal modelling.

We calibrated a total of 27 models using Large Scale Mean Shift (LSMS) optimised image segmentation and Random Forest as classifier. The highest values of balanced accuracy (BA > 0.68) were obtained for both the total and the green part, using the combination of RGB and multispectral camera data. There was no significant difference when increasing the calibration area from 20% to 40% of the total area, while the prediction of the total and green parts was significantly better than that of the flowers in all models.

These results demonstrated the effectiveness of drones as monitoring methods for herbaceous alien species such as *Carpobrotus* in complex landscape systems such as dune systems. This evidence sets the stage for an expansion of the monitoring protocol with the possible involvement of even metric-scale satellites such as PlanetScope that can be used to project drone results onto larger dune areas.

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The role of "Plant Functional Traits" (PFTs) in the monitoring of woods and habitats: a case of study

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Climate change is a threat that puts at risk the development and existence of the species that make up habitats at national and European level. For this reason, it is necessary to have useful tools to assess the state of health of plants, which can return a comparable data between populations of the same species and between the same types of habitats.
Plant functional traits (PFTs) are defined as any morpho-, physio-, and phenological plant characteristics affecting overall plant fitness through their influence on survival, growth, and reproduction. They have often been used to interpret plant-environment relationships, to make previsions on possible consequences and to quantify a wide range of natural and/or human-driven processes in terms of species or community ecology [1-3]. To propose a protocol useful to be applied in different habitat and condition, five PFTs were applied to a case study of three *Quercus cerris* L. woods subjected to different forestry practices in Molise region. Specific Leaf Area (SLA), Leaf Mass per Area (LMA), Leaf Dry Matter Content (LDMC), Leaf Thickness (Lth), Leaf Water Content (LWC) and Leaf Chlorophyll content (CHL) were measured on a total of 270 seedlings in the tree stands (Selva di Castiglione – SC; Bosco della Ficora – BF; Bosco San Leo – BSL).
Specific leaf area (SLA) measurements showed the highest mean value in the SC stand (265.551), which differed significantly from the BF stand (215.078) and the BSL stand (205.224) ($p=0.000$ and $p=0.0001$ respectively), the values of the latter being very similar ($p=0.508$). Leaf dry matter content (LDMC) measurements showed that the mean value of the SC stand (339.652) differs significantly from those of the BF stand (407.723) and the BSL stand (416.036), with the SC displaying the lowest mean value ($p=0.001$). For Lth, the low values obtained for the SC stand (1.362), differed significantly from those obtained for the BF stand (1.983) and the BSL stand (2.301) ($p=0.001$). The SC stand exhibited the highest average value for CHL (0.022 mg/cm^2). This value differs significantly from those displayed by the BSL (0.016 mg/cm2) and BF stands (0.014 mg/cm^2).
High levels of SLA are correlated with a greater propensity for development by the plant, indicating a carbon investment oriented towards faster growth rather than to store material for longevity [4-6]. High levels of SLA also indicate the lower presence of dense tissues in the leaf that expose the plant to being more palatable to grazing herbivores. On the contrary, the high LDMC values show a greater propensity of plants to longevity [7-9]. The other PFTs according with the results of SLA and LDMC.
The *in situ* studies on plant functional traits of seedlings and plants of forest species, providing crucial information on their ecology, may assume a great importance to address forest management practices. This information is likely to prove useful in the near future, precisely in the light of recent Italian and European policies linked to the containment of global warming. The guidelines for the "urban and extra-urban forest plan" of the National Recovery and Resilience Plan (Mission 2; Component 4; Investment 3.1) recently published by the Italian Ecological Transition Ministry require specific references to natural forest communities and native woody flora to propose models of new urban forests that are consistent with the environmental characteristics of the sites and with the natural potential vegetation types. Yet, the new EU forestry strategy, which plans to plant 3 billion more trees in the European territory by 2030, can become an opportunity to apply the principles of ecology and vegetation science to sustainable land management.

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Skull variation of Eurasian otter (*Lutra lutra*)

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The Eurasian otter (*Lutra lutra*) is a Mustelidae belonging in the subfamily Lutrinae [1]. This species has a wide range, occurring in North Africa ,Europe, Russia and Asia [2]. The Eurasian otter is considered a semi-aquatic mammals living in a wide variety of aquatic environments, including rivers, lakes, reservoirs, swamps, and marshes. The diet of Eurasian otter is mainly composed of fish, but it can also include crustaceans, amphibians, reptiles, birds, and mammals [3].

Hung and Law [2] recognized 11 extant and one extinct subspecies: *L. l. angustifrons, L. l. aurobrunnea, L. l. barang, L. l. chinensis, L. l. hainana, L. l. kutab, L. l. lutra, L. l. meridionalis, L. l. monticolus, L. l. nair, L. l. seistanica* and the extinct *L. l. whiteleyi*.

Despite wide spectrum of habitats and the high number of subspecies, the otter was thought to have low only low degree of phenotypic and genetic variation. However, genetic and phenotypic variability was investigate mainly across Europe[4, 5].

Moreover, recent geometric morphometric studies revealed differences in skull shape across Great Britain [6] and Scandinavia [7]. These new findings suggest that geographic and adaptive variation in the Eurasian otter might be more extended than previously considered.

To explore the intraspecific variability in size and shape of the skulls among subspecies of Eurasian otter we used a 3D geometric morphometric approach. We specifically aimed at to identify skull size and shape variation among subspecies.

We examined 235 (F = 102; M = 132) skulls of adult Eurasian otters belonging to 10 over 12 subspecies. Information on sex, age, locality, and subspecies were extracted from the museum's labels. When information on age was missing, this was assigned by checking the status of the sutures. We calculated the topographic distance among each localities and then we pooled localities into 42 geographic areas.

We photographed each skull in the vertical, ventral, and dorsal projections for 36 times (one photo every 10°), for a total 108 pictures and using the photogrammetry technique we reconstruct 3D models of each skull. For the NHM of London we obtained the 3D models directly from the skulls using a surface scanner. Previous studies showed that these two techniques give similar results in geometric morphometric analyses [8,9].

On each 3D model we placed 30 3D landmarks and nine curves with sliding semi-landmarks using Stratovan Checkpoint (Stratovan Corporation [Davis, California, USA]). To remove variation due to rotation, translation and isometric size, landmarks and semi-landmarks were subjected to General Procrustes Analysis superimposition [10]. The natural logarithm of centroid size (lnCS, the square root of the sum of squared distances of all the landmarks) was used as a proxy of size [10].

To account for the highly unbalanced sample size of subspecies, we estimate the mean shape and mean lnCS for every thirty-two geographic areas of the subspecies *L.l.lutra,* obtaining a final 77 geographic areas. A Principal Component analysis (PCA) was run on the aligned coordinates to explore shape variation among subspecies. ANOVA on lnCS and Procrustes ANOVA on shape coordinates were run to test for differences among subspecies. Results of ANOVA a Procrustes ANOVA indicate that subspecies differ significantly in size and shape.

The PCA showed a clear difference of the nominal subspecies *L.l. lutra* from all other subspecies but *L.l. whiteleyi* and *L.l.kutab*. Also, the North African *L.l. angustifrons* and the Near East *L.l meridionalis* differed from all Asian subspecies, i.e. *L. l. aurobrunnea, L. l. barang, L. l. chinensis, L. l. monticolus and L. l. nair* .

Our results suggest that similar selective pressures may act at large scale in different portions of the Eurasian otter range, resulting in different evolutionary pathways. Different types of diets can lead to variations in the shape of the skull in the Eurasian otter [6]. We speculate that one of the main drivers in the variation of skull shape in various subspecies could be the different type of diet. In fact, the diet of *L. l. lutra* is mainly composed of fish [3],

while in the diet of *L. l. angustifrons* is mainly composed of amphibians [11] and in the diet of *L. l. nair* is mainly composed by crabs [2].

The outcomes of our extensive investigation also suggest the existence of at least one highly divergent taxon, i.e. *L. l. nair* that might be considered an Evolutionary Significant Unit. To our knowledge no genetic studies have been carried on Eurasian otters from Sri Lanka, and there is a general gap of knowledge on the Asiatic populations of *L. lutra* [2]. Investigations on these populations are particularly urgent considering the many threats Asian biodiversity is facing [12]. Whereas some Himalayan recognized subspecies should be carefully reconsidered, especially *L. l. monticolus* and *L. l. kutab*. However, we are aware that the small samples size limit the strength of our conclusions and claim for the need of a more extensive sampling and analyses to be concentrated on these taxa in the future.

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A territorial supply chain for a sustainable Blue Economy in Molise

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Plastic is the primary source of pollution in the seas, oceans and coasts: in fact, according to UNEP (United Nations of Environment Programme, 2021), at least 85 per cent of total marine waste is plastic [1], moreover, there are currently an estimated 150 million tons of plastic in the ocean, and by 2050 the weight of marine plastics will exceed that of fish [2].
The dispersion of plastic in the oceans affects not only the quality of marine biodiversity, but also the economic activities linked with marine resources altering the quantity and quality of demersal resources. Globally, the estimated market value of marine and coastal resources and industries reach approximately the 5% of global gross national product, and more than 3 billion people rely on marine and coastal biodiversity for their livelihoods [3].
Under these conditions, it is now more important than ever to develop sustainable economies that combine economic activities with environmental protection and biodiversity conservation.
In this perspective, the concept of Blue Economy, used for the first time during the UN conference in Rio in 2012 [4], turns out to be of fundamental importance where aims to "*the improvement of human well-being and social equity, while significantly reducing environmental risks and ecological scarcities*" [5], or rather, "*the sustainable use of ocean resources for economic growth, improved livelihoods, and jobs while preserving the health of ocean ecosystem*" [6].
The European Union has always played a key role in developing the concept of the Blue Economy, which includes all those activities that are marine-based or marine-related, not only those activities which traditionally contribute to the Blue Economy (e.g. marine living resources, marine non-living resources, marine renewable energy, port activities, shipbuilding and repair, maritime transport and coastal tourism), but also emerging and innovative sectors (e.g. ocean energy, floating solar energy and offshore hydrogen generation, blue bioeconomy and biotechnology, desalination, maritime defence, security and surveillance, research and infrastructure) [7].
Even in Molise region, the Blue Economy, if applied with the instruments of economic and social sustainability described so far, could give rise to projects of great territorial importance.
The positive effects of adopting in Molise a sustainable Blue Economy approach could be very important. In Molise the 17% of the population is concentrated in the four coastal Municipalities (Petacciato, Montenero di Bisaccia, Termoli and Campomarino), and in the Region, the economic activities connected to the blue economy are 938 [8]. Moreover, Molise coast has high naturalistic value, as the protected areas of Natura 2000 network covers 45% of the coastline [9].
In the framework of the SMART ADRIA Blue Growth project (https://smartadria.italy-albania-montenegro.eu/), funded by the Interreg IPA CBC Cooperation Programme and involving Italy, Albania and Montenegro, local authorities, SMEs, civil society and research bodies, the University of Molise provided the basis to develop in Molise a territorial supply chain linked to the world of fishing, aquaculture, restoration and coastal tourism, which considerably engage itself in reducing the use of disposable plastic.
In particular, the University of Molise, through a series of formal and informal meetings with local stakeholders that work in different Blue Economy sectors has established a multi-level collaboration that has resulted first in the drafting of a "*ROADMAP for a Molise-based single-use plastic free industry, from the sea to the table*" (http://envixlab.unimol.it/smart-adria-blue-growth-verso-la-riduzione-della-plastica-monouso/), and then in the ratification of an expression of an interest.

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Fig. 1. Trend Watching Event - Termoli (CB) - 28/04/2022.

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Remote Sensing for studying Invasive Alien Plants in coastal ecosystems: a state of the art contribution

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Coastal environments are among the most endangered ecosystems worldwide [1], and invasive alien plant species (IAPs) are one of the main threats to these habitats [2], requiring effective tools for early detection and mapping. Remote sensing (RS) has become one of the most efficient methods to deal with biological invasions [3], and even though studies on alien plant invasions have been done using remote sensors in different habitats [3-8], there is still a lack of an overview of the progress and extent of RS applications in monitoring IAPs in coastal ecosystems. This contribution on the state of the art is specially needed to support the development of more operational RS frameworks that can enhance the management of coastal IAPs invasions.

Here, we conducted a systematic literature review of 86 research publications that implemented, recommended, or discussed RS tools for IAPs management in coastal environments from 2000 to 2021. Specifically, our work focused on: i) analyze the main characteristics of the research articles that have employed RS tools to survey IAPs in coastal ecosystems; ii) examine how features, instruments and methodologies of the RS studies applied to IAPs in coastal ecosystems have evolved over time; iii) analyze the interaction between different RS features (e.g., instruments, sensors, resolutions, etc.) and the coastal ecosystem types where the studies were conducted.

Our research evidenced that most of the research has been carried out in China (22) and the USA (11), with “invasive species”, “hyperspectral”, “random forest”, “Acacia longifolia”, “maxent”, “invasive plant species”, “invasive alien species”, “phenology”, “Landsat”, “support vector machine”, and “hyperspectral remote sensing”, being the most mentioned keywords in the selected articles. Sporobolus (17.3%) was the most studied genus, while North America showed the highest frequency provenance of IAPs (31.4%). As for life forms, phanerophytes (33%) and geophytes (31.9%) were the most studied in our research. Although the number of studies increased from 2000 to 2021, this rise was more pronounced from 2015, and while most of them have primarily focused on the detection of IAPs, interest in modelling has increased at this time. The most used instrument at the beginning of the analyzed period were aircrafts, but satellites have increased since 2005, while the use of UAVs has taken importance around 2014. Multispectral resolution not only occurred in a large number of studies, but also indicated a significant increase from 2005 to 2021. Ultra-high resolution, followed by very high resolution, were the most commonly used spatial resolutions in the analyzed studies. However, ultra-high resolution showed a significant decrease, whereas very high resolution remained constant over time. Although frequentist inference was the most widely adopted classification approach in the early years, machine learning increased significantly after 2009. Even though Mediterranean seacoasts have been the most studied areas, research on subtropical littoral ecosystems increased over time. Interactions between different RS characteristics and coastal ecosystem types showed that machine learning was used in all types of coastal ecosystems, while frequentist inference was used in more studies than machine learning, but its use was mainly limited to Mediterranean coastal ecosystems.

Our results highlighted certain preferences in considering certain aspects of RS in relation to the type of coastal ecosystem under study and highlighted the great possibilities of RS data to further improve the monitoring of IAPs. Our account provides a benchmark of the available literature useful in supporting the creation of targeted approaches to address IAPs using RS techniques in coastal ecosystems.

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The sea and the coastline: development of the territory

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The aim of this research work is to understand the role of the port of Termoli in the territorialisation process, focussing mainly on the XVIII and XIX centuries when the countryside in Molise became one of the new target markets for the Kingdom of Naples' grain trade. The changing conditions of the grain trade, caused by the serious grain crisis in 1764, had in fact brought to the fore production areas such as the Molise countryside and, consequently, stimulated coastal town activities from which grain could be easily transported to the areas of usage. The maritime route was preferable to the overland route due to the lack of roads and the poor viability of those available, that were often, near the many waterways, lacking bridges. While the Capitanata roads were impractical, the ones in Molise were downright dangerous. The decision to transport via sea was almost mandatory.
Until the end of the nineteenth century, despite its limits and weaknesses, the port of Termoli managed to keep its role as a convergence point between the urban landscape and the Molise hinterland, making up for the overland viability in the transport of goods and people, but in the next century things started to change. With the railway development and the improvements to the road network conditions, but most of all following the changes made in the whole country, the connection between "the fields and the sea" became weaker and weaker until it faded completely. The inland areas will go through a marginalisation process that will be even more pronounced after the second world war, despite the anthropized coastline, and the port, regardless of the reconstruction project in the twentieth century, will lose the commercial role it held in the previous centuries. In Molise the phenomenon was also linked to the development of an industrial area right by the town of Termoli, but also to the use of the sea more for bathing activities rather than the more traditional commercial activities.

Fig. 1. Project of the 1905. ASCb, Prefettura, b.15, fasc. 51.

Nowadays, a port such as the one in Molise, especially after the radical changes made to the Mediterranean routes in the last two centuries, has inevitably lost its importance. Although the connection between the coast and the inland area in the future could never be the same as it was in the XVIII and XIX centuries, it may nevertheless be patched up with different functions, that could restore a past linked to the sea, in order to regenerate it according to the demands of the present day. In this perspective the implementation of a sea Eco Museum could represent a way to provide documentary evidence and also to qualify and manage an area that needs to return to function in an integrated system.

Fig. 2. Termoli. ©Maurizio Perrotta.

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Pro-Food-IV project and sustainability in food industry: Life cycle assessment of innovative solutions for fresh-cut production systems

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An exponential growth of fresh-cut sector has been observed in the last decade [1]. In Italy, this sector represents a large part of agro-food supply chain, with approximately 6,500 ha of plastic tunnels producing 110 mln Kg year^{-1} of fruits/vegetables for an overall value of 744 million € [2]. As they are free of waste and ready for consumption, fresh-cut products require many inputs in their production systems. As a consequence, concerns regarding environmental impacts of fresh-cut production systems have increased [3]. Chiefly, soil management, product washing and food packaging becomes crucial due to i. the use of pesticides [4], ii. the water consumption/contamination [5] and iii. the adoption of controversial techniques to ensure an optimal shelf-life [6]. Accordingly, fresh-cut sector undergoes important changes in order to enhance product quality, food safety and environmental performances [1]. Life Cycle Assessment (LCA) is the most suitable method to assess sustainability of fresh-cut production systems [7], allowing the estimation of environmental impacts of a product along its whole life cycle [8]. Within this framework, ProFood-IV project aims at the development of innovative and low environmental impact solutions to improve safety and quality of fresh-cut products. Present work focuses on the assessment of the environmental performances of innovative solutions developed in ProFood-IV project in accordance with the standards of ISO 14040 series. Specifically, environmental impacts of a conventional and an innovative production system were compared through an LCA method. As the project is still in an early stage, the production systems have been designed in a simplified way but taking into consideration all the processes of real systems.

Comparative LCA is carried out using SimaPro software 8.5.2.0 developed by Pre consultants. Due to the strong corporate imprinting of ProFood-IV project, the inventory of the conventional system is mainly based on primary data collected through questionnaires to chain employees. Inventory of the innovative system was built with secondary data from Ecoinvent v3.0 databases. Main differences between the two compared systems regard soil disinfestation with Metam-Na that is replaced by solarization before sowing in the innovative system. The conventional sanitization through oxidizing role of chlorine is replaced by ozone in the innovative system. Finally, traditional packaging is replaced by a biopolymer packaging combined with a functional film based on Na-Acetate, in order to decelerate product deterioration. The IMPACT 2002+ 2.15 method was chosen for results' interpretation. The functional unit is a 120gr pack of IVG baby-leaf salad. Systems' boundaries refer to a "from cradle to gate" LCA.

Results show that innovative system reduces the overall impacts by 43% respect to the conventional one. In particular, soil disinfestation impacts by solarization decrease by 97% compared to Metam-Na use. The use of ozone reduces impact by 54% compared to chlorine sanitization. In particular, innovative sanitization regulates water consumption/contamination, reducing impacts on "climate change" and "resource depletion" (respectively, -80% and -82% compared to conventional sanitization). Finally, innovative packaging reduces overall impacts by 14% respect to conventional one.

Innovative solutions result in a better environmental profile respect to a conventional system. Above all, we highlight the importance of impact reduction by solarisation. Soil disinfestation represents half of the overall impacts of agricultural phase, which represent the most impacting phase (87% versus 13% of impacts of manufacturing phase). Although sanitization and packaging are not equally influential on the whole production system, it should be emphasized that innovative processes lead to an increase in product shelf-life, implying a

substantial reduction in overall impacts. Our findings could support decision-making while evaluating the introduction of innovative products and techniques in fresh-cut production systems. Therefore, this study moves toward a more sustainable production of fresh-cut products. Within ProFood-IV project, results will also be supplemented by economic analyses in order to validate the practical feasibility of the innovative solutions.

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(Im)mobilities: Discourses produced by objects

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Outlooks on (im)mobilities rest on games of perceptions and territorial realities. Not only symbolic infrastructures but also material infrastructures, intersect the imaginary value of places [1]. Posthuman perspective help to get out of these dynamics. They are looking to go forward utterly anthropological sight, to understand how bodies interact whit non-human objects in shape and enable human and sensorial faculty [2].
Especially, immobility collides whit the daily needs of interconnected lives. Indeed, a better comprehension of migration reasons is possible thanks to the consequences and causes of immobility [3]. These assumptions are also the basis of current research works about the migration propensity of inner areas youth, to explore the motivations and needs that drive them to stay in these territories [4]. The propensity to stay or leave and the voluntariness of taken choice [4] are powerful facts for analysing migratory imaginaries [5], tied to local realities and typical transnational communities of many Italian emigration regions. Consequently, immobility must be understood as the continuity that an individual pursues in a precise space-time context, in a place of residence, which is not to be understood as absolute immobility because, daily, people move for various needs [6]. For example, this may be the case of commuting inhabitants, for study or work reasons [7]. In these daily practices, it's possible to search pieces of "resident" and "migrant" imaginaries, for reading immobility between both structural migration restrictions and aspirations to stay [8]. Analyzing these aspects might be put into discussion and/or tip over an expected regime of truth [9].
From recent interviews conducted on a people sample from Molise, through which the narrative-territorial identity of Molise was investigated, among the problems that emerged was the difficult accessibility of the places [10]. A prime example is the *Sente-Longo viaduct*, disused since 2018[1]. This arterial road is essential to connect *Alto Molise* and *Alto Vastese*, revoking the classical function of these preponderant elements of mountainscapes, like economic, social and cultural links, keys for the vitality of village communities. According to the recent perspective of non-human [11], the infrastructure and the resulting dynamics of its inactivity raise various considerations. In terms of affordances [9], the viaduct no longer offers the possibility of mobility for which it was built and therefore also prevents daily community relationships. The issue opens up space for the theme of accessibility, central to social justice in marginal areas [12]. Secondly, being a road located on the border between Abruzzo and Molise, the border agency [13] emerges powerfully in the influence of daily bodily practices, generating imaginary cross-scale negotiations, capable of influencing territorialization and deterritorialization processes in the so-called borderlands [13]. The border agency is combined with the agency of the viaduct, understood here as a geographical object [11, 14], which is expressed through various discursive practices, potentially capable of anthropomorphizing the object in question [11, 15]. A sense is attributed to the sign, understood here as the border. We can argue that the sign denotes a useful infrastructure for the mobility of things, ideas and people. Nevertheless on a connotative level, it takes on a secondary meaning, namely that of immobility, bound or desired by those who practice border processes. Given that, according to architectural geography, it is possible to understand objects, practically, through aesthetic activities [11]. The approach of discourse analysis is proposed here, in the context of visual methodologies [11], to read the narratives generated by the different degrees of the significance of the *viaduct Sente-Longo*. To bring out these discursive dynamics, the audiovisual sources produced online, from 2018 onwards, by the various

[1] For the reasons that led to the closure of the viaduct and the relevance that the issue is assuming for the local population, see the following links: https://www.rainews.it/tgr/molise/video/2020/07/mol-viadotto-sente-chiusura-783994c4-3e96-497c-a078-8f61bac39d6e.html;
https://www.rainews.it/tgr/molise/video/2021/09/mol-viadotto-Sente-chiuso-2fe1dded-9ff3-480c-b0e7-226b4f7baf26.html;
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local broadcasters, can highlight the liminal [16] and local dimensions of the stories on (im)mobility.
In conclusion, the ultimate goal of this analysis is to understand how the difficult mobility experienced, both materially and virtually, affects the phenomena of commuting between *Alto Molise* and *Alto Vastese*, influencing, in the long term, also the migratory phenomena from the aforementioned zones.

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Literature review on nature-based solutions and their long-term feasibility

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Nature-based solutions (NBS) have recently gained a lot of momentum, among scientists and decisionmakers, as a response to the current and projected changes of environmental and climate conditions [1]. This umbrella concept embraces a series of territorial planning approaches, to mention a few: Ecosystem-based Adaptation, Disaster Risk Reduction, Climate Change Adaptation and Mitigation [2]. These approaches propose the search of a concerted relationship with ecosystems in different fields and with different scopes, principally to improve or restore ecological conditions and services, and increase the resilience of people and places to climate change (CC). Multiple benefits and positive examples have recently sprawled in research and applications [3, 4], together with NBS implementation scenarios and socioeconomic trajectories [5].

In addition to planning scales, the temporal scale is fundamentally part of the definition of such measures. The envisioned lifespan of an NBS matters to the type of climate-related issue that is being addressed, as well as to the capacity of generating environmental and socioeconomic benefits.

The relation between NBS and climate change is conceptualized in the form of 'time to deliver benefits' [6], 'stability over time of benefits-delivery' [7], scenarios of socioeconomic developments *e.g.* business as usual, green transitions [5]. Timeframes of territorial planning also deal with the progressive shifts in local administrations and political agendas [8], the economic side of NBS planning and the temporality of their return of investments [9]. These aspects of NBS planning try to act in response to the uncertainty that characterises any future state.

Few studies directly address the need to further investigate on the effectiveness and feasibility of NBS across temporal scales and environmental changes. The benefits associated to NBS still have to be thoroughly studied, tested and quantified [10]. Climate and biological changes are highly likely to shift the functioning and temporal dynamics of ecosystems, with impacts on the performance of NBS [7] [11], and on their tolerance and adaptability [12]. Hence, in addition to delivering multiple benefits and adaptive capacity, nature-based solutions are also understood to be vulnerable to future changing conditions, as they are made of natural components.

Two aspects emerge from the above: 'climate change impacts *addressed by* NBS' *vs* 'climate change *impacts on* NBS'. In order to analyse how the literature on nature-based solutions and climate change features time, future horizons and impacts, we performed a systematic literature review. The aim is to understand how these concepts relate, frame and act with regards to temporal dynamics, future climatic and environmental conditions, the framing of impacts, and long-term planning needs.

The knowledge gap addressed in this work is the lack of information on nature-based solutions effectiveness across temporal scales and environmental changes.

The review was performed through the Scopus search engine, gathering a total of 515 documents through the combination of the entries 'nature-based solution(s)'and 'NBS', with at least one entry revolving around climate change, temporal dynamics, feasibility and effectiveness, in order to sort the documents discussing the changing features of territorial planning with NBS in time.

Preliminary results are articulated on the differentiation between documents supporting NBS feasibility to face climate change ('CC impacts addressed by NBS') representing 98% of the total, and the ones supporting the fact that NBS themselves face the risk of climate change ('CC impacts on NBS') which is found in 13% of literature. After the previous differentiation, the documents are currently being analysed based on the treated aspects of NBS and climate change relation in time; at the same time, they are categorised based on the challenges mentioned, along with spatial-temporal-knowledge scales, climate classification, landcover type, methods, type of NBS considered. Expected results include the gathering of available knowledge on proved or unproved effectiveness of NBS, which, considering the singularity of every NBS setting and application, may pose some challenges to comparability. Further research should focus on assessing the real potential of NBS to deliver benefits and co-benefits in the future.

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The integration of resilience to socio-natural disasters in territorial planning. The link between preparedness and post-disaster recovery in landslide risk management in fragile areas

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The study aims to contribute to the integration of principles, policies and strategies that are aimed at building community resilience to "natural" disasters in the tools of ordinary planning, particularly in territories characterized by depopulation, starting from deepening of the link between preparedness and post-disaster recovery. The project take place in fragile territories, rural and inland areas, for their higher vulnerability and because they need a response at the policy level [1]. Particular attention is given to landslides because they affect those areas [2] and because the types of disasters that are most closely linked to climate change have seen the greatest increase in the frequency and destructive extent in recent years [3, 4].

The use of terms such as "Natural Disaster", "Resilience", "Vulnerability", "Hazard", has changed greatly over the last few decades and has been the subject of critical literature that led to the attribution of new meanings or to the abandonment of some terms instead of others. A study conducted by Pagliacci [2], which examines floods, landslides and earthquakes in Italy, shows how risks tend to be concentrated mainly in rural and peripheral areas of the country. Rural areas represent 85% of the total areas at risk in Italy and are proportionally more exposed to landslides and earthquakes and less to floods.

The topic of demographic decline, often declined in shrinking cities, shrinking rural areas, Inner Areas, has acquired ever greater relevance to the point of establishing itself as an autonomous field of specialization for those who do research in the fields of planning, geography, social sciences, and same can be said about the branch of disaster studies. However, it is not clear what happens when depopulation and disasters are explicitly overlaid. Are there interferences, coincidences, mutual influences? How many voices take the two phenomena into consideration jointly, exploring their interaction? A review of the scientific production (social, environmental and earth sciences) has been expressed to date regarding the reconstruction/re-inhabitation of places affected by a disaster and undergoing depopulation. It seems reasonable to state that the interest around the proposed theme has grown particularly in the last five years, in which the most substantial studies are concentrated: books; book chapters; long-reads, and this increase is only partially explained by the general increase in academic production. It seems also possible to say that the growing interest is evidently located: it is more marked in those countries that experience a long-term negative demographic trend and that are prone to disasters (Japan [8], Italy [7], USA); but this is clearly not surprising. Since the research project crosses three macro-topics, i) connection between preparedness and reconstruction in disaster risk reduction, ii) depopulation, iii) landslides; the study of bibliographic sources is done for each of the areas.

The study makes use of the methodology of multiple case studies [5], compared through an abductive procedure, i.e. based on an operation of juxtaposition of even heterogeneous case studies, aimed at bringing out not only the relationships of hidden similarity but above all to reveal in this way the specific transformative potential, the unexpected "virtuality" of change [6]. The case studies are analysed: 1) in the light of current regulations and plans and their evolution 2) in the dynamics of the event, the behavior of the authorities and the (prepared or not) reaction of the community, 3) in the media narratives 4) in the ways in which post-disaster recovery is expressed.

The reasonings resulting from the comparison of case studies are then placed in dialogue with the results of another parallel operation: interviews addressed to experts in the field, policy makers, planners, scientists or activists on the questions that drive the research. Case-study analysis can thus confront hypothesis, and verify or deny it. A case study that has been taken into consideration so far is that of the mudflows of 4-5 May 1998 in Sarno (Italy) through desk research, evaluation and re-elaboration of information already collected from official sources.

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LIFELINES SAFETY: A TECHNICAL AND TECHNOLOGICAL CHALLENGE

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As part of the project SICURA (caSa Intelligente delle teCnologie per la sicURezza- l'Aquila), namely Smart House of the technologies for safety, funded by the Italian Ministry of Economic Development, the Construction Technologies Institute of the National Research Council of Italy, ITC-CNR in L'Aquila, as a partner of the project contributes, among others, to the design and coordination of an experimental living lab, its implementation in a digital/virtual environment, and to the innovation in structural and earthquake engineering. Recalling the key concepts of the project, the living lab is established as a research, experimentation, and innovation laboratory aimed at providing users with competitive solutions through approaches, tools, and services. The core of the project addresses the safety of infrastructures, prevention, and mitigation of the effects resulting from critical events, and support in emergency management, by exploiting the network capacity offered by the new 5G technology, and the possibility of connecting multiple devices through the Internet of Things (IoT)-based solutions. Within this framework, the ITC-CNR focuses on seismic hazard and lifelines safety and achieves its goals by setting up a technological laboratory with scaled-down models, representative of a typical urbanized context, including the soil deposit and the underground and aboveground structures interacting with the soil. Specifically, the physical small-scale models consist of lifelines systems, such as communication networks, underground conduits, and phone towers. The presence of a soil deposit allows users to integrate structural engineering problems and monitoring with soil-structure interaction. This last aspect is often neglected but can have a significant impact in specific contexts, therefore efforts are necessary to establish its relevance under various conditions and characterize the best way to accurately account for such an interaction. Lifelines have a complex structure with multiple components connected and interacting with other systems or components, with a hierarchical asset and specific functions. This makes the system vulnerable due to the consequence of possible damage that could compromise the whole functionality. This would certainly be a problem, also in the phase of an emergency, because critical infrastructures ensure essential services that are needed during the management of an emergency state and should remain operational. The vulnerability is further accentuated by the variety of component types and materials used, and the spatial variability and exposure to different geological and geotechnical contexts. An exhaustive case history of damages to the road, telecommunication, and gas system was experienced during the 1994 Northridge (Mw 6.7) earthquake [1].
In complex soil conditions, the effects of earthquakes can be devastating, as for the case of soil liquefaction (Fig. 1), with a loss of shear resistance of the superficial saturated sandy soil deposit of low relative density that can lead to translational failure of slopes and undermining of foundations and infrastructures, to the floatation of buried structures, ground settlements, bearing failures with differential settlements, tilting or overturning, lateral spreading and embankment failures damaging buildings and infrastructures [2].

Fig. 1. Effects of soil liquefaction on buildings and infrastructures [2].

This laboratory is being designed with a 5-ton shake table apparatus that would reproduce earthquakes on small

physical models to address the lifelines' performance in a soil liquefaction environment. A soil box will be mounted on the table, a sandy soil deposit will be prepared at medium relative density and saturated, and the underground and aboveground lifelines will be installed during the preparation of the soil or from the top depending on the planned experiment. The experimentation will support the knowledge of how these systems would react to various conditions, explore new possibilities for prevention and mitigation of earthquake-related effects, and improve the early warning systems for emergencies and the activation of safety measures. The soil deposit and the structures are instrumented with accelerometers, strain gages, displacement sensors, and soil pore water pressure transducers for continuous monitoring of the structure, the soil, and the soil-structure interface. Small shakers will also be installed to induce, in addition to the seismic excitation, a source of external noise to the system to reproduce the ambient noise in the real environment. The monitoring allows for the calibration and improvement of numerical tools to consolidate and enhance the existing approaches. Moreover, a focus on the soil-structure interaction is helpful to understand in which cases it must necessarily be considered, how best to characterize the interface, and what simplified approaches can be used. Referring to the early warning systems, usually, key parameters are monitored, and exceeding a set threshold triggers the alert [3]. However, based on the high-speed capacity of the new 5G technology, the ability to collect, process, and integrate a large amount of data, and the capabilities of networking many devices, it enables more comprehensive alert systems to be used for problems showing some complexity. In this case, the idea is to use a Digital Twin of the monitored system to support the early warning procedures (Fig. 2). This implies good numerical modelling to exactly reproduce the real phenomena happening on site and the necessity to manage huge datasets with analysis of large computational domains, running in parallel, and exploiting multiple processors. In such a framework, multiple components can interact and exchange data through the potentiality of the internet connection, the networked devices can be remotely controlled and accessed, thus creating an integrated and automated system with increased efficiency and performance, enhancing the Internet of Things applied to the early warning systems for seismic hazard by including the subsurface component in complex contexts.

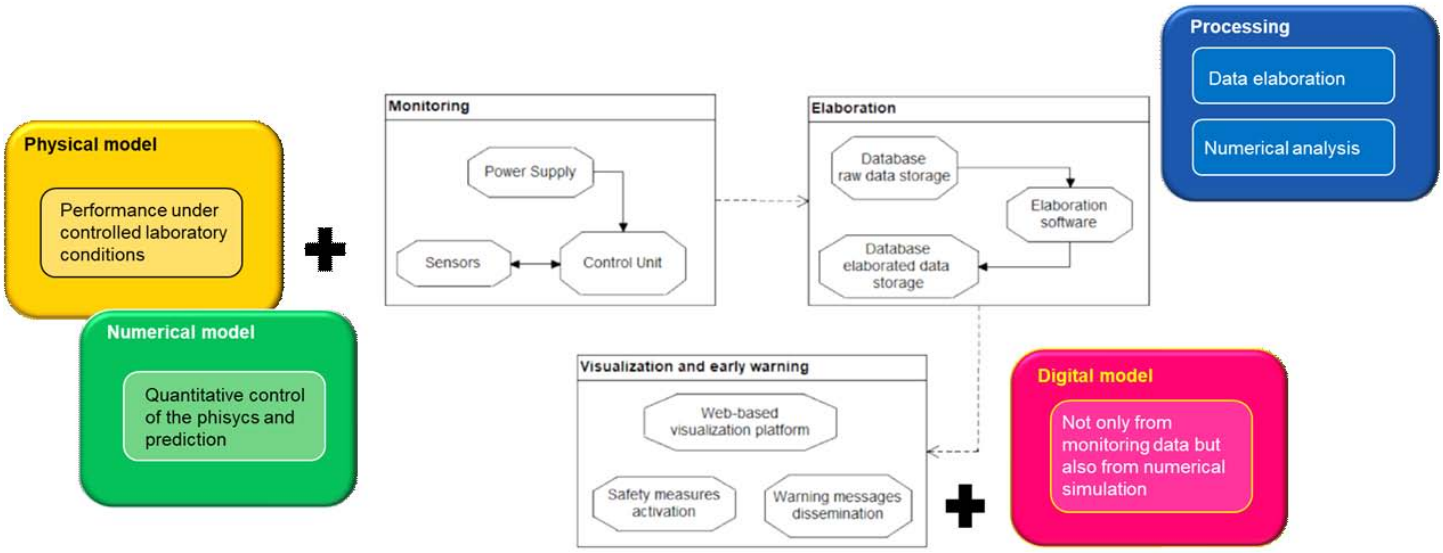

Fig. 2. Early warning procedure for complex systems (modified after [3]).

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A new tool for seismic fragility assessment of minor historical centers in the Inner Areas of Italy

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Seismic events of the past highlighted the high seismic fragility of the Italian territory. In particular, minor historical centers of Inner Area of Italy, located in the central and south Apennine areas, represent the zones most affected by earthquakes. Due to the presence existing unreinforced masonry and reinforced concrete buildings designed in absence of seismic code provisions or according to obsolete design codes, significant damages to buildings, economical losses and casualties verified in these areas following to earthquakes.
In reason of this, different methodologies devoted to large scale seismic fragility assessment have been developed in the last years, based on empirical, mechanical, expert judgment or hybrid approaches [1]. Large-scale seismic performances encompass a statistic treatment of a great number of data concerning building typologies, damage survey, number of victims and/or monetary losses whose final goal is the development of *fragility curves*. They envisage the exceedance probability of a given Damage State (DS) or Limit State (LS) threshold of the building stock included in an area as a consequence of scenarios earthquakes characterized by Intensity Measure (IM) representative of the strong-motion. Common to each method, the level of shaking is quantified through different earthquake IM parameters, including peak-ground acceleration/displacement/velocity, spectral accelerations/ velocity/displacements, or macroseismic intensity measures.
Learning from earthquakes, severe damage to buildings have been also observed as a consequence of far-fault earthquakes, depending on both building features and earthquake intensity. For instance, earthquakes occurred in the Inner Apennine Area of Italy proved that minor historical centres have been seriously damaged (with local or local collapses) due to earthquakes having epicentre located to more than 30 km away [2].
Literature overview highlighted that no methodologies devoted to predict probabilistic structural safety at large scale, accounting for multiple source-to-site effects of earthquakes randomly distribute over the territory (i.e., far-fault earthquakes), are available.
In the research undergoing herein presented, a novel probabilistic-based seismic hazard disaggregation analysis (PSHA) has been implemented within a fragility model. The method is devoted to predicting likelihood damage scenarios associated to the achievement of a Limit State threshold of the building stock included in an area of interest, under the effects of conventional scenario earthquakes selected through PSHA [3]. For a given area, constituted by a building population, a set of most probable scenario earthquakes is provided by PSHA in terms of M-R-ε disaggregation of the hazard, delivering the combination of parameters that contribute the most to the ground motion at the site. Fragility matrices exploit such a feature of the PSHA analysis, since whatever is the reference intensity measure of the selected fragility curves, they can be expressed as a function of the M-R-ε disaggregation parameters by means of the introduction of the attenuation laws. In particular, a change of variable of the IM has been applied moving from one-dimensional fragility curves to fragility surfaces presented in terms of matrices, named Urban Fragility Matrices (UFM). UFMs provide the probability of exceedance of a certain Damage State (DS) or Limit State (LS) threshold for the building stock included in an area of interest, relative to a set M-R-ε representing the expected scenario earthquakes selected through PSHA.
UFM are defined with the following matrix operation:

$$[U]_{nxm} = \sum_i \alpha_i \, [T]_{i,nxm} \qquad (1)$$

where [U] and [T] are the UFM and TFM respectively, both having *n* x *m* dimension (*n* are the intervals of magnitude and m and R that of distances); instead, α_i is a scalar value representing the percentage of each building class included the area of interest. TFMs represent the typological fragility matrices--i.e., relative to the single building classes included in the area of interests- whose terms indicate the probability of exceedance of a DS or LS threshold

of building stock for a given set M-R-ε. Each term of the TFM can be obtained with the following equation:

$$CDF = P[DS > ds|\overline{PGA}] = \int_0^{\overline{PGA}} f(im)\mathrm{d}im = \int_0^{PGA(M,R,\varepsilon)} f(im)\mathrm{d}im \quad (2)$$

where the function PGA(M,R,e) represents the chosen Ground Motion Predictive Equation (i.e., the attenuation law) of the shaking. The *f*(*im*) is the probability density function (typically the lognormal one) defined by median (μ) and standard deviation (β) of the IM available in literature with reference to specific structural type and/or building classes.

The effectiveness of the proposed methodology has been validated with reference to the case study of Pozzuoli (Campania region, Southern Italy), struck by M 4.0 seismic event in 1983, for which results of the seismic damage survey referred to about 3500 buildings were available: damage survey highlighted that about 20% of the buildings achieved a damage state corresponding to Ultimate Limit State (ULS). In Fig. 1 is shown the UFM for Pozzuoli, obtained with reference to ULS threshold. Outcomes of the research showed that the damage scenarios predicted through UFMs of Fig. 1 good match with that observed in the aftermath of 1983: in correspondence of M 4-5 and R 0-5 km, less than 20% of buildings had to achieve the ULS.

M	R (km)																				
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
3,0	2,96E-06	4,47E-08	4,53E-11	1,16E-13	7,34E-16	9,01E-18	1,85E-19	5,66E-21	2,40E-22	1,33E-23	9,15E-25	7,62E-26	7,45E-27	8,38E-28	1,07E-28	1,51E-29	2,36E-30	4,03E-31	7,42E-32	1,47E-32	3,11E-33
4,0	6,96E-03	5,58E-04	3,64E-06	2,45E-08	3,34E-10	8,87E-12	3,76E-13	2,23E-14	1,70E-15	1,59E-16	1,76E-17	2,26E-18	3,27E-19	5,30E-20	9,45E-21	1,84E-21	3,85E-22	8,66E-23	2,07E-23	5,25E-24	1,40E-24
5,0	2,29E-01	8,95E-02	7,82E-03	3,76E-04	1,75E-05	9,60E-07	6,68E-08	5,97E-09	6,76E-10	9,36E-11	1,53E-11	2,87E-12	5,99E-13	1,37E-13	3,38E-14	8,92E-15	2,50E-15	7,39E-16	2,29E-16	7,43E-17	2,51E-17
6,0	8,22E-01	5,15E-01	2,38E-01	7,59E-02	1,80E-02	3,68E-03	7,23E-04	1,44E-04	2,97E-05	6,50E-06	1,51E-06	3,74E-07	9,92E-08	2,81E-08	8,51E-09	2,74E-09	9,35E-10	3,36E-10	1,27E-10	5,00E-11	2,05E-11
7,0	9,99E-01	9,93E-01	8,38E-01	4,85E-01	3,10E-01	1,84E-01	9,95E-02	4,97E-02	2,35E-02	1,07E-02	4,77E-03	2,11E-03	9,30E-04	4,12E-04	1,84E-04	8,30E-05	3,79E-05	1,75E-05	8,25E-06	3,94E-06	1,91E-06
8,0	1,00E+00	1,00E+00	9,99E-01	9,91E-01	9,33E-01	7,29E-01	5,37E-01	4,23E-01	3,37E-01	2,63E-01	2,01E-01	1,50E-01	1,10E-01	7,89E-02	5,55E-02	3,86E-02	2,65E-02	1,80E-02	1,21E-02	8,16E-03	5,46E-03

Fig. 1. UFM for the city of Pozzuoli.

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REDESIGNING THE SEISMIC BEHAVIOR OF TRADITIONAL CLT BUILDINGS

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Due to the satisfactory seismic performances and the high environmental sustainability of material, multi-story Cross-Laminated Timber (CLT) buildings become a valid building technology in seismic-prone regions [1]. Panel-to-panel and panel-to-foundation connections of traditional CLT walls consist of Hold-Downs (HDs) and Angle Brackets (ABs) (Fig. 1). They are steel-to-timber mechanical joints, made with thin-steel plates nailed or screwed to the vertical panel and bolted in foundation. HDs are devoted to withstanding tensile forces at the base of the panel due to flexure, while the ABs to resist shear forces. Instead, the compression forces, due to both horizontal and gravity loads, are resisted by the timber-to-timber or timber-to-foundation contact at intermediate and ground storey, respectively.

Several studies highlighted that the steel-to-timber connections made with HDs and ABs have an appreciable ductile and dissipative response, but they involve a significant damaging in the connection zones. In fact, they are designed to fail according to Johansen's mechanisms – i.e., a combination of timber embedment and yield of metal fasteners (Fig. 1)- which envisage an unavoidable damage in metal parts end in timber panels too. This does not match with the modern Performance-Based Design philosophy which requires damage reduction and ease of repair of the dissipative elements, in addition to their load-bearing and dissipative capacity.

Damaging of timber components represents a serious issue, because they can be replaced or repaired with difficulty. In the last few years, some research focused on developing alternative low-damage connection systems for CLT walls, with the common goal of (i) concentrating the hysteretic behavior in the metal parts only, and (ii) eliminating possibilities of damaging in timber components.

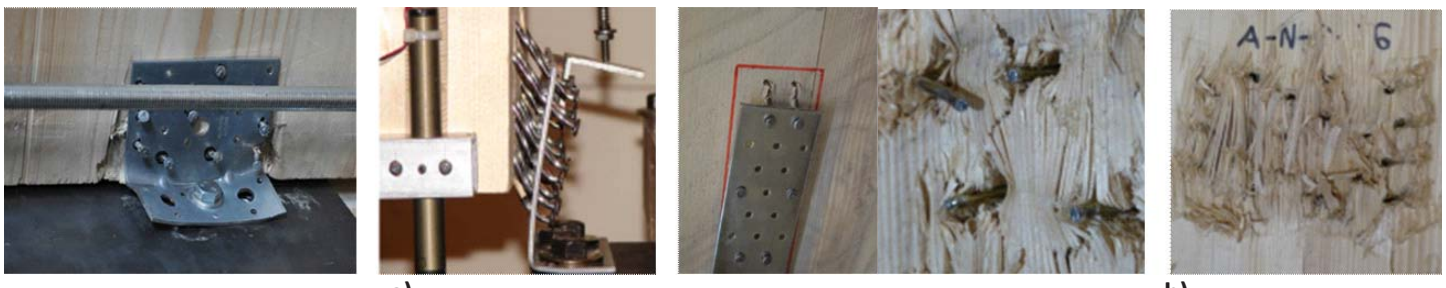
Example of innovative low-damage connections matching with the points (i) and (ii) simultaneously have been developed in New Zealand for post-tensioned timber walls [2]. Instead, in Europe, connections for traditional CLT buildings matching with the point (i) only can be found for instance in Latour and Rizzano [3] and Scotta et al. [4].

Another source of permanent damage is related to the platform construction technique used to build traditional CLT buildings. It provides horizontal floors (made with CLT panels) inserted between two vertical panels: under seismic actions, the rocking motion of the vertical panels compresses in orthogonal to grain direction the floor panels which remain permanently deformed.

The undergoing research herein presented is aimed at redesigning the role of the seismic behavior of traditional CLT buildings, addressing them toward low-damage structural systems. In fact, the idea under development is that of introducing the concept of '*2.0 CLT buildings*' which is based on twofold objectives:

a) To include low-damage dissipative connections.
b) To adopt the balloon-type constructive techniques (which involves full-height CLT walls with floor that do not crosses the vertical panels).

Within this framework, the authors started to work on the point a) designing a new connecting system to withstand tensile forces at the base of the panel. It comprises a fuse-type dissipater made with a threaded steel bar, with



a) **b)**

Fig. 1. Typical failure modes of the mechanical connections made with HD and AB.

reduced diameter in its central part (dog-bone profile) and connected to a stiff steel plate joined to the CLT panel (Fig. 2). The system has been conceived to concentrate the plastic deformations in the steel bar only, while the CLT panel and the stiff plate will remain elastic under seismic actions (i.e., strong metal plates and weak steel bar). As further advantages, the system should be easy-to-repair because substituting the steel bar is sufficient to make the connection functional again after its damaging and the threaded bars are readily available on the market.
To date, first experimental tensile tests, under displacement control, have been conducted on both single dog-bone shaped threaded bars (six tests) and bars coupled with steel plates (four tests), aimed at investigating load-bearing capacity, ductility, and failure mechanisms of the connection.

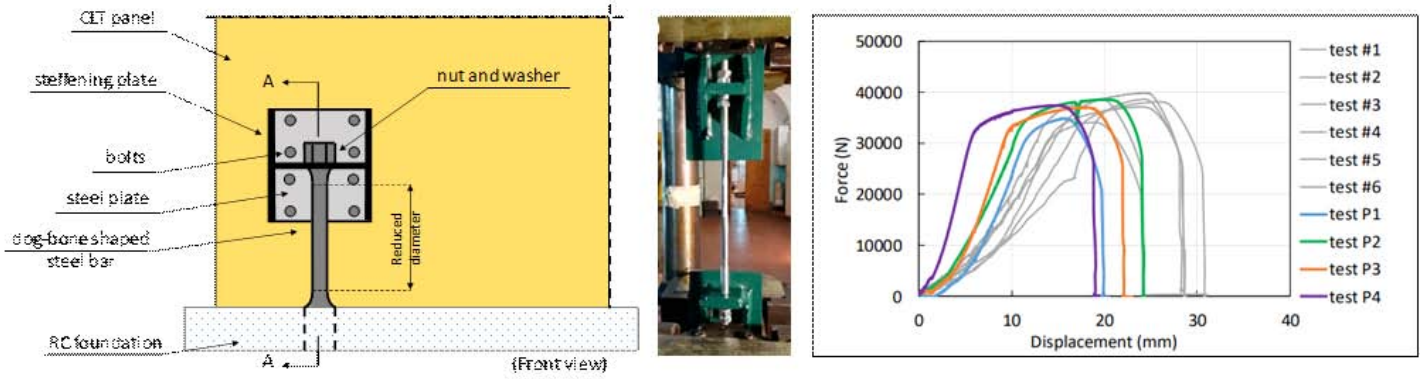


Fig. 2. Arrangement of the connection at the base of the CLT panel, connection system and test results.

In Figure 2, the force-displacement diagrams for the single bars (from test #1 to #6) and that for bars coupled with plates (from test P1 to P4) are reported. Test results highlighted that the system was able to concentrate the plastic deformations over the fuse length of the bar only, with rupture of the specimens occurred in a length equal to 5D measured with respect the middle of the bar. Moreover, a significant ductility is resulted from the tests, with an ultimate to yielding displacement ratio about 1.80. Such a ductility represents a fundamental source to allows significant top displacement capacity due to rocking motion of the CLT panels.
The experimental results are useful to design an experimental set-up for testing CLT panels under cyclic loadings equipped at the base with the proposed connection system.

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Determination of site-to-source distance for events included in the liquefaction case-histories databases

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In the framework of liquefaction assessment, simplified methods have been developed to evaluate the triggering of the phenomenon by estimating a safety factor. In these approaches, the seismic demand is scaled through a magnitude scaling factor (MSF) that, in turn, depends on the magnitude and converts the seismic load to the load equivalent to an earthquake of magnitude 7.5 [1]. The rationale of this approach is based on the concept that the higher the moment magnitude Mw, the higher the number of equivalent cycles N_c induced by the seismic demand. However, scientific evidence highlights that the correlation between N_c and Mw is weak, and a more reliable estimation of the seismic demand should directly account for N_c. The latter is strongly influenced by the site-to-source distance. Empirical predictive relationships to evaluate the N_c consider often both the minimum distance between the site and the failure plane (R_{RUP}), and the epicentral distance (R_{EPI}), and the closest distance between the site and the surficial projection of the fault, the so-called Joyner-Boore distance (R_{JB}), see [2]. For this reason, we calculate both the above-mentioned distances for the events from three case-histories databases adopted for the development of liquefaction resistance curves (Boulanger and Idriss [3], Moss et al. [4], and Andrus et al. [5]). Being both R_{RUP} and R_{JB} dependent on the focal mechanism, first of all, for each event, the focal mechanism that produced the shaking has been obtained by carrying out literature research and interpreting the so-called beach balls (Fig. 1). The latter are stereographic projections of the lower part of the focal mechanism: in particular, they are spheres whose center coincides with the hypocenter of the earthquake and which synthesizes the two mathematically equivalent planes that may have produced the earthquake. Furthermore, it shows the deformation of the surrounding rock mass through different colors. Thus, the faults were classified as strike-slip, normal, reverse or unknown.

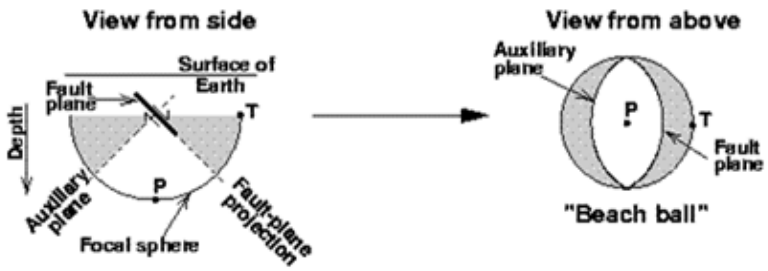

Fig. 1. Beach-ball.

Subsequently using the "ps2ff" software developed by Thompson and Worden (2017) [6], the two average distances R_{JB} and R_{RUP} of the case histories of the three databases were calculated. The software is developed in the LINUX environment and does not have its own GUI. For this reason, the development of scripts from the command line is required to assign preconfigured input files that characterize the analyzed earthquake and the geometrical parameters of the fault that produced it.

In this regard, deep literature research concerned other parameters of the rupture, such as the tectonic environment of the earthquake (ACR active crustal region or SCR stable continental region), the depth of the hypocenter and the dip angle of the fault. When these parameters were unknown, the hypotheses made available by the two authors [6] extrapolated from the analysis of the NGA-West2 database and the CEUS-SSC 2012 report were adopted. Consequently, 420 configuration files were implemented to extrapolate about 3700 R_{JB} and R_{RUP} distances. Fig. 2 and Fig.3 report the trend of the average ratio R_{JB}/R_{REPI} and R_{RUP}/R_{REPI} with R_{REPI} for strike-slip (a), normal (b) and reverse (c) faults, respectively with dip angles of 90°, 50° and 40° for different values of magnitude. The dashed red lines indicate the average epicentral distances of the three databases, belonging to the range 40-70 km. About the average magnitude of the databases, 7, it can be observed that R_{JB} is 80% of R_{EPI} for strike-slip faults and 70% of R_{EPI} for both normal and reverse faults, while R_{RUP} is about 0.75 R_{REPI} considering all the mechanisms.

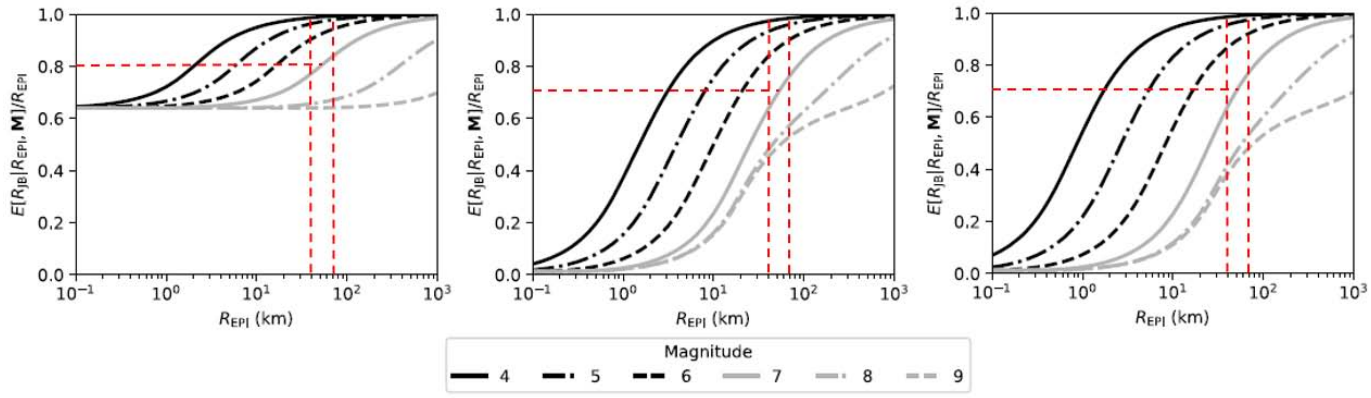

Fig. 2. R_{JB} mean /R_{REPI}; (a) curves for strike-slip, (b) normal, and (c) reverse mechanisms, assuming average dips angle of 90°, 50°, and 40°, respectively.

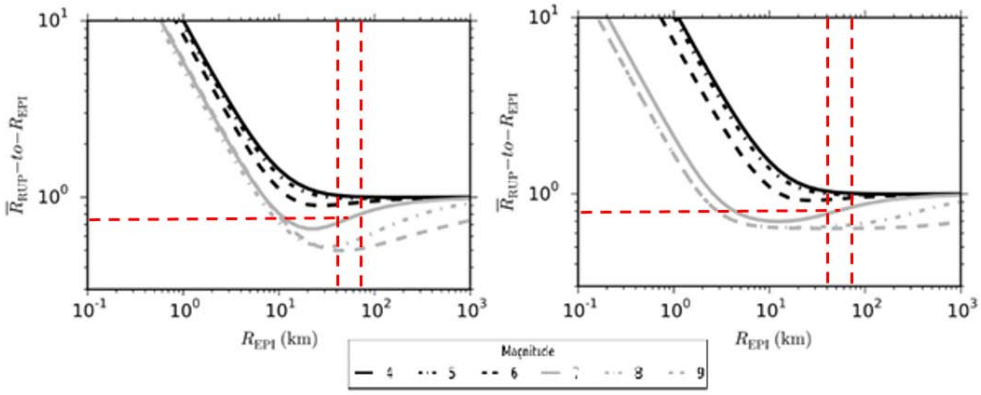

Fig. 3. R_{RUP} mean /R_{REPI}; (a) curves for unknown mechanism, (b) strike-slip, with assuming dips angle variable in range 10°- 90° and 75°-90°, respectively.

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Posters:

Late-stage or advanced studies with solid results

NGF MODULATES CHOLESTEROL METABOLISM AND STIMULATES ApoE SECRETION IN GLIAL CELLS CONFERRING NEUROPROTECTION AGAINST OXIDATIVE STRESS

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Cholesterol is essential to ensure proper functioning of the central nervous system (CNS), as it plays a crucial role in differentiation, synaptogenesis and neurotransmission [1,2]. Cholesterol metabolism in the CNS is particularly regulated in astrocytes [3]. Indeed, in the adult brain, neurons reduce or abandon cholesterol biosynthesis, ensuring the requirement of this important sterol from lipoproteins derived from astrocytes, in which cholesterol biosynthetis remains intense [4,5]. Cholesterol transport from astrocytes to neurons involves a horizontal transport mechanism, through which cholesterol is incorporated in ApoE-rich lipoproteins and then extruded by the ATP-binding cassette transporter A1 (ABCA1). Once released into the extracellular environment, ApoE-containing lipoproteins are taken up by neurons through the low-density lipoprotein receptor (LDLr) and LDLr-related protein 1 (LRP1). Literature data indicate that cholesterol contained in ApoE-rich lipoproteins significantly increases dendrite differentiation and synaptic function. Furthermore, some reports suggest that ApoE increases neuronal resistance to oxidative stress, thus preventing apoptosis and neurodegeneration [6-8]. Therefore, the homeostatic maintenance of cholesterol in the brain is essential to ensure the proper functioning of nerve cells, and the regulation of this important sterol should be finely controlled to guarantee different neurophysiological processes [9].

Several growth factors can influence the activity of CNS cells, including glial cells. NGF is part of the neurotrophin class, a small family of growth factors importantly implicated in the regulation, development and function of the nervous system [10,11]. In 1951, the Nobel laureate Rita Levi-Montalcini discovered this growth factor, capable of improving the growth of sensory and sympathetic neurons [12,13]. Although initially identified as a signal molecule strictly involved in neuronal survival and differentiation, recent evidence reveals that NGF plays a key role in the regulation of metabolism in different cell types. Indeed, NGF influences the metabolic processes implicated in glucose homeostasis, autophagy, oxidative stress and energy expenditure. Nevertheless, the activity of NGF on cholesterol metabolism is not fully elucidated [14].

Thus, the aim of this work was to evaluate whether the trophic support mediated by NGF could also involve the regulation of cholesterol homeostasis in astrocytic cells, as well as its impact on neuronal differentiation and neuronal survival under oxidative stress conditions.

For this purpose, an astrocyte-derived cell line, U373, was used as an experimental model. These cells were treated with recombinant NGF (100 ng/ml) for 48 hours and then collected to perform Western blot analysis on proteins belonging to cholesterol metabolism network. As shown in Figure 1, NGF administration increases the expression of several proteins and enzymes involved in cholesterol biosynthesis, intracellular transport, and efflux. Notably, there is a significant increase in both full-length (SREBP-2 FL) and nuclear active (nSREBP-2) forms of SREBP-2, the major transcription factor of cholesterologenic genes. Coherently, NGF also induces the expression of 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR), the key and rate-limiting enzyme of cholesterol biosynthesis, as well as the expression of Niemann–Pick disease, type C1 protein (NPC1), which is involved in cholesterol intracellular trafficking. Furthermore, ABCA1 significantly increases following NGF treatment (Fig. 1). Conversely, no changes were observed for ApoE expression.

Considering that, upon their synthesis, ApoE-rich lipoproteins are extruded by glial cells into the extracellular environment, cholesterol content and ApoE secretion in the culture medium were analyzed. As illustrated in Figure 2, cholesterol levels significantly rise in the culture medium of NGF-treated cells. This increase is mediated by a concurrent buildup in both the free and the esterified cholesterol fractions. Additionally, this event was

accompanied by an enhanced ApoE secretion in the culture medium. These data confirm the hypothesis that NGF promotes cholesterol biosynthesis and its secretion from glial cells through ApoE-rich lipoproteins.

Experimental data highlight that astrocyte-derived ApoE-rich lipoproteins can increase neuronal resistance to oxidative stress. Thus, we investigated whether NGF-stimulated secretion of ApoE from U373 cells could protect N1E-115 neurons from oxidative insult. To reach this objective, U373 cells were pre-treated with or without NGF for 48 hours. Simultaneously, fully differentiated N1E-115 cells were seeded on coverslip and treated or not with rotenone, a specific mitochondrial complex I inhibitor. After 16 hours from the treatment, the coverslips seeded with N1E-115 were transferred onto the U373 layer to set up co-cultures. In particular, N1E-115 cells were cultured in fresh medium (Ctrl), or co-cultured

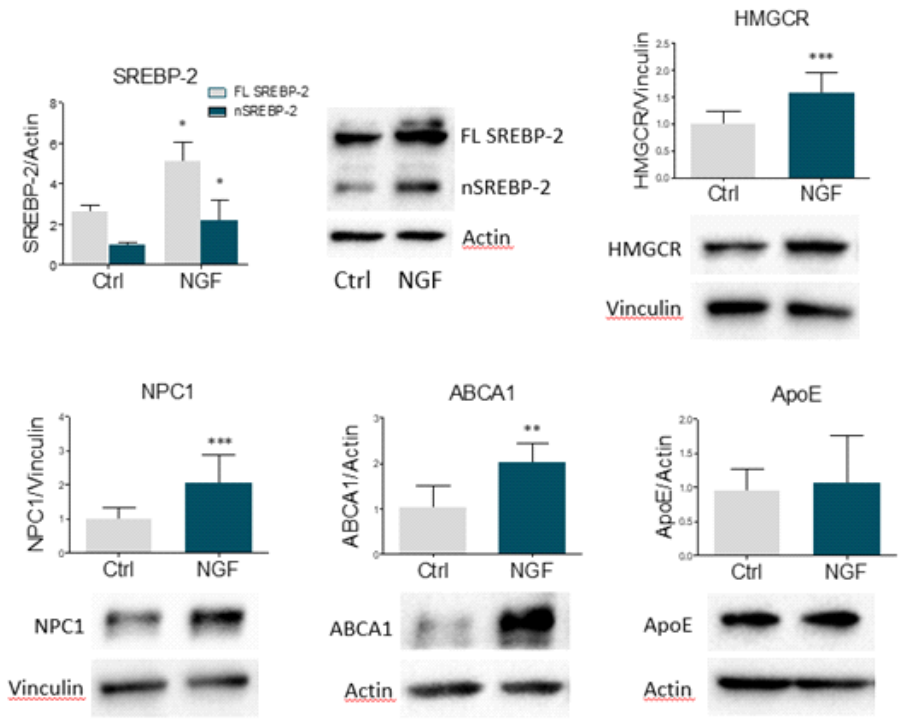

*Fig. 1. NGF administration increases the expression of proteins and enzymes involved in cholesterol biosynthesis, intracellular transport and efflux. Representative Western blot and densitometric analysis of SREBP2, HMGCR, NPC1, ABCA1 and ApoE in U373 control cells (Ctrl) or NGF (100 ng/ml) for 48 hours. n = 6 independent experiments. Actin and Vinculin were employed as housekeeping proteins to normalize protein loading. Data are expressed as means ± SD. Statistical analysis was assessed by unpaired Student's test *p <0.05; ** p < 0.01; *** p < 0.001.*

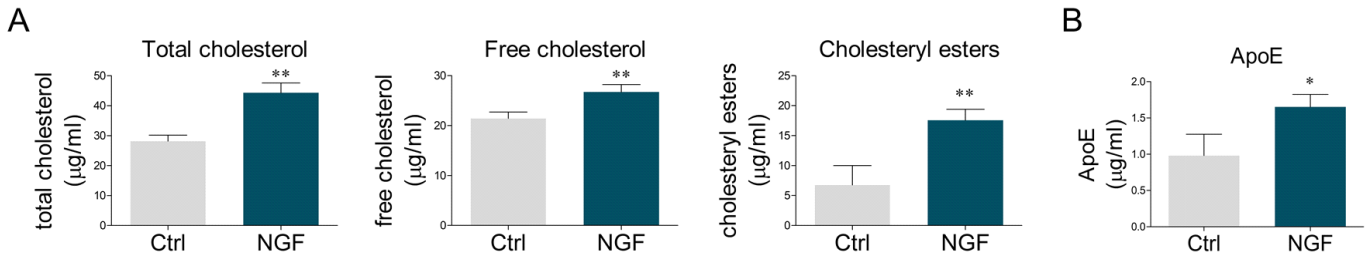

*Fig. 2. NGF significantly increases the amount of cholesterol released by U373 in the culture medium, corroborating the increased expression of ABCA1 and the enhanced ApoE secretion. (A) Graphics show cholesterol (total cholesterol, free cholesterol and cholesteryl esters) concentration in the culture medium of U373 control cells (Ctrl) or NGF (100 ng/ml) for 48 hours. (B) Quantification of ApoE levels (µg/mL) by ELISA assay in the culture medium of U373 cells treated with vehicle (Ctrl) or NGF (100 ng/ml) for 48 hours. n = 6 independent experiments. Data are expressed as means ± SD. Statistical analysis was assessed by unpaired Student's test *p <0.05; ** p < 0.01.*

with control U373 (U373-Ctrl), U373 pre-treated with NGF (U373-NGF), and U373 pre-treated with NGF but silenced for *apoE* gene (U373-NGF ApoE siRNA) for 48 hours. Figure 3 shows that rotenone strongly affects cell survival and determines neurite retraction in N1E-115, resulting in a loss of neurite-bearing cells. Similar results are also observed in N1E-115 co-cultured with U373-Ctrl. This suggests that control astrocytes are not sufficient to protect neurons from oxidative stress. Conversely, neuronal death (as well as the reduction of neurite-bearing cells and neurite length) is prevented when N1E-115 are co-cultured with U373-NGF. It is also important to note that, when N1E-115 were co-cultured with ApoE-silenced U373, the beneficial effects shown by NGF are completely lost. This suggests that NGF-mediated ApoE secretion into the culture medium exhibits neuroprotective effects against rotenone-induced oxidative stress.

Although these experiments need to be confirmed on primary cell cultures, the data collected in this study identify NGF as a key modulator of cholesterol homeostasis in astrocytes, demonstrating how this neurotrophin can mediate neuroprotective effects through ApoE secretion. This evidence is of particular interest for biomedical

research. In fact, a more complete and detailed understanding allows us to identify new molecular targets that can be exploited in pharmacological approaches useful in the treatment of neurodegenerative diseases [9].

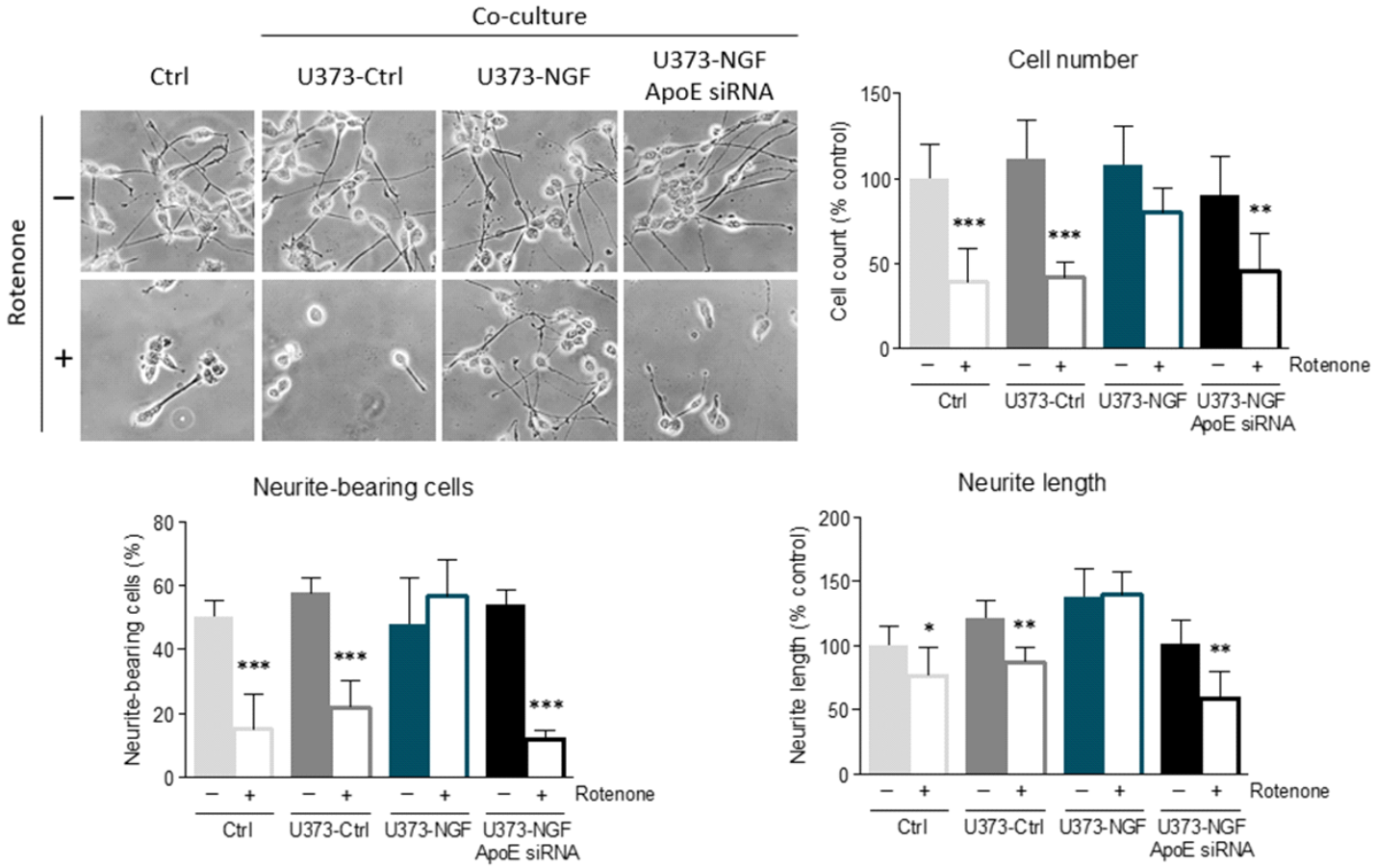

*Fig. 3. ApoE secretion from U373-NGF protects neurons from oxidative stress. Brightfield images and quantitative assessment of neuronal morphology of N1E-115. Cells were previously treated (+) or not (-) with rotenone (0,1 µM) for 16 hours. Then N1E-115 were cultured in fresh DMEM (Ctrl), or co-cultured with control U373 (U373-Ctrl), U373 pre-treated with NGF (U373-NGF), and NGF pre-treated U373 silenced for ApoE (U373-NGF ApoE siRNA) for 48 hours. n = 5 different experiments. Data represent means ± SD. Statistical analysis was assessed by using two-way ANOVA, followed by Bonferroni's post hoc test. **p < 0.01; ***p < 0.001.*

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Bioinformatic Detour: Hub Genes Identification via Network-Based Analysis

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Background

Plants exhibit a remarkable root plasticity, whereby root undergo morphogenetic changes to react and/or to adapt to environmental stress conditions [1]. Indeed, several factors, including alteration of gravity direction, touch, wind and bending, occurring in the environment may induce a mechanical stress condition and strongly affect plant stability especially in perennial species. However, studying woody plants is frequently difficult, due to the long development time of the plant and the logistical induction of stress in controlled conditions. Advanced technologies have improved our understanding and insight into the stress-response mechanisms that plants use as adaptive and survival tools. While we have a good understanding of the stem response, i.e., increase of the stem diameter and reduction of the height due to wind impact [2] and reaction wood (RW) formation [3], the knowledge of the woody root response to mechanical stress is obscure and fragmentary. Similar to the stem, RW formation is also part of the root strategy to adapt to the mechanical stress, along with reduced elongation and modification of the lateral roots developmental patterns [4–7]. Bent poplar tap roots are characterized by three adjacent sectors, above bending sector (ABS), bending sector (BS) and below bending sector (BBS), on both the convex (cx) and the concave (cv) side of the bent [5–7]. While compression forces on the concave side induce RW formation similar to that of gymnosperm stem (elevated number of cambial cells and increased lignification) [6,8], tension forces induce the formation of new lateral roots. These anatomical differences are supplemented by differences in the main phytohormones (i.e., auxin, cytokinin, and abscisic acid) and several functional proteins that appear to be highly involved in mechanical stress perception and response [6]. The signalling pathways and molecular activities that coordinate this fine-tuned root response to mechanical stress are currently unknown. Given the logistical challenges that arise when working with perennial roots, alternative solutions that produce accurate results are required. Bioinformatic breakthroughs based on network-based pipelines can be used to highlight hub genes of interest [9-12]. Presently, for the first time, a comprehensive network-based analysis of proteomic signatures was used to identify functions and pivotal genes involved in the coordinated signalling pathways and molecular activities that asymmetrically modulate the response of different bent poplar root sectors and sides [13].

Methodology

The proteomic signatures of *Populus nigra* woody bent root obtained from our previous study [6] were used as input for the bioinformatic pipeline. In detail the normalized abundance of 66 differentially represented proteins [the concave- cv and convex-cx sides of three bent root sectors - above bending sector located just above the bending zone (ABS); the bending sector of the maximum radical bend (BS); and below bending sector located just below the bending zone (BBS)] , were used to construct the protein abundance profiles (PAP) [13]. k-means analysis [14] was successively employed to group the proteins in six clusters and determine the sector-specific PAP peaks (≥0.3 score). For each cluster, a high-confidence set of protein-protein interactions (≥0.7) was selected from the *Populus trichocarpa* interactome, obtained via the STRING database [15,16]. The cluster-specific proteins, their direct interactions and the first direct neighbours, were used to construct six cluster related sub-networks. The subnetworks were identified and visualized with the open software Cytoscape, version 3.8.2 [17]. The 'Maximal Clique Centrality' (MCC) algorithm, from the Cytoscape plugin *cytoHubba* (version 0.1), was used to identify the three top hub genes in each subnetwork [18]. All 18 hub genes were then explored through UniProt [19], Blast [20] and PopGenie [21].

Results

The constructed bioinformatic pipeline grouped the 66 proteins in six main clusters which were then correlated with at least one of the six sectors (ABS-cx, ABS-cv, BS-cx, BS-cv, BBS-cx, BBS-cv) of the bent poplar taproot. In detail, cluster I with ABS-cx (score: 0.758156; 12 proteins); cluster II with BBS-cv (score 0.726657; 4 proteins); cluster III with BS-cx and BBS-cx (score 0.793721 and 0.46037, respectively; 11 proteins); cluster IV with ABS-cx, BS-cv and ABS-cv (score 1.019561, 0.864264 and 0.464451, respectively; 3 proteins); cluster V with ABS-cx and ABS-cv (score 0.44414 and 0.422867, respectively; 16 proteins) and cluster VI with BBS-cx (score 0.300643; 20 proteins). For each cluster, the MCC was used to identify the top three hub-genes, which were always directly connected between each other forming a triangle. In cluster I, the top three hub genes were all 60s ribosomal protein L5 (POPTR_0013s13220, POPTR_0014s17230, and POPTR_0019s13040); in cluster II- glutathione reductase chloroplastic isoform X1 (POPTR_0001s14480 and POPTR_0003s17670) and a probable phospholipid hydroperoxidase glutathione peroxidase (POPTR_0003s12620); in cluster III - dihydrolipoyl dehydrogenase 2 (POPTR_0008s10700 and POPTR_0010s15200 as chloroplastic isoform and POPTR_0010s16120 mitochondrial isoform); in cluster IV - V-type proton ATPase catalytic subunit A/d2 (POPTR_0008s00560, POPTR_0017s11530, and POPTR_0017s11540); in cluster V - proteasome subunit alpha type−6/beta type-2-A (POPTR_0006s14260, POPTR_0008s15530, and POPTR_0016s14640); and in cluster VI- glucose-6-phosphate isomerase 1 chloroplastic (POPTR_0002s10420), an uncharacterized protein LOC7477096 (POPTR_0005s07990), and a phosphoglycerate mutase-like protein 4 (POPTR_0007s11330).

Discussion and conclusion

Woody plants undergo to a series of morphological, physiological, and biochemical adaptations to ensure their survival under mechanical stimuli. Despite many targets that have been proposed to trigger this response, an understanding of the comprehensive and synergistic effect of the antistress spatially related pathways is still lacking. Our network-based analysis reveals new insight on spatially related strategies, commonly or specifically activated in the different bent root sectors (ABS, BS, and BBS) and sides (cx and cv). In particular, the ABS-cx was correlated with the cluster-subnetwork I, where the three 60S ribosomal protein L5 were identified as the hub genes, indicating that protein synthesis is one of the crucial adaptive mechanisms that ensures stability and optimization of the protein homeostasis [22, 23]. The hub genes of the subnetwork V, characterized as 'proteasome subunit α and β, indicate that both ABS-cx and ABS-cv ensures the plasticity via the degradation of misfolded targeted protein [24]. Interestingly, the hub genes identified in the subnetwork IV are shared between ABS-cx, ABS-cv and BS-cv. As these three sectors have significant differences, in terms of the anatomical and phytohormonal response to mechanical stress [6] and their positional relationship varies (opposing sectors, adjacent sectors and no direct connection), we assume that the identified V-type ATPase genes are involved in type of a continuous response to mechanical stress. Considering the position of the three sectors, it is possible that short-distance chemical and electrical signaling which involves Ca^{2+} signature [25,26] through the plasma membrane network [27] is required, to induce the biochemical response. The BBS-cv sector was previously associated with ROS signature that contributes to increased lignification as a strategy for tissue reinforcement under compression forces [6]. The present study further confirms the role of the ROS gene network in ensuring homeostasis [28], by identifying the cluster II hub genes glutathione reductase or glutathione peroxidase. Their role in the reduction of oxidative stress, protection of the plasma membrane and prevention of lipid peroxidation has been previously noted for various abiotic stresses [29], and they are probably part of the 'standard' stress response mechanism of plants. In the opposing BBS-cs sectors, the identified hub genes from cluster VI allude that here, the tension forces have significant impact on energy and metabolic processes. This is furthermore supported by the hub genes identified in cluster III which are shared between the BBS-cx and BS-cx. Two of these genes (POPTR_0010s15200, POPTR_0010s16120) have been, respectively, associated with early stem development and progression of poplar though dormancy-release stages, processes with dependent on intensified activity of the energy metabolism [30,31].

In conclusion, the comprehensive network-based approach presently used has allowed us to scratch the surface of what is most likely a complex and ongoing interplay between stimuli-signals-response. In particular, the analysis revealed novel information regarding the response coordination, communication, and potential signaling pathways

asymmetrically activated along the main root axis, delegated mainly to Ca2+ (for new lateral root formation) and ROS (for gravitropic response and lignin accumulation) signatures. Furthermore, some of the data indicate that the concave side of the bent sector, where the mechanical forces are most intense, communicates to the other (neighbor and distant) sectors, inducing spatially related strategies to ensure water uptake and accompanying cell modification. This information could be critical for understanding how plants maintain and improve their structural integrity—whenever and wherever it is necessary—in natural mechanical stress conditions. Further research focusing on identifying the role of these hub genes at earlier time points, as well as other collaborative components of the response, would provide a better understanding of plant cell communication and strategies under stressful conditions.

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A multi-level approach as a powerful tool to identify and characterize some Italian autochthonous common bean (Phaseolus vulgaris L.) landraces under a changing environment

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Agro-biodiversity is a vital element for human life since it is responsible of food security, ecosystem functions, sustainable development and climate change resilience [1]. In recent years, environmental and socio-economic changes, including extreme climatic events, change in land use, urbanization and rapid extension of industrial agriculture, have deeply affected natural ecosystems, producing an unprecedented loss of plant genetic diversity [2]. A key component of this diversity is represented by landraces, which are genetically variable, locally adapted and precious cultural heritage resources able to meet the needs of future global climate scenarios due to their adaptability to unfavorable conditions [3, 4]. However, despite their paramount importance, they are on the verge of extinction due to changes in market trends leading to increased demand for the more productive modern varieties [5]. Nevertheless, this valuable genetic material still survives in some remote marginal areas, where they constitute an important source of profitability for agricultural activities and economic development of these rural territories [6]. Therefore, concerted efforts are required to protect landraces biodiversity and to investigate their relevant traits, in order to raise awareness of this unexplored locally adapted germplasm and undertake effective conservation strategies to avoid their genetic and cultural erosion.

Based on these premises, in the present study [8], the diversity of seven Italian common bean (*Phaseolus vulgaris* L.) landraces (Ciliegino–CV, San Michele Rosso–SMR, Monachella–MO, Mascherino–MA, Pinto–PI, Tuvagliedda Rossa–TR and Suocera e Nuora–SA) was assessed and explored by using a multi-level approach able to integrate morphological, genetic, and phaseolin pattern characteristics, along with their ability to counteract two types of stress (salt and osmotic stress) that frequently occur in the Mediterranean basin and continuously increase due to the changes in the climate and anthropogenic activities.

Most of landraces was collected from Molise marginal areas (CV, MO, PI and SA), while the remaining from two Italian Apennine Regions, Tuscany (MA) and Basilicata (SMR and TR) (Fig. 1).

Morphological and genetic characterization were performed by using seed descriptors and ISSR molecular markers, respectively, while phaseolin analysis [a seed storage protein used to identify common bean gene pool (Andean or Mesoamerican)] was performed through 2-DE and SCAR technique. Stress response was investigated at plant level, evaluating the main morphological parameters, together with physiological ones, like proline, malondialdehyde (MDA), total chlorophyll and carotenoids.

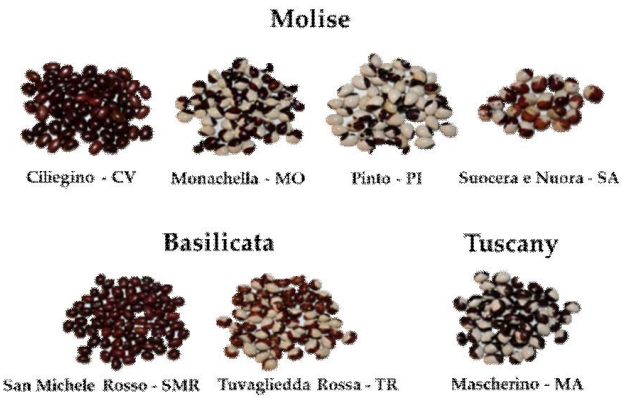


Fig. 1. Autochthonous common bean populations analyzed in the study.

Results of morphological characterization separated the landrace populations into two main groups: CV and SMR, showing full red seed coat color, and MO, SA, MA, TR and PI, with a bicolored (light and dark) seed coat pattern (Fig. 2 a, b).

On the other hand, genetic analysis grouped populations into two new groups, highlighting a genetic relatedness among Molise common bean populations and the ones coming from other Italian regions (Fig. 2 c, d). All the populations belong to Andean gene pool, as they exhibited the four typical Andean phaseolin patterns (C-, H-, T- and A-types) (Fig. 2 e, f).

Stress response analysis showed morphological and biochemical changes at organ level strictly related to the

genotype and the stress typology. Despite osmotic stress which only impacted the overall biomass accumulation of SA and leaf biomass of SMR and MA, salt stress reduced biomass accumulation in all the three organs in SMR and SA, in root and stem in PI and only in leaf in MA (Fig. 3 a, b, c).

Inhibition of biomass accumulation under salt stress was found to be correlated with the high levels of proline, identifying this osmolyte as a suitable biochemical marker to screen salt-sensitive bean landraces (Fig. 4 a, b) [7].

Decrease in stem and leaf biomass was also associated with a decrease in stem diameter, branching, and/or height, along with a decrease in leaf number and/or area (Fig. 3 d, e, f).

Unchanged or decreased MDA levels were reported in stress affected bean populations, as well as in stress tolerant populations CV, MO, TR, where no variations in morphological and biochemical parameters were detected (Fig. 4 a, b, c, d), suggesting the presence of an effective antioxidant activity, as proven by their high levels of carotenoids and chlorophylls found (Fig. 4 e).

The multi-level characterization approach performed in this study demonstrated how these common bean landraces constitute a valuable pool of diversity, which may be proven useful in global climate change mitigation and particularly promising towards a sustainable-oriented development and socio-economic promotion of marginalized areas of origin.

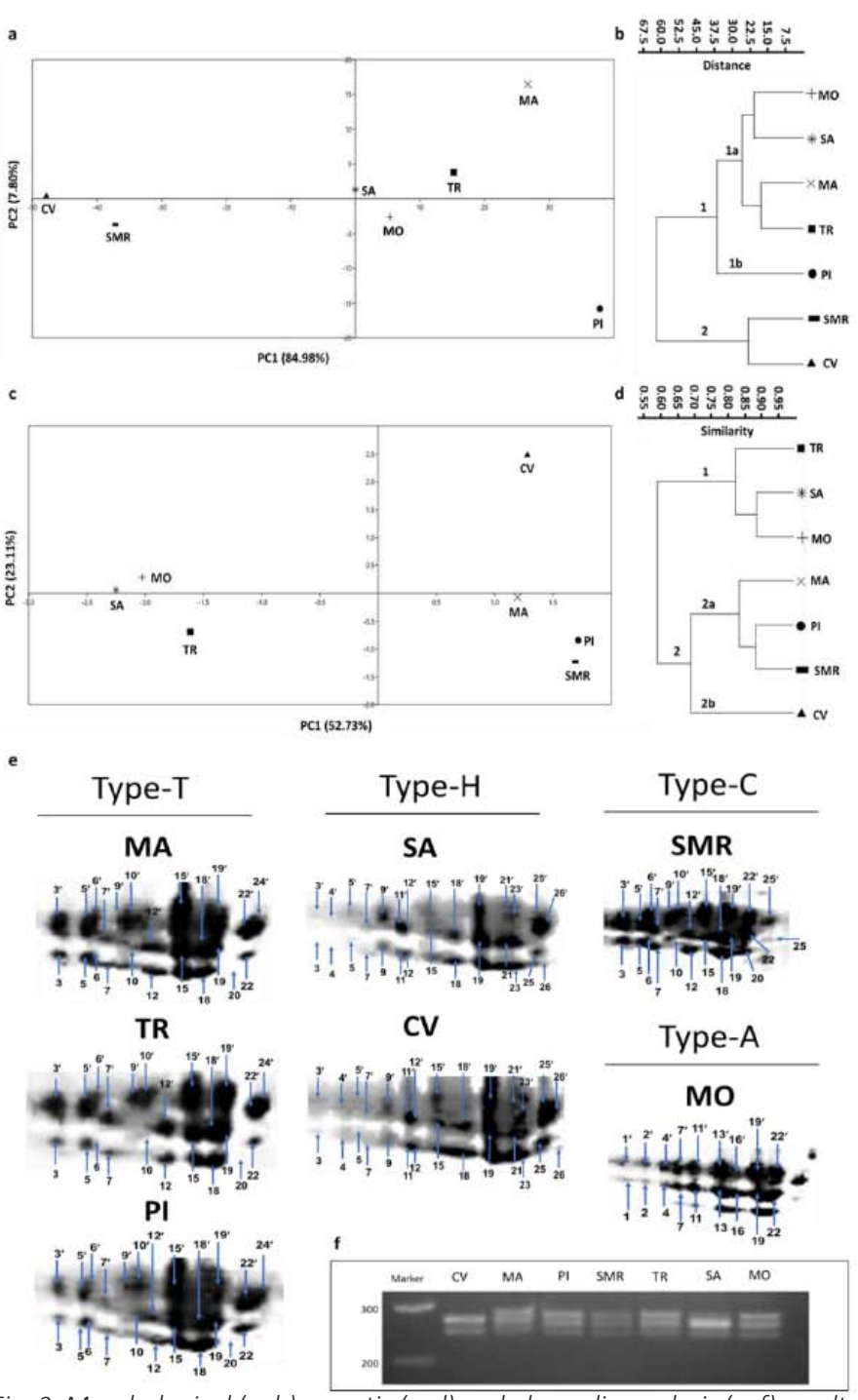


Fig. 2. Morphological (a, b), genetic (c, d) and phaseolin analysis (e, f) results obtained from the characterization activity performed on common bean populations.

Furthermore, evaluation of the nutritional potential of the analyzed common bean populations could be considered of pivotal importance in order to unveil the possible presence of specific phytochemical markers and/or nutraceutical compounds relevant for human health, resulting in agri-food qualities valorization, with a consequent strengthening of conservation activities.

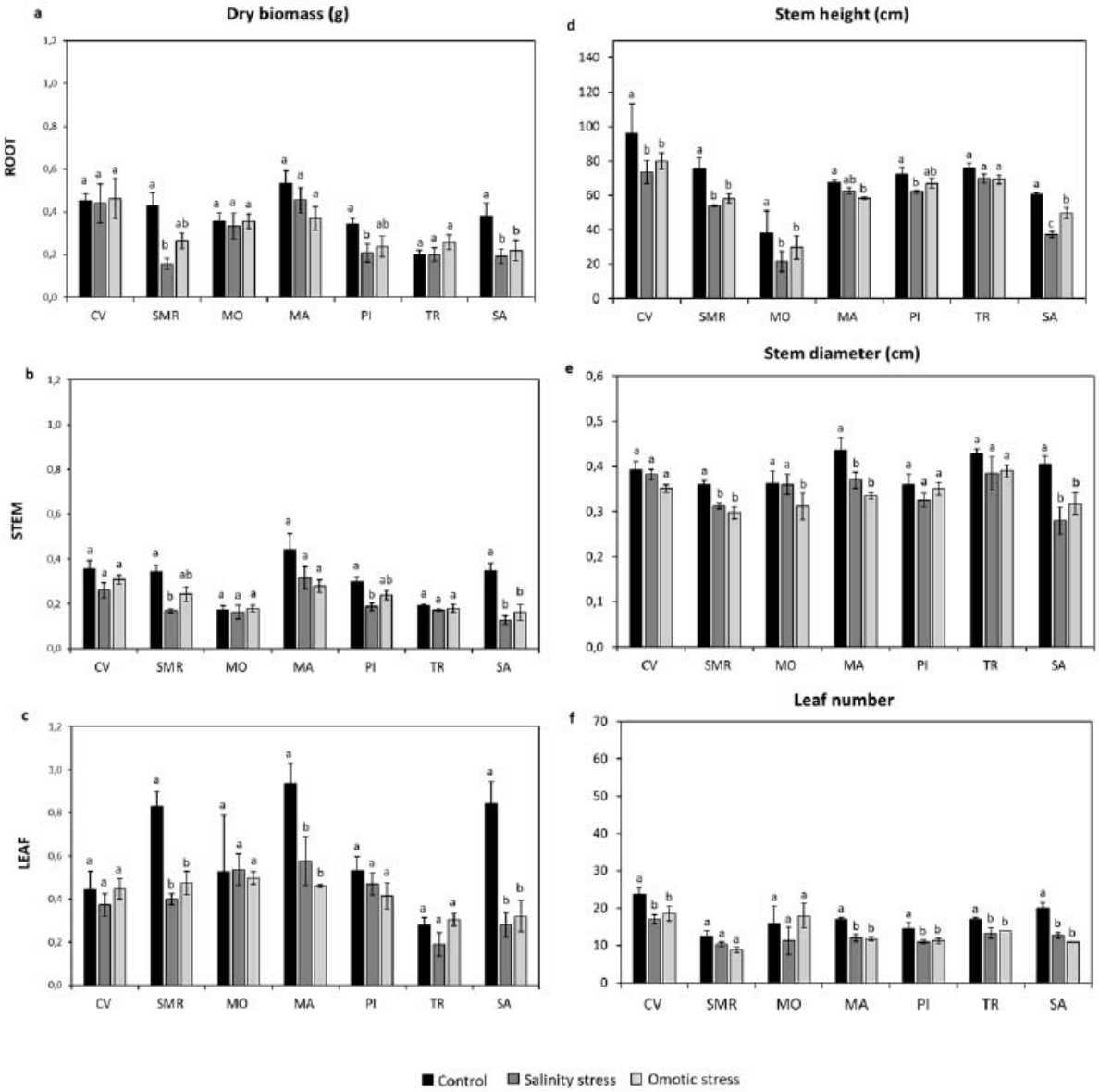
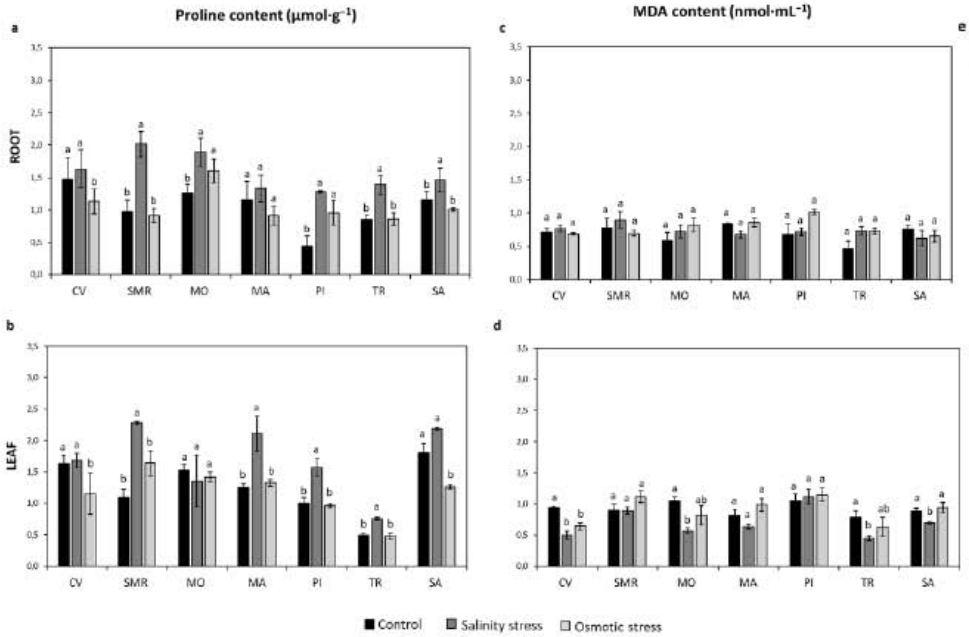


Fig. 3. Results of stress response analysis, evaluating morphological parameters of common bean populations.



e

		Control	Salinity stress	Osmotic stress
Total Chl (μg·mg⁻¹)	CV	1.34 ± 0.05 a	0.98 ± 0.04 b	1.11 ± 0.02 b
	SMR	1.12 ± 0.06 a	1.13 ± 0.14 a	1.08 ± 0.03 a
	MO	0.92 ± 0.02 b	1.14 ± 0.06 a	1.09 ± 0.03 a
	MA	1.61 ± 0.09 a	1.53 ± 0.06 a	1.72 ± 0.10 a
	PI	2.19 ± 0.08 b	2.56 ± 0.03 a	1.98 ± 0.17 b
	TR	1.41 ± 0.03 b	1.73 ± 0.03 a	1.15 ± 0.11 b
	SA	1.21 ± 0.06 a	1.41 ± 0.11 a	1.39 ± 0.09 a
Chl A/Chl B	CV	3.28 ± 0.05 a	3.22 ± 0.08 a	3.27 ± 0.01 a
	SMR	4.02 ± 0.05 a	3.71 ± 0.10 b	3.57 ± 0.07 b
	MO	3.19 ± 0.38 b	3.63 ± 0.26 a	3.44 ± 0.04 a
	MA	3.39 ± 0.04 a	3.22 ± 0.05 a	3.34 ± 0.04 a
	PI	3.54 ± 0.03 a	3.62 ± 0.27 a	3.02 ± 0.33 b
	TR	3.54 ± 0.02 a	3.33 ± 0.01 a	3.43 ± 0.04 a
	SA	3.23 ± 0.03 a	3.18 ± 0.03 a	3.28 ± 0.07 a
Carotenoids (μg·mg⁻¹)	CV	0.19 ± 0.01 a	0.18 ± 0.01 a	0.19 ± 0.00 a
	SMR	0.16 ± 0.01 a	0.18 ± 0.02 a	0.15 ± 0.01 a
	MO	0.12 ± 0.00 b	0.18 ± 0.01 a	0.16 ± 0.01 a
	MA	0.26 ± 0.02 a	0.25 ± 0.01 a	0.26 ± 0.02 a
	PI	0.31 ± 0.01 b	0.39 ± 0.01 a	0.29 ± 0.03 b
	TR	0.23 ± 0.01 b	0.29 ± 0.00 a	0.18 ± 0.02 b
	SA	0.17 ± 0.01 b	0.21 ± 0.02 a	0.21 ± 0.01 a

Fig. 4. Results of stress response analysis, evaluating physiological parameters of common bean populations.

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Insight into the organ pivotal pathways and genes of Arabidopsis halleri - An in silico study

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From recent years, the ability of *Arabidopsis halleri* to accumulate metals and to be constitutively tolerant specifically to cadmium (Cd) and zinc (Zn) attracted the researcher's concerns to use this trait in the remediation of metal polluted soils [1, 2]. Several studies focused on *A. halleri* to assess the expression level of genes involved in root uptake, root-to-shoot translocation and vacuolar sequestration or vein accumulation [3-5]. However, despite of these establishments, a comprehensive understanding of the mechanisms laying *A. halleri* as a multi-metallicolous hyperaccumulator is still lacking. It has been described that the difference between hyperaccumulator and non-hyperaccumulator plants is not due to the presence of novel genes but resides in the different expression patterns of the genes involved [6]. Thousands of molecules are involved in these complex responses and, among them, proteins act in most of the bio-chemical reactions. Some proteins function alone, but many proteins interact with other proteins to fulfill their function modulated by a specific stimulus, signals, or a particular cell state [6]. Thus, it is very important to understand how proteins function in a coordinated manner to accomplish vital cellular activities or give response under certain circumstances such as metal stress. Several experimental techniques are established to detect protein–protein interactions (PPIs), but these *in-wet* approaches are labor-intensive and time-consuming. Moreover, they do not work well in identifying weak transient interactions [7]. In this regard, the weighted gene co-expression network analysis (WGCNA) has been identified as a novel and effective bioinformatics application on expression data for exploring the relationships between different gene sets (modules), or between genes providing a straightforward biologically functional interpretations of gene network modules [8]. Currently, the WGCNA has been successfully used to construct gene co-expression networks to answer various biological questions, identifying centrally connected hub genes as promising biomarkers or therapeutic targets [9, 10]. Additionally, WGCNA transforms gene expression data into co-expressed modules allowing to explore relationships between genes and phenotypes in different tissues or data sets. This feature also assists in comparing differentially expressed genes (DEGs) and modules [11] and it has been widely used for the identification of candidate genes to specific traits in many fields of plant biology, included response to abiotic stresses [12, 13]. Thus, for the first time, the present study aimed towards a comprehensive and systematic analysis of root and shoot of *A. halleri* by WGCNA, highlighting key pathways and genes crucial to organ functions, identification and screening of hub and bottleneck genes for their possible involvement in plant metal stress responses. The results from this study could be used as a reliable set for experimental studies/validations or further computational analysis and hub and bottleneck genes may serve as potential candidates to improve knowledge about hyperaccumulator plants and to push phytoremediation technologies.

To accomplish the aim, the RNA-seq dataset of *A. halleri* ssp. *halleri* (*Linnaeus*) root and shoot were obtained from Corso and colleagues [14] analysis. To construct co-expression networks from RNA-seq data of root and shoot in *A. halleri*, the WGCNA was applied. All the parameters, included those related to adjacency matrix, soft-thresholding power, and connectivity between genes, were screened prior the WGCNA module analysis. The adjacency matrix was constructed by using soft threshold power of $\beta = 14$ for root and $\beta = 8$ in case of shoot based on scale-free topology with $R^2 = 0.97$. A total of 19,653 genes of root and 18,081 genes of shoot were categorized into 14 modules, respectively. Module eigengenes (MEs) were used as the most notable component representing the module's gene expression profile (Figure 1). Module size in root ranges from 44 to 2762 genes, while in shoot from 38 to 5797 genes.

Further, we categorized the modules for significantly enriched molecular functions (MFs), biological processes (BPs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways which were identified by integrating the information coming from the genes comprised into the modules with the Gene Ontology (GO) terms and KEGG

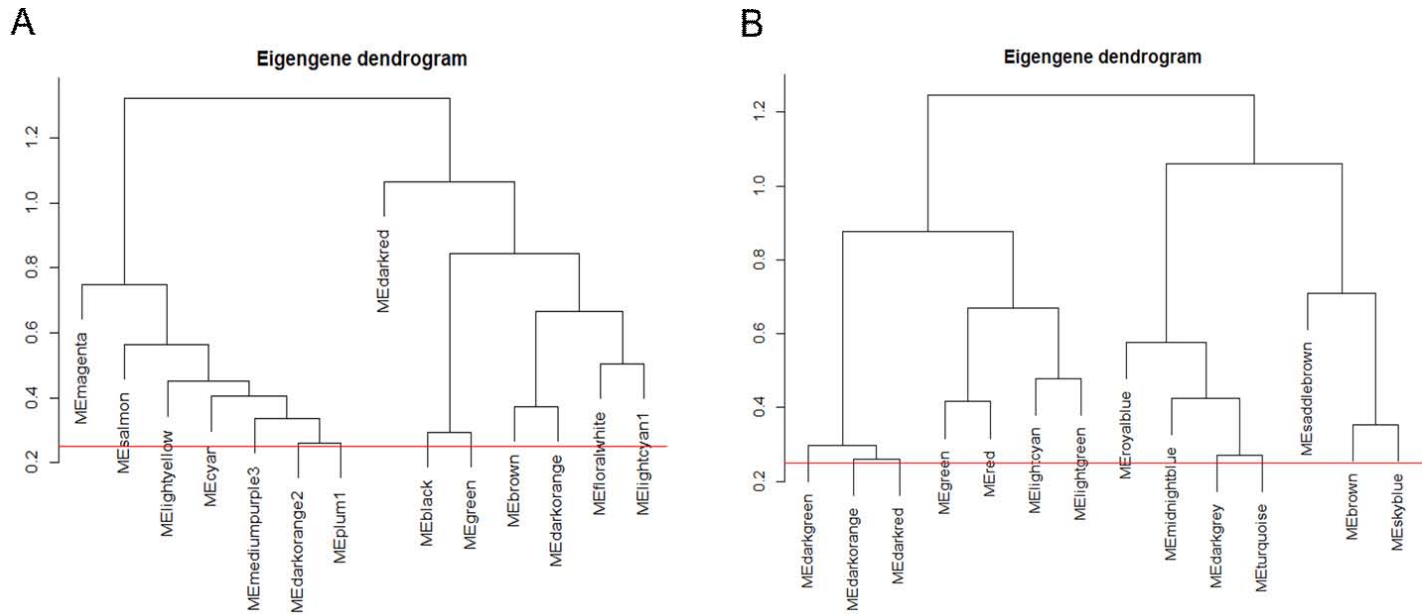

Fig. 1. Dendrograms representing the tree-based clustering of module eigengenes in root (A) and shoot (B). Red line represents the merging threshold.

pathway annotations. Top three MFs, BPs and KEGG pathways significantly enriched by this analysis ($P < 0.05$) were identified in each module of root and shoot. We found "photosynthesis" (KEGG:00195) and "photosynthesis–antenna proteins" (KEGG:00196) among the most enriched pathways in both root and shoot. The pathway "circadian rhythm—plant" (KEGG:04712) was identified as uniquely enriched in *A. halleri* shoot. While some pathways were enriched only in the root including "glucosinolate biosynthesis" (KEGG:00966), "autophagy–other" (KEGG:04136) and "SNARE interactions in vesicular transport" (KEGG:04130).

Additionally, PPI network derived from WGCNA of root and shoot were imported to Cytoscape to predict the top 10 hub genes and the top 10 bottleneck genes in each module by maximal clique centrality (MCC) and "Bottleneck" algorithm, respectively. Further, most differentially expressed modules across treatment (Cd-Zn) and control groups were identified in root and shoot based on accumulative expression of multiple gene in that module by using Limma package in R. Based on this analysis, magenta (p value < 0.0001) and floralwhite (p value < 0.010) were the top two most significant differentially expressed modules in root (Fig. 2A and 2B), and for shoot saddlebrown (p value < 0.02) and sky-blue (p value < 0.02) modules were selected (Fig. 2C and 2D). These results were also confirmed by assessing the association between module with the Cd-Zn treatment by correlating module eigengenes with the transcriptional profiling of the traits under analysis.

Hub and bottleneck genes of these modules were searched in public databases to assess their possible involvement in plant stress and visualized within the module together with their expression pattern across control and treatment groups. All hub and bottleneck genes of magenta (Fig. 3A) and saddlebrown (Fig. 3C) modules and all but one of the hub

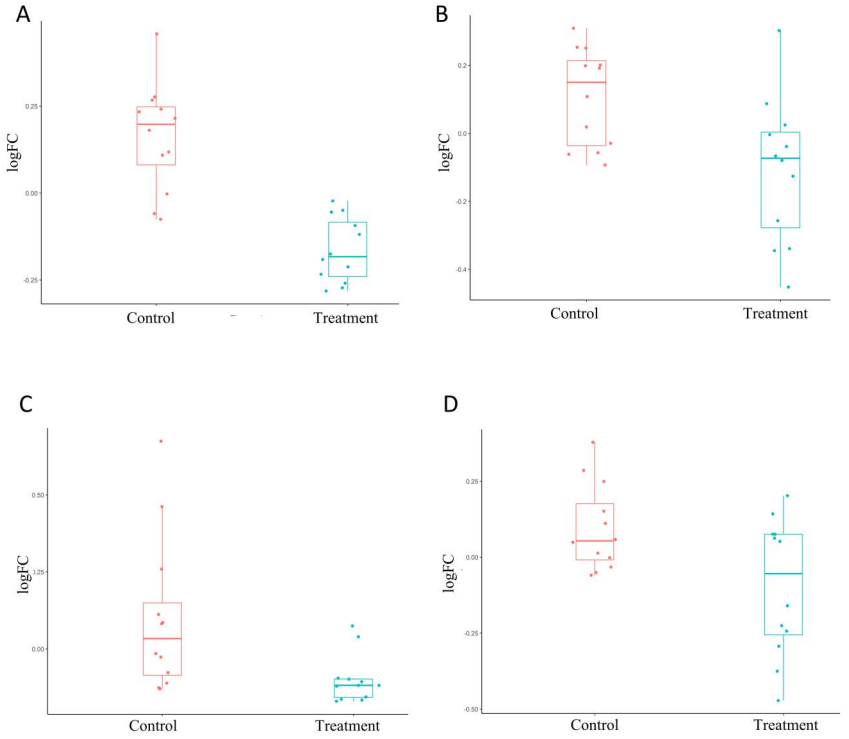

Fig. 2. Boxplot of the expression patterns of the genes within the modules associated to the most significant differential expression between control and treatment in root (A, B) and shoot (C, D). A, B, C, and D to identify magenta, floralwhite, saddlebrown and skyblue modules, respectively.

and bottleneck genes in the floralwhite module (Fig. 3B) showed a pattern along with a decreased gene expression in treatment groups with respect to the control. In the case of skyblue module, instead, the expression values of some hub and bottleneck genes showed an increase under Zn treatment with respect to the control which does not follows the same trend in the case of Cd treatment (Fig. 3D). However, most of the genes showed no significant alteration between control and treatment for both Cd and Zn.

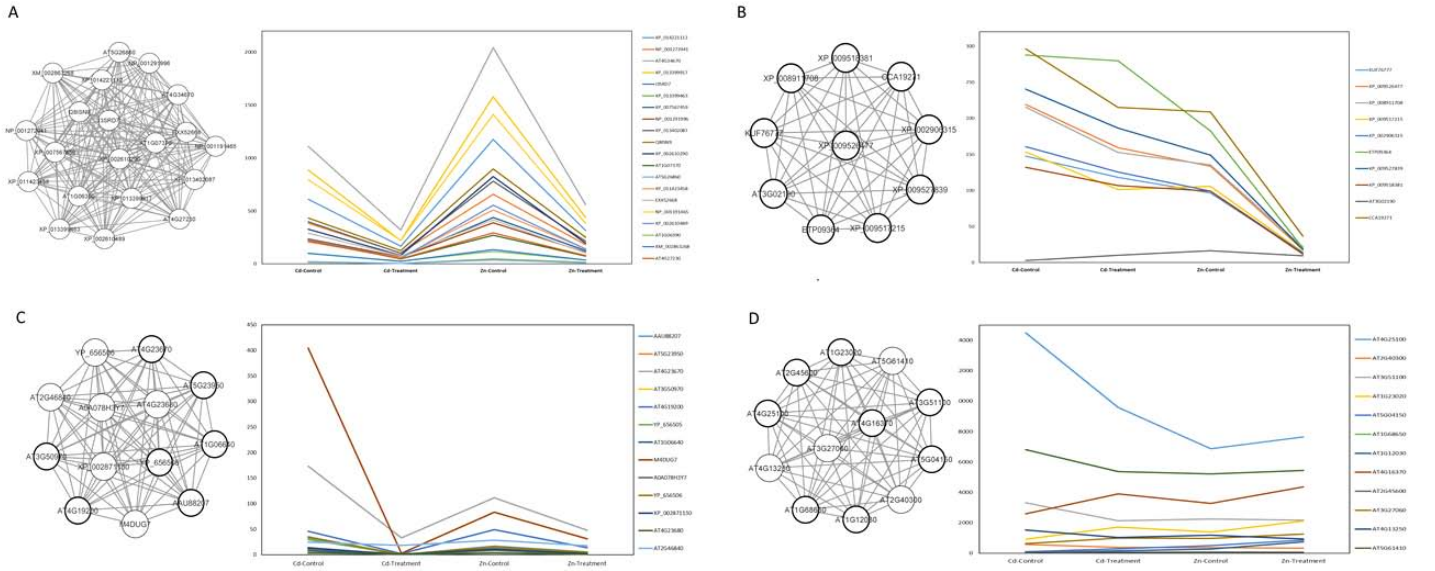

Fig. 2. Visualization of top hub and bottleneck genes and their connectivity into the modules of interest together with their validation at expression level. Bold border nodes in the networks for hub-bottleneck genes. Expression values of genes in control and treatment of Cd an Zn, respectively. A, B, C, and D to identify magenta, floralwhite, saddlebrown and skyblue modules, respectively.

The biological characteristics of the differentially expressed modules were examined deriving the respective networks by an interolog approach and analyzing existing data on PPIs to identify hub and bottleneck genes to be compared with those identified from WGCNA. Floralwhite and saddlebrown modules showed no intersecting genes possibly due to the presence among the top hubs and bottlenecks from WGCNA of a high number of predicted and hypothetical genes which have no mapping in STRING. Instead, in the case of the magenta and of the skyblue modules there were two (AT1G06390 and AT4G34670) and five over-lapping genes (AT1G23020, AT2G40300, AT4G16370, AT5G04150 and AT5G61410). These genes may deserve as promising biomarkers for metal stress of root and shoot of *A. halleri*.

Despite of certain limitations of this study including restricted sequencing performed so far on the species and do not perform in wet validations, the results from this study indicate that this analysis is reliable and further convincing that the identified genes can be considered candidates for *in-wet* strategies as targeted genes in *A. halleri* root and shoot in response of metal stress. This type of approach can also be useful to hypothesize the function of unknown-function proteins and to drive experimental research.

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Bovine Lactoferrin affects Tat-induced oxidative stress and iron dysregulation

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1.Introduction

Iron is a fundamental nutrient for all living organisms, participating to cellular respiration, oxygen metabolism, DNA synthesis, drug metabolism and steroid synthesis [1]. Perturbation of iron homeostasis plays a role in a wide range of diseases [2] including viral infections. Here, iron metabolism is altered and worsened by the action of pro-inflammatory cytokines, leading to higher intracellular iron content, which facilitate viral spreading [3]. As recently demonstrated, Tat protein of Human Immunodeficiency Virus-1 (HIV) elicits intracellular oxidative stress in a human astrocytoma cells line [4]. This state triggers a compensatory cellular response implemented by increased expression of Nrf2, which translocates into the nucleus and promotes the transcription of genes containing the antioxidant response element (ARE), including Glutathione Peroxidase 4 (GPx4), Glutamate-cysteine ligase (GCL) and a subunit of System Xc-cystine/glutammate antiporter (SLC7A11). As evidenced, oxidative stress, inflammation and iron overload are closely related and viruses take advantage by this alteration, exacerbating pathogenesis. In this context, Lactoferrin (Lf), an iron-binding glycoprotein of the innate immunity possessing pleiotropic functions such as anti-microbial, anti-viral as well as anti-inflammatory and antioxidant activities, could exert a protective role [5,6,7,8]. In almost all studies, bovine Lactoferrin (bLf) is used as bioequivalent of human Lf because of sequence similarity and shared functions. Depending on its iron content, Lf is present as native (Nat-Lf) and holo forms (Holo-Lf), with an iron saturation rate of 10-20% and >95%, respectively. The protein is classified as a "generally recognized as safe" (GRAS) substance by the USA Food and Drug Administration. Lf ability to modulate iron homeostasis has been demonstrated in different in vitro studies [8, 9, 10], where it has been shown to revert dysregulation of iron proteins, including Transferrin Receptor, Ferritin, Ferroportin (Fpn) and Ceruloplasmin (Cp), proteins involved in iron entrance (TfR1), storage (Ftn) and export (Fpn/Cp). In addition, Lf can maintain physiological ROS levels by both modulating the expression of the main antioxidant enzymes, such as SOD and GPX4 and by binding free ferric ions. Therefore, based on these evidence, we evaluated the protective role of bLf, both in its native and iron-saturated forms, against Tat-mediated oxidative stress and iron dysregulation in the astrocytoma cell line U373.

2. Results

2.1 Subcellular localization of native and holo Lactoferrin

In this study, U373 pcDNA3.1 (U373), as control cells, and U373 pcDNA3.1-Tat (U373-Tat) cells were treated with 100 µg/mL of either Nat- or Holo-bLf. To prove whether Lf could enter cell nucleus, bLf subcellular localization was tested out through Western blot.

Both Nat and Holo forms were able to enter cell nucleus of U373 cells, expressing or not Tat protein, with Holo-bLf showing higher levels than the native form in both compartments (Fig. 1).

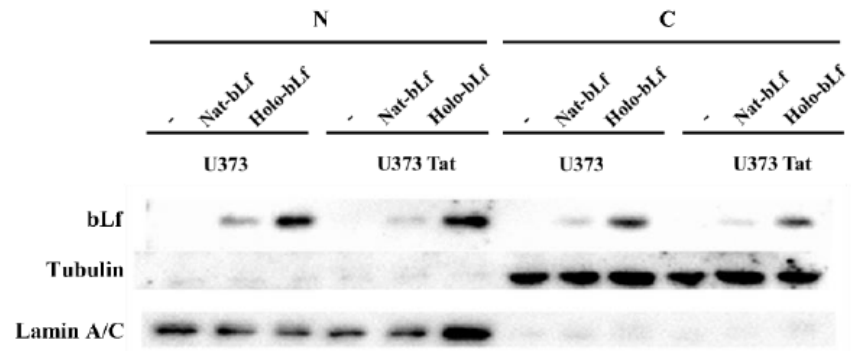

Fig. 1. Analysis of bLf internalization and sub-cellular localization in U373 and U373-Tat through Western blotting and densitometric analysis of bLf in cytosolic (C) and nuclear (N) fractions on total cell protein extracts after 24 h of treatment with 100 µg/ml of Nat-bLf or Holo-bLf.

2.2 BLf boosts the cellular anti-oxidant response

To better clarify the role of bLf in maintaining physiological ROS balance, we analyzed the expression of Nrf2, System Xc, GPX4 and GCL by Western blot in both U373 and U373-Tat cells.

Nrf2 expression levels were measured in nuclear extract after 2h of treatment. The results show an increase of Nrf2 expression and, consequently, of System Xc in U373-Tat cells with respect to the control (Fig. 2A, B). BLf treatment exerted a positive regulation on Nrf2 nuclear translocation and, therefore, on System Xc expression. Interestingly, the holo form was more active than the native one. In addition, the expression of GPX4 and GCL is significantly induced in U373-Tat cells compared to control cells. In this case, both Nat- and Holo-form significantly boost GPX4 expression in the presence of the viral protein, whereas only Holo-bLf exerts this effect in control cells (Fig. 2C). On the other hand, no significant reduction in the expression of GCL was recorded after bLf treatment (Fig. 2D).

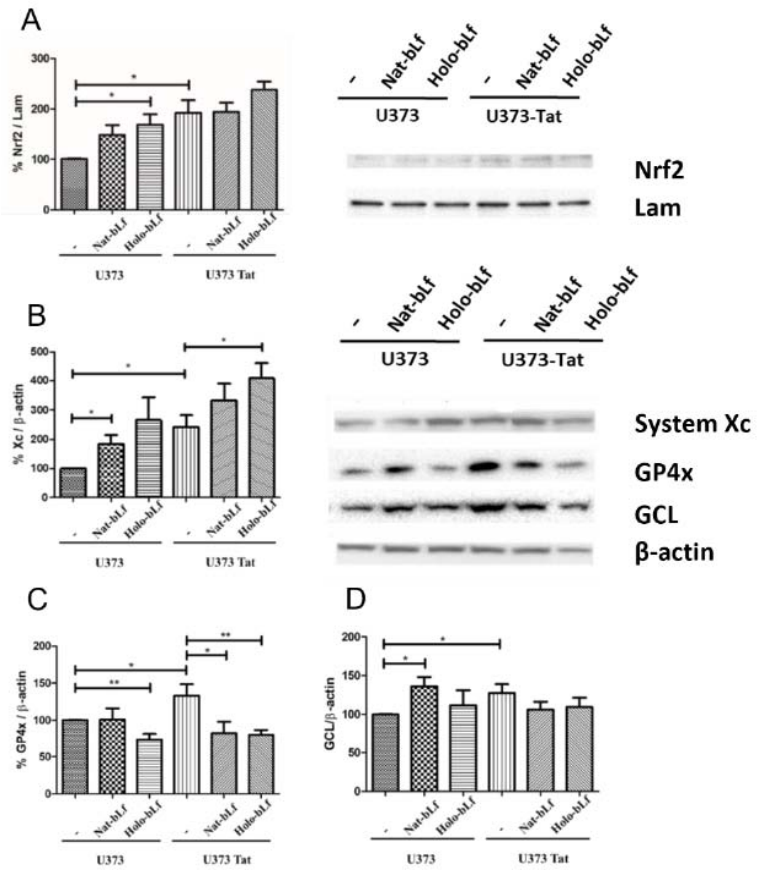

*Fig. 2. Western blot and densitometric analysis of Nrf2 nuclear translocation (A), System Xc (B), GPX4 (C) and GCL (D) in U373 and U373-Tat cells treated with 100 µg/ml of Nat-bLf or Holo-bLf. Error bars: standard error of the mean. Statistical significance: *: p <0.05; ***: p <0.001 (one-way ANOVA with post-hoc Tukey test).*

2.3 BLf potentiates cell iron release and reduces iron uptake

Next, we analyzed bLf role on the expression of the main proteins involved in iron homeostasis.

The analysis showed that Fpn levels increased (Fig. 3A) and TfR1 levels decreased (Figure 3B) in Tat-

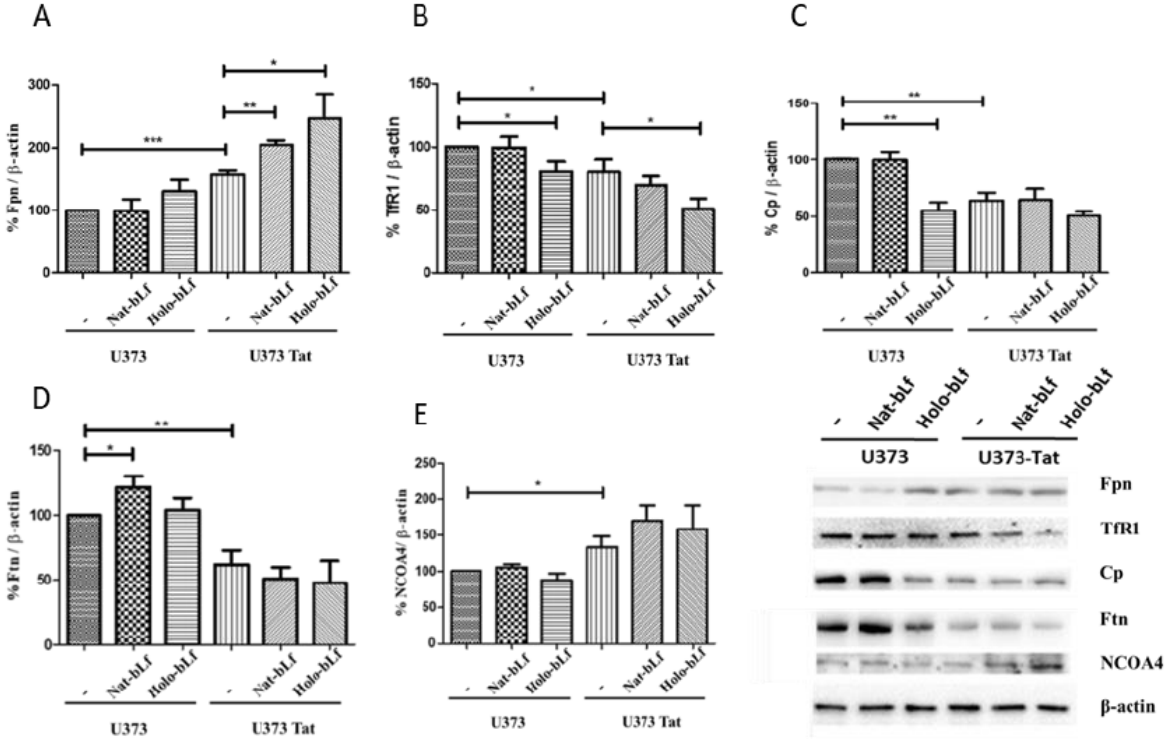

*Fig. 3. Western blot and densitometric analysis of Fpn (A), TfR1 (B), Cp (C) Ftn(D), NCOA4 (E), in U373 and U373-Tat cells treated with 100 µg/ml of Nat-bLf or Holo-bLf for 48h. Error bars: standard error of the mean. Statistical significance is indicated as follows: *: p <0.05; **: p <0.01; ***: p <0.001 (one-way ANOVA with post-hoc Tukey test).*

expressing cells compared to CTRL. BLf significantly potentiated such effects, in both Nat- and Holo-forms for Fpn and in the sole Holo-form for TfR1, in U373-Tat cells. In addition, levels of Cp decreased in presence of the viral protein (Fig. 3C). Of note, Holo-bLf significantly reduced Cp expression in control cells, whereas it did not influence the effect of Tat on Cp expression. In addition, a significant reduction of Ftn levels associated with the up-regulation of nuclear receptor coactivator 4 (NCOA4), a cargo receptor for the selective turnover of ferritin, was recorded (Fig 3 D, E). This last process was closely related to Tat viral protein expression, whereas bLf treatments did not show any influence on it.

3. Conclusions

In conclusion, bLf has been shown to boost the antioxidant response of astrocytoma cells to HIV-Tat challenge, also potentiating cell iron release and reducing iron uptake.

Taking these data, the effect of bLf could be ascribable to both its iron-binding activity and to its ability to enter into the cell nucleus, thus possibly modulating gene expression. With regard to the latter aspect, the higher modulatory efficacy of the Holo-form vs. native bLf could be due to its higher structural stability. Although more studies are needed to corroborate and deepen the present findings, the efficacy of bLf in counteracting Tat-induced oxidative stress makes this glycoprotein an interesting candidate for the treatment of HIV-infected patients in association with standard therapies.

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A MULTILEVEL CHARACTERISATION OF WHITE TRUFFLE FROM MOLISE REGION

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Truffles are a polyphyletic group of hypogeous fungi, which complete their life cycle underground [1]. Species belonging to the genus *Tuber* (the so-called true truffles) are ascomycetes of the *Pezizales* order, a large group of ectomycorrhizal fungi growing in symbiosis with the roots of several plants and shrubs, bringing benefits to the forest ecosystems and to the host plants [2-5]. Besides their ecological role, some *Tuber* species are edible and have a considerable economic and commercial importance. Among these, *T. magnatum* Picco, known as the Italian white truffle, is the species of greatest interest and with the highest economic value. Indeed, *T. magnatum* fruiting bodies can be considered one of the most expensive foods in the world, reaching a cost of thousands of euros per kilogram [6, 7, 8]. In addition to the valuable culinary properties, the exorbitant prices of *T. magnatum* ascomata reflect their low availability on the market, which is due to both the difficulties in cultivation and the limited distribution range [2, 9, 10]. In fact, this prized truffle species, which has long been considered endemic to Italy, grows spontaneously only in a few European countries [11, 12]. It is notable that Molise region (Central-Southern Italy) is one of the most productive areas (30-70 quintals per year), even if, to date, Molise truffle has received very little scientific attention [7, 13, 14]. Accordingly, the present research aimed at filling the gap of knowledge currently existing, providing a multilevel characterisation of two populations of Molise white truffles. More specifically, 21 *T. magnatum* ascomata harvested in two different areas of the region (Figure 1) were analysed from a (1) morphological, (2) genetic, and (3) microbiological perspective. Nine samples (indicated with progressive numbers from 1 to 9) were collected in November 2019 in the study area 1, between Carovilli and Vastogirardi municipalities, whereas other twelve samples (from 10 to 21) were harvested in January 2020 in the study site 2, a more extensive area on the border with Abruzzo region. With regard to the morphological investigations, basic analyses for species identification and assessment of fruiting body maturation degree were performed.

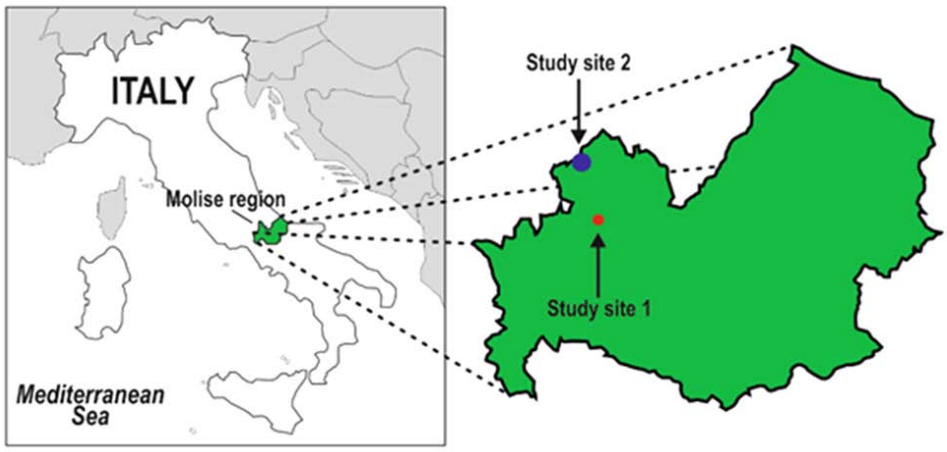

Fig. 1. Study area: the study site 1 is located between Carovilli and Vastogirardi municipalities (Isernia province) whereas the study site 2 covers a more extensive area on the border with Abruzzo region.

Moreover, the thickness of the peridium (the external surface of fruiting bodies) – a parameter for which no comprehensive information was available – was measured by light microscope observations. Overall, morphological analyses showed significant differences between the two *T. magnatum* populations but also a certain variability within them. As reported in Table 1, truffles collected at study site 1 were bigger, riper, and with a thinner peridium compared with those from study site 2. However, analysis with Pearson's correlation coefficient highlighted no strong linear correlation between the examined morphological parameters. Therefore, differences observed between the two groups could be related to the different harvesting period of the fruiting bodies (white truffles collected in January are usually smaller due to ripening in winter period, under frost and ice, and partially immature with asci often lacking in spores), as well as site-specific factors (e.g. microclimatic conditions and soil properties), truffle geographic origin, and genetic features [7, 14]. As a starting point to assess the genetic variability of the 21

ascomata, the Sequence-Characterised Amplified Region SCAR A21-inf, a single locus marker, was considered. This choice was due to a previous research carried out by Mello et al. [15], who demonstrated that the SCAR A21-inf region was polymorphic in *T. magnatum* by identifying two SNPs (single nucleotide polymorphisms) that generated three haplotypes (I, II, and III). Interestingly, they found that the haplotype III was present only in two samples from Molise region and absent in samples collected in other geographic areas. Our results confirmed the presence of two SNPs that generated the three known haplotypes, which were randomly distributed within the two *T. magnatum* populations, irrespective of truffle collection sites (Table 1). Haplotype III was the most frequent, followed by haplotypes II and I [14].

Table 1. List of codes assigned to the 21 ascomata, collection site, truffle weight and maturity, peridium thickness, and haplotype at SCAR A21-inf locus.
[a]Ascomata were classified into three maturation stages: (I) immature, (II) intermediate, (III) mature.

Sample code	Collection site	Weight (g)	Maturity level[a]	Peridium thickness (µm)	Haplotype (SCAR A21-inf)
1	1	6.78	III	298.75	III
2	1	4.68	III	271.25	III
3	1	5.19	III	287.68	III
4	1	4.75	III	521.36	II
5	1	7.01	III	487.00	III
6	1	4.79	II	667.50	III
7	1	2.45	III	482.22	I
8	1	2.89	III	533.50	III
9	1	3.68	III	522.50	III
10	2	5.11	III	1231.25	II
11	2	2.88	I	813.75	I
12	2	3.64	I	985.16	III
13	2	1.93	I	444.29	III
14	2	3.61	I	798.75	II
15	2	2.17	II	653.13	III
16	2	3.34	III	757.50	I
17	2	2.56	II	394.17	III
18	2	4.30	II	637.67	II
19	2	2.36	I	854.46	II
20	2	4.01	I	615.91	II
21	2	2.51	II	811.03	I

Another important goal of the present study was to characterise the Molise white truffles from a microbiological standpoint. For this purpose, the bacterial communities associated with the 21 ascomata were analysed by using the 16S rRNA gene amplicon high-throughput sequencing. Consistent with previous research [16-20], the bacterial communities retrieved in the investigated white truffles were made up almost exclusively of *Proteobacteria*, mainly belonging to the *α-Proteobacteria* class. *Bacteroidetes* and *Firmicutes* phyla were, overall, poorly represented (Figure 2), whereas *Actinobacteria*, microorganisms normally associated with *Tuber* fruiting bodies [13, 16, 17], were not detected. Although *Bradyrhizobium* was the most represented genus, some truffles showed "unusual microbial profiles", with *Flavobacterium*, *Mesorhizobium*, *Pedobacter*, *Phyllobacterium*, *Polaromonas*, and other bacterial genera as dominant taxa (Figure 2). Principal Coordinate

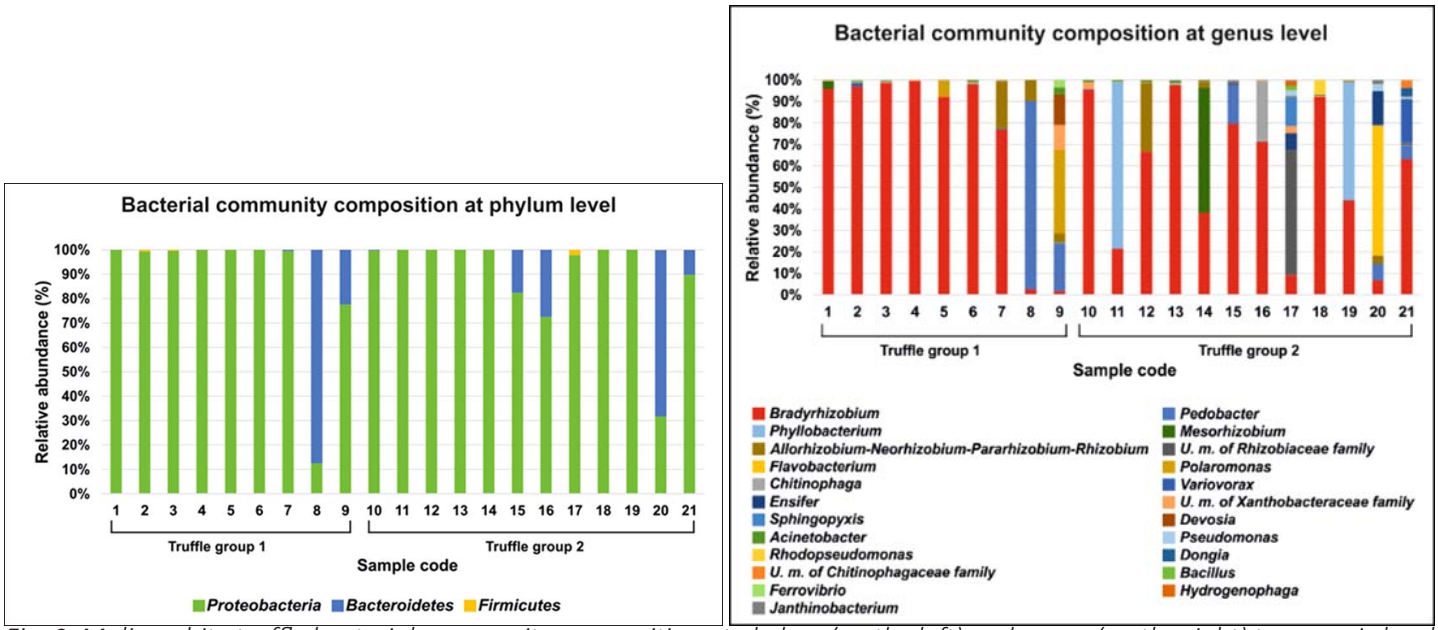

Fig. 2. Molise white truffle bacterial community composition at phylum (on the left) and genus (on the right) taxonomic level.

Analysis (PCoA) based on Bray-Curtis metric revealed a greater dispersion among bacterial communities associated with ascomata harvested at study site 2, which also showed a higher microbial diversity. Differences observed in the microbiota composition between the two *T. magnatum* populations could be related to differences between the two study areas in terms of extension, microclimatic conditions, soil properties and microbial communities. Moreover, as a small-scale heterogeneity exists in the soil [18], it is likely that the microbial communities of the soil surrounding the fruiting bodies (from which truffle bacteria are selected) differed not only between the two collection sites but also within them, contributing to determine the variations observed among samples belonging to the same population. In conclusion, this work allowed to obtain very interesting results on the Molise white truffle, revealing a surprising heterogeneity of the analysed populations that makes them ideal for further studies. One of the main future goals will be to carry out complete and more in-depth investigations of Molise truffles, in order to identify peculiar traits that could be useful to define specific markers of *T. magnatum* geographic origin, contributing to the conservation and promotion of this resource of utmost importance for the local economy.

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Examples of Interdisciplinary Geoarchaeological research Approaches: The Ancient Cities of Pompeii and Isernia

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Southern Italy hosts several very important archaeological sites, covering a wide chronological range spanning from prehistorical to historical times. Some of them are very famous Archaeological Parks, visited by several thousands of visitors and well known all over the world, such as the Pompeii and Paestum-Velia Archaeological Parks. Other archaeological sites, corresponding to limited sectors of ancient cities, are only partially accessible to visitors because localized in subsoils of historical centres, as Aesernia, Bovianum and other ancient cities.
Recently, Archaeological Parks, Local Archaeological Superintendences, National and International Archaeological Research Projects and Archaeologists freelance have implemented their expertise teams with geologists and natural sciences researchers. Progressively, the multidisciplinary interactions between archaeologists and geologist are aimed to the reconstruction of ancient landscapes and environments. Starting from this approach, the study of natural and anthropogenic changes that affected the birth, decline, burying and disappearance of ancient cities and settlements are one of the main tools of the archaeological environmental researches, also aimed to the preservation of the archaeological heritage and its scientific divulgation as well as for the evaluation of related seismic, volcanic and hydro-geological hazards.
Since several years, the Research Group of the GeoGisLab of the Bioscience and Territory Department, thanks to several interdisciplinary collaborations, is involved in geoarchaeological studies of some of the most important archaeological sites of Southern Italy. These studies are aimed to characterize the ancient coastal landforms and environments and the evolution during the long-life of the historical cities of Pompeii, Stabiae, Salernum, Picentia, Poseidonia-Paestum, Elea-Velia, as well as to identify the environmental and anthropogenic changes that affected important cities and settlements localized in the hilly and mountainous sectors of the Southern Apennine such as Aesernia, Bovianum, Benevento and Abellinum. The main results of the performed studies were reported in several international and national peer-review journals [1-7].
In this abstract, we synthesize the main research approaches used to study the territory within and around the ancient cities of Pompeii and Isernia, and major results obtained.
The ancient city of Pompeii is one of the most important Archaeological Parks of the world for both its rich cultural heritage and the number of visitors. Here, the Roman ruins are well preserved under a thick succession of pyroclastic layers of the 79 AD Vesuvium eruption that caused the total burying of the city and strong environmental and landforms changes of a large sector of the southern Campania Region. The Ancient Pompeii was founded on a volcanic hill (Pompeii Volcano, [1]) located between the southern slope of the Vesuvius Volcano and the Sarno River alluvial coastal plain. The western and southern sectors of the Pompeii volcanic hill present steep scarps (also over 10m high), that were sea-cliffs at ca. 6.0ka BP when the of the Post Glacial rapid sea level rise caused a wide marine transgression within the Sarno River plain. During Roman ages, the coastal sector was characterized by articulated landforms and environments made mainly of wide dunal ridges and sandy beaches alternated with back-ridge depressed areas hosting lagoonal and marshy environments [2]. Recently, the geological and geomorphological studies of our group improved the knowledge both on the landforms that were important for the settlement and development of the city and on the adjacent coastal environment and especially on the localization of the Pompeii harbour.
The urban landforms were highlighted by a detailed geomorphological study based on available topographic maps and new GPS data acquired within the urban area.
Figure 1 shows the main landforms of the urban sector of the Pompeii Archaeological Park, testifying that the city adapts itself to the natural landscape. Southern and western city walls and most important *villae* and buildings were constructed directly on the relics of the ancient paleo-seacliff. Several quarters were built taking into

consideration ancient impluvium, small streams and relics of ancient volcanic landforms such as crater rims, ridges and peaks.

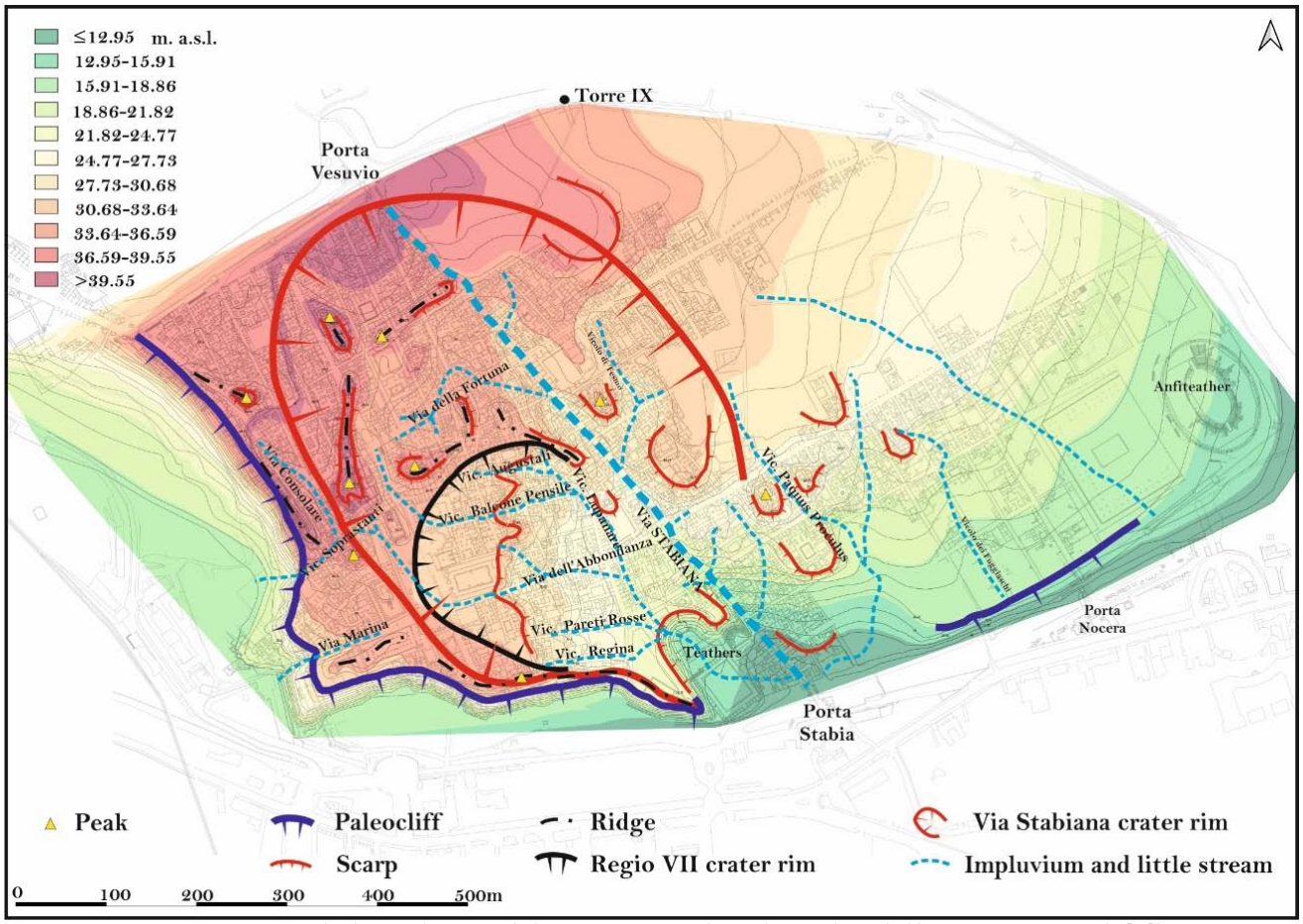

Fig. 1. Geomorphological map of the Pompeii Archaeological Park (from Amato et al. [1]).

The reconstruction of the environmental setting of the coastal sector in 79 AD was hypothesized thanks to the stratigraphical, paleoecological and tephrostratigraphical analyses of several boreholes carried out within a depressed back-ridge area localized just outside the southern sector of the ancient city. Also published stratigraphical and new archaeological data were taken in consideration for the reconstruction of the ancient shoreline (Figure 2). The main results of these studies are the identification of marine environments active in 79 AD within the more depressed sectors of the Bottaro back-ridge, testified by lithofacies and fossil assemblages. This important data opens new research outlooks for the location of the Pompeii ancient harbour that is still unknown therefore representing a big scientific challenge.

The city of Isernia is in the Molise Region at about 420 m a.s.l. on a terraced and stretched hilly ridge, limited by the incisions of the Carpino and the Sordo rivers (Figure 3). Isernia territory is historically and archaeologically very important. It hosts consistent traces of a very old human frequentation and occupation, witnessed by the Isernia La Pineta Site dating back to the Lower Paleolithic Age (ca. 600 ka BP). Furthermore, the area of Isernia city is characterized by a multilayered historical center, preserving Samnitic, Roman and Middle age ruins, which are mainly located in the subsoil but well reflect the ancient shape of the Roman colony which was founded in 263 BC (Liv., XVI, 48). The city and its urban design were characterized by a complex distribution of artificial terraces connected by a principal axis (the actual Corso Marcelli, Figure 3) that crossed the entire residential area. The city walls, mainly made of large travertine blocks quarried in local travertine outcrops, were built along the borders of the elongated ridge lying on a travertine substratum.

Recently, our research group improved the geological, geomorphological and stratigraphical knowledge thanks to the realization of a new geological-geomorphological map that takes in consideration also the main paleoenvironmental features and their changes along the times.

Starting from the archaeological and chronological data available for Isernia La Pineta Site, the collection and

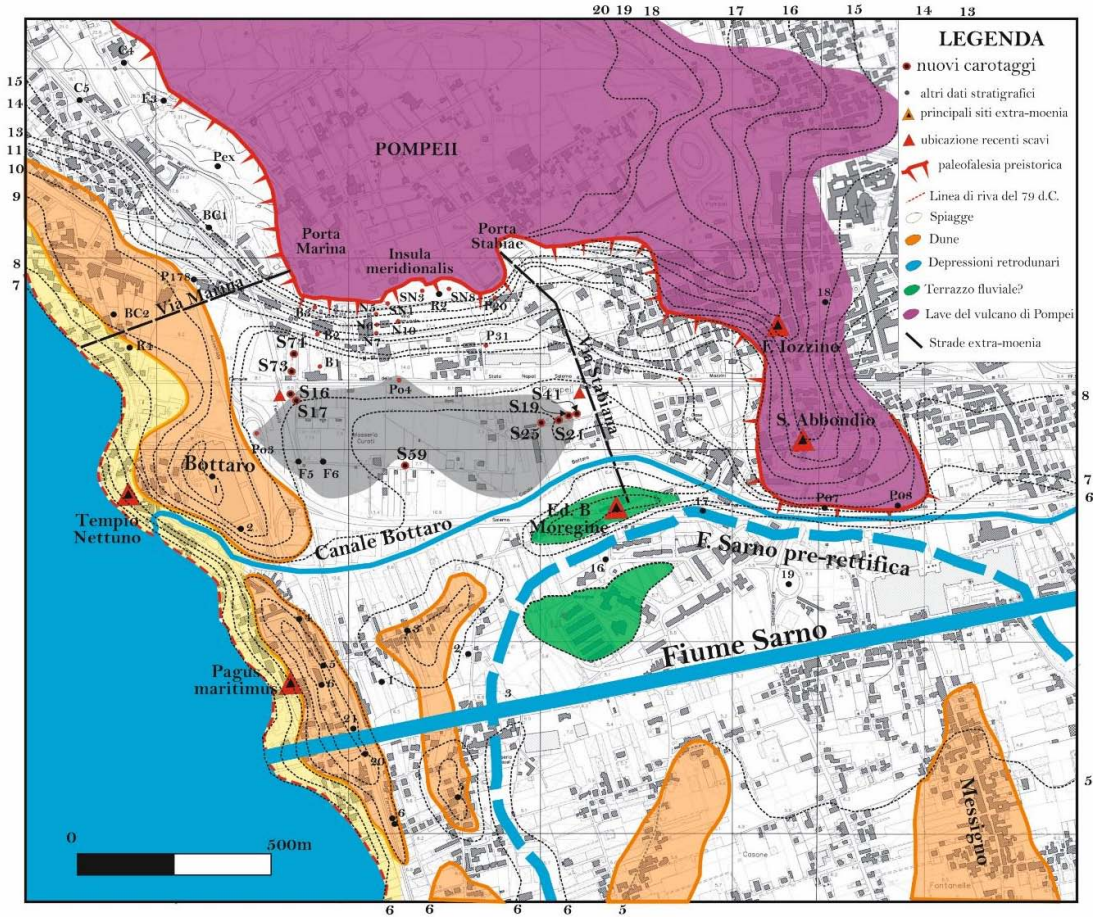

Fig. 2 79 AD paleoaenvironmental reconstruction of the Pompeii coastal sector (from Amato et al. [1]).

interpretation of ca. 200 boreholes and the realization of a new deep borehole in the Isernia Le Piane tectonic depression, supported by new Ar/Ar datings on tephra layers intercalated within the drilled succession, allowed to propose a detailed reconstruction of the entire Isernia territory [7].

Figure 3 shows that fluvial-marshy environments characterized the Isernia intermontane basin during Lower and Middle Pleistocene times when a group of humans settled down there very close to the Carpino River. During Roman times, when the Aesernia colony was founded, the ancient city of Isernia occupied only the narrow and stretched southern portion of the travertine terrace, while the other parts were used for agriculture and farming. A local aqueduct was built for the Aesernia colony, which captured the waters of some springs located along the N-NE borders of the Isernia Le Piane sector and conveyed them via subsoil to the historical center serving public houses and fountains. Over the centuries, many natural events, especially earthquakes, along with human interventions and historical events have led to profound modifications of the urban fabric. During the Middle Ages and until 1800 AD, the urban shape remained confined within the ancient city walls bordering the southern part of the travertine terrace. The remaining part of the territory was under marshy conditions, such as the Isernia Le Piane sector, used for local agriculture and farming purposes or covered by dense forests. Only from 1800 AD onwards, precisely after the 1805 AD (S. Barbara) earthquake, Isernia city progressively expanded occupying also other sectors of the travertine terrace. Intense phases of urbanization and building constructions occurred especially during the last century, mainly from the 1970s to the 1990s.

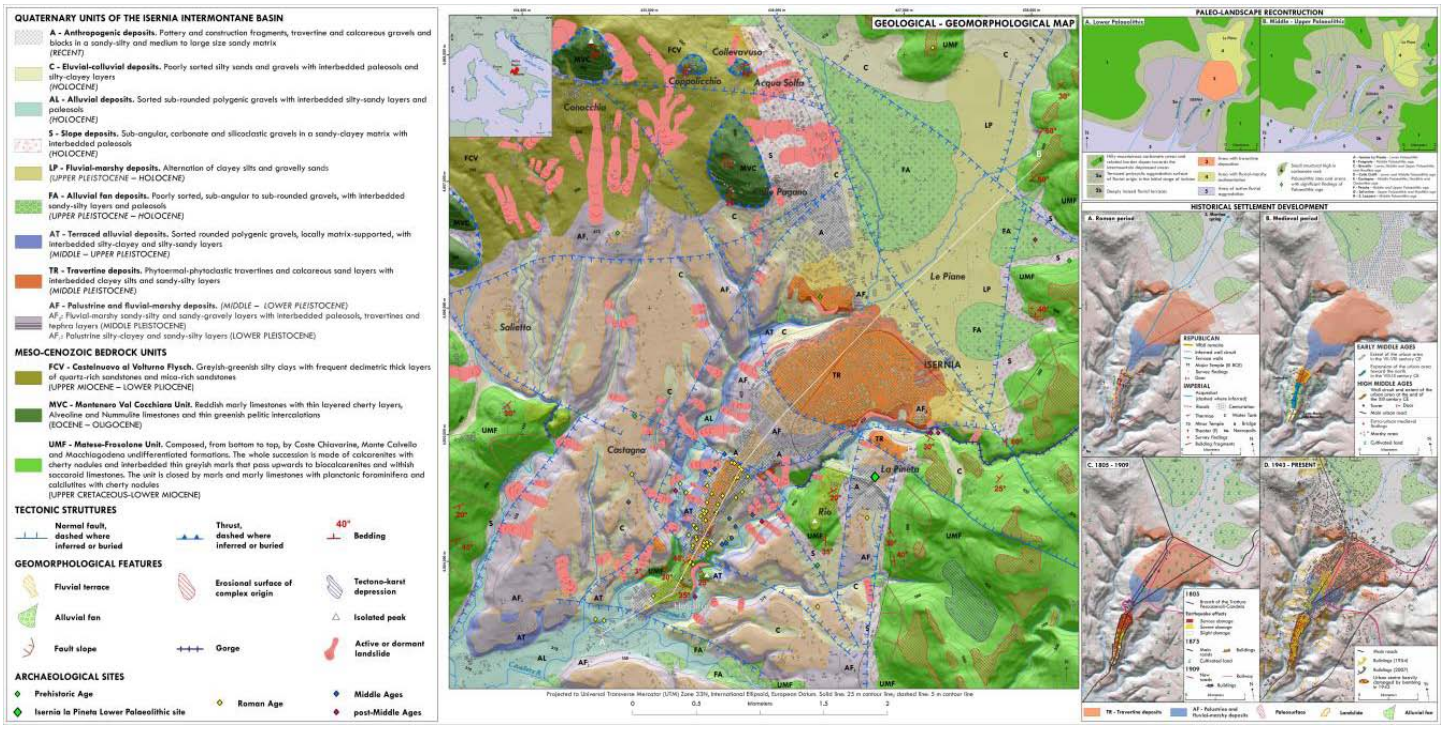

Fig. 3. Geological and geomorphological map of the Isernia territory. On the right palaeolandscape and paleoenvironmental reconstruction in relationship with historical-archaeological data.

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CARG Project as a basic tool for territorial planning and prevention of natural and man induced hazards: Isernia and Campobasso 1:50.000 geological sheets

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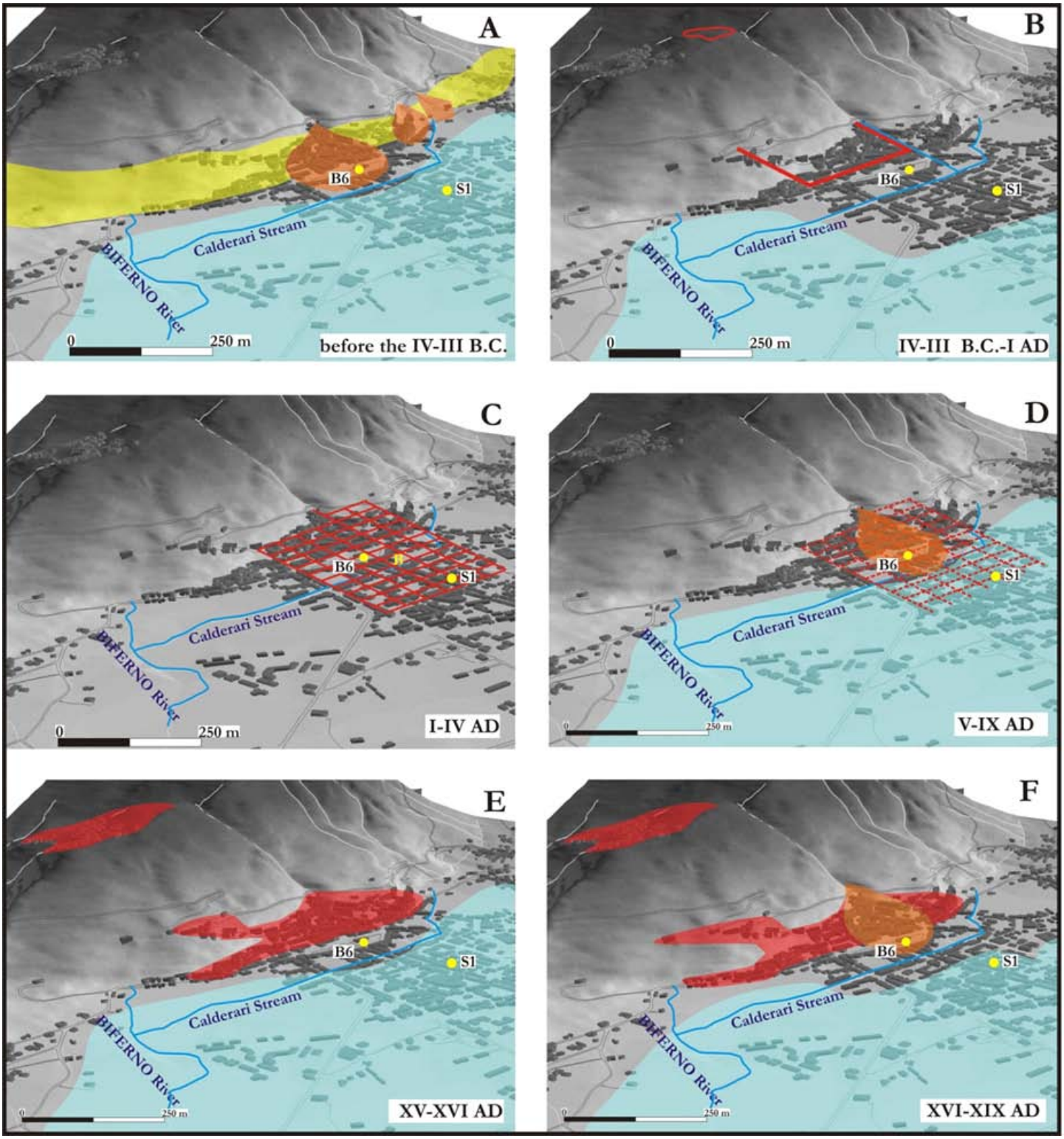
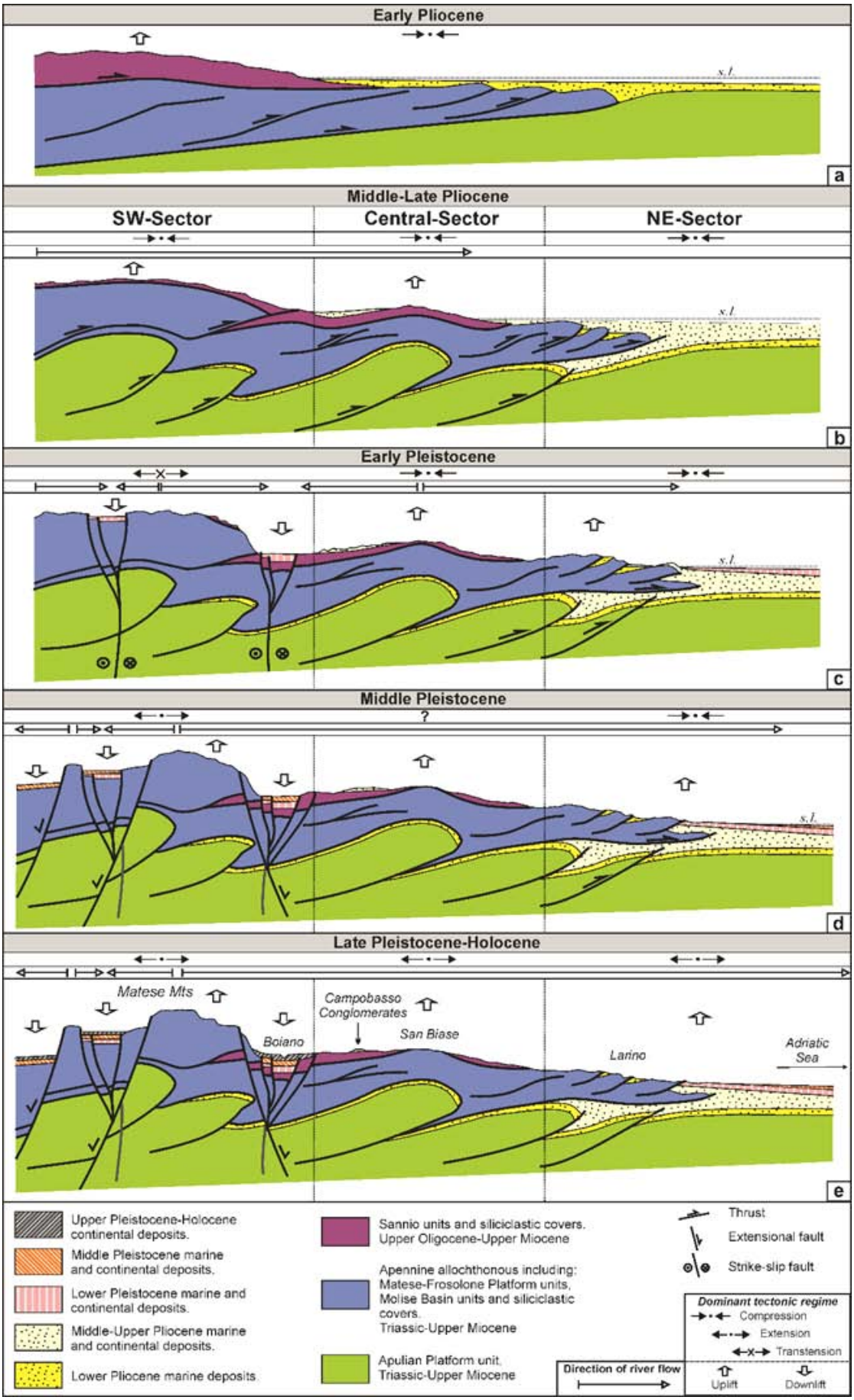
Geological knowledge of a country with a high structural complexity such as Italy is a central objective for the mitigation of natural or man-induced hazards. By locating and understanding past and present natural processes, it is possible to estimate their future evolution, also in the light of climate change. This is a fundamental step that contribute to territorial planning, by a sustainable management of natural resources and secure infrastructure design, aimed to risk prevention, ecological transition, and sustainable development.
The CARG project leads to a complete knowledge of our country through the production of high-quality data, cartographies, and 3D geological models essential for environmental, urban, and infrastructural planning activities. CARG Project cartography is the geological base map used not only by geologists but usually used by technicians of Local Administrations (eg. Engineers, Architects, Planners, ecc.), Research Institutions (University, CNR, ecc.) and National and Local Civil Protection Offices, in order to project plans of risk mitigation from flooding, landsliding, coastal erosion and seismic prevention. Also, other specialists, as archaeologists, botanists, tourists, currently use CARG geological maps in order to support their researches and works. Each sheet is on-line available for consultation at https://www.isprambiente.gov.it/Media/carg/index.html. This cartography consists of 636 geological and geothematic sheets at the scale 1:50,000: since the end of the 80's when the project was started, a total of 281 geological sheets, equal to about 44% of the total coverage, have been realized. Among these, the Campobasso and Trivento sheets are the only two completed in Molise region, for a total coverage of about 40%. In particular, the GeoGisLab of Department of Biosciences and Territory realized the Campobasso sheet. This sheet provides new data about the structural pattern of the Boiano basin, a NW-SE oriented morphotectonic depression associated with the quaternary evolution of an about 40 km long fault system with the same direction. These faults are responsible at least of two historical disruptive earthquakes in Molise area: the December 1st 1456 (M 7.19) and the July 26th 1805 (M 6.68) [1,2]. Many evidence for these earthquakes and related environmental changes occurred in archaeo-stratigraphical successions of the Bovianum ancient city, now partially in the underground of the Boiano town, where several layers are made of anthropogenic and marshy deposits [3]. Also a detailed chronostratigraphic and paleoenvironmental study for Campochiaro alluvial fan system was carried out in order to better understand the articulated fault systems intersecting the Boiano intermontane basin [4] and Matese mountains [5]. CARG project data were also used for reconstruction of the long and short terms paleolandscape evolution of the Molise Apennine [6], also aimed to identify the sectors of the regions mainly susceptible to landslidings and floodings.
Starting from this expertise, the research team of the GeoGisLab works to realization of the new 1:50.000 geological map of Isernia sheet. The preliminary field geological data, also with boreholes stratigraphy, are allowing us to understand the quaternary evolution of the Isernia and Venafro intramontane basins, also in relation to the paleoenvironmental evolution [7, 8], the geological and structural arrangement of the western side of Matese mountains, and the geometry, cinematic and seismic potential of the active faults recognized in this area in the last years [9, 10].



Location and extension of the urban area of Boiano, the waterlogged area and the ongoing debris production along the piedmont area during various periods, reconstructed by means of archaeostratigraphical data coming from cores S1 and B6 (yellow circles) and the examined archaeological excavations (for location see Fig. 3). Polygons in blue and orange indicate the presumed extension of the waterlogged area and the ongoing debris cone sedimentation, respectively. A) the Boiano area before the IV-III century B.C.; in yellow the aggradational piedmont zone. B) the boundaries of the Samnitic Bovianum and Roman Municipium during the time period IV-III century B.C.-I century AD, marked by the defense walls (red lines). C) the layout hypothesis of the Roman Colonia (red grid) with its centre, the Calderari archaeological area (B), during the I to IV century AD. D) the urban area of Boiano in the time period V-IX century AD, partially affected by debris cone sedimentation and waterlogging. E) the hypothetical expansion upslope of the modern village of Boiano (red polygon) during the XV to XVI centuries AD. F) the hypothetical extension of the modern village town of Boiano (red polygone) during the last centuries.



Schematic geological cross sections across the Molise Apennine to the Adriatic coast (not drawn to scale) illustrating the overall strati-graphic-tectonic setting along with tectonic events and tectonics regimes that have characterized its SW, Central and NE sectors during various time intervals (a – e) starting from the Early Pliocene and the related landscape evolution.

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Wild Coast Cascade: a citizen science tool for monitoring coastal-marine biodiversity in the Central Adriatic

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Introduction

Coastal areas host unique biodiversity that deserves to be protected and for which continuous monitoring activities are needed [1]. The impossibility of continuously monitoring vast areas of territory, has promoted the development of integrative techniques for collecting biodiversity data such as that of Citizen Science (here after CS). CS, intended as scientific research conducted with the support of the public, may have a key role in collecting large quantities of data with reduced costs and time, covering very large areas compared to standardized samplings [2, 3]. CS also favours the enhancement of public awareness on scientific fields (e.g. biodiversity) and applied techniques (such as conservation planning) [4].

In this context, the present study aims to preliminary explore the potential of CS for monitoring coastal-marine biodiversity in the Central Adriatic coast supported by a dedicated iNaturalist project: Wild Coast CASCADE (www.inaturalist.org/projects/wild-coast-cascade - Fig. 1). iNaturalist is one of the most effective networking services for Citizen Science initiatives for biodiversity observation and data storage (www.inaturalist.org).

Fig. 1. QR Code of the project Wild Coast CASCADE in iNaturalist.

Materials and methods

The study area consists of a 166km long coastal strip of Abruzzo and Molise regions including two LTER (Long Term Ecological Research Network) sites “Foce Saccione-Bonifica Ramitelli” (IT20-003-T) and “Foce Trigno-Marina di Petacciato” (IT20-002-T; www.lteritalia.it).

To raise public awareness of coastal biodiversity issues and to encourage the field data collection, 15 Citizen Science field campaigns have been carried out on central Adriatic coast, during the last two years, in collaboration with local nature conservation associations (e.g. Ambiente Basso Molise) and protected areas authorities (e.g. “Torre del Cerrano” Marine Protected Area).

Each observation uploaded in the iNaturalist database, includes geographic coordinates, a picture taken in the field and a proposal of taxonomic identification. The quality of each observation is accurately evaluated assigning the new data to a lower quality category: ‘Needs ID’ which indicates the need to be checked and eventually corrected by iNaturalist community members specialized on taxonomic groups. When more than 2/3 of the specialists agree and confirm the organism’s identification (at the specie level), the observation is assigned to the higher quality category: “Research”, and the data can be used for scientific purposes.

For preliminary explore the data collected on Wild Coast CASCADE, we extracted on November 2022, all the observations, grouped them on taxonomic categories defined automatically by iNaturalist and quality level.

Results

Wild Coast CASCADE, registered 3613 observations referable to 728 species. The most frequent taxon is Reptiles (28.5%) followed by Plants (20.9%), Insects (11.4%) and Birds (11.3%). The least represented categories (<1%) were Amphibians, Fungi and Lichens and Chromists (Fig. 2).

81% of the observations are of “Research” quality (Fig. 3) with Reptiles, Amphibians and Mammals presenting the most Research level data (>99%), most of the other categories half observations of good quality (50%) and Fungi and Lichens with few data of quality (23%).

Focusing only on observations of research level, the category with the higher number of species are Plants (227 taxa) and Insects (156 taxa). While the poorer categories are Chromists (2 taxa) and Fungi and Lichens (2 taxa) (Fig. 4).

The taxonomic reports of several species of conservation concern are noticeable with 19 species included in the IUCN Red List (1 Critically Endangered: *Pinna nobilis*, 4 Endangered: *Caretta caretta*, *Testudo hermanni*, *Chelonia mydas* and *Claodocora caespitosa*, 9 Vulnerable: *Physeter macrocephalus*, *Passer italiae*, *Streptotelia turtur*, *Balaenoptera physalus*, *Epinephelus marginatus*, *Palinurus elephas*, *Aythya ferina*, *Cerambix cerdo* and *Dentex dentex* and 5 Near Threatened: *Haematopus ostralegus*, *Calidris ferruginea*, *Aythya nyroca*, *Raja asterias* and *Sciaena umbra*).

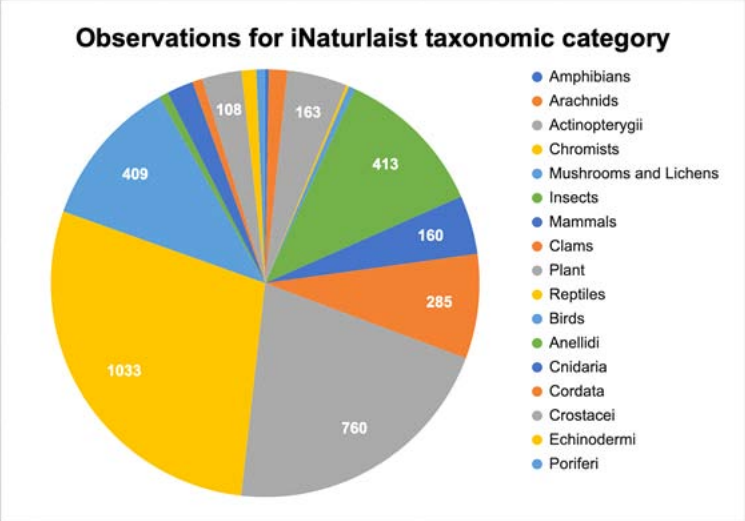

Fig. 2. Total number of observations for each iNaturalist taxonomic category.

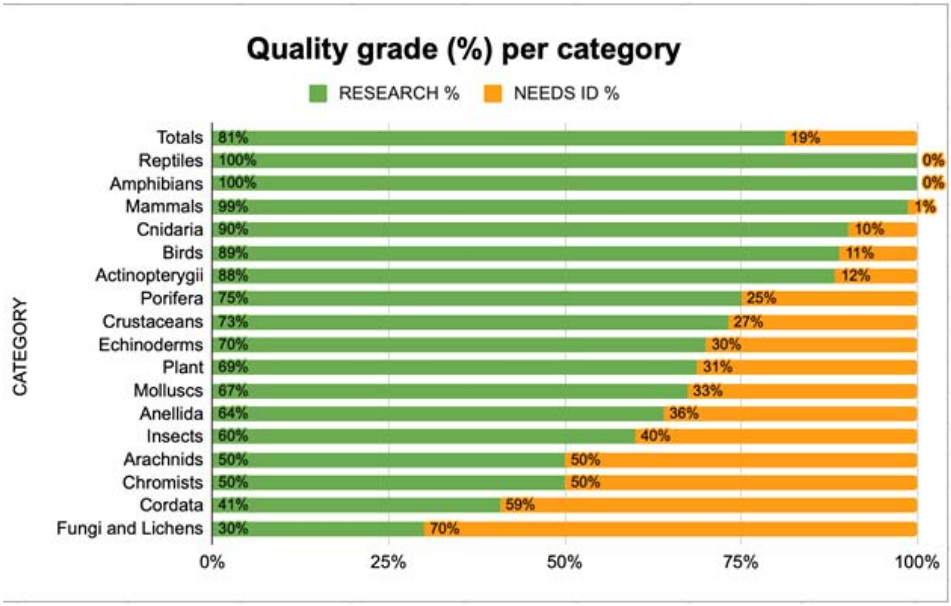

Fig. 3. Percentage of "Research" level (green) and "Needs ID" (orange) observations per each iNaturalist macro category. "Needs ID": observation that need to be checked; "Research"; observations confirmed by iNaturalist community members specialized on taxonomic groups.

Among the introduced species Wild Coast CASCADE reports 48 taxa, with the most observed one, the Invasive *Myocastor coypus* (11 observations, Mammals), *Acacia saligna* and *Carpobrotus edulis* (respectively 8 and 3 observations, Plants) and *Trachemys scripta* (4 observations, Reptiles).

Discussions and conclusions

The large number of observations reported in a short period confirms the ability of CS activities to collect biodiversity data. Furthermore, the high number of observations at a "Research" grade demonstrates how much the data collected by citizens can also be useful in scientific studies.

The presence in the Database of numerous reports concerning threatened and alien species suggests the importance of citizens as "sentinels" of biodiversity in large areas, often not explored by experts [5]. Wild Coast Cascade is linked with the Citizen Science program of the E-LTER network (European Long Term Ecological Research) [6].

As the database is constantly

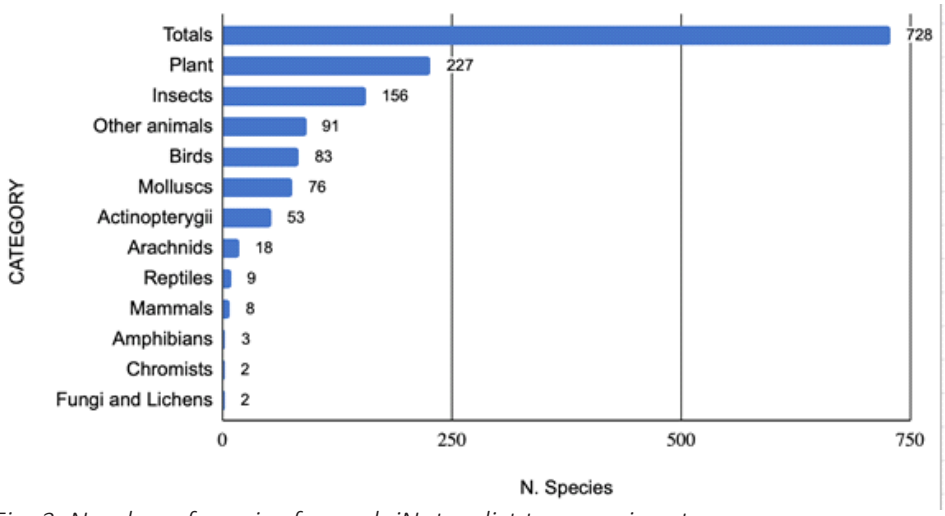

Fig. 3. Number of species for each iNaturalist taxonomic category.

updated, an increase in biodiversity data in the coastal-marine ecosystems of the Central Adriatic is expected for the future. Such information could provide valuable support for identifying integrated strategies for the management and conservation of these fragile ecosystems.

Acknowledgements
The research was partially funded by the Project INTERREG V-A IT-HR CBC Programme – 'Strategic' project: 'CoAStal and marine waters integrated monitoring systems for ecosystems proteCtion AnD management' (CASCADE – ID: 10255941), INPS (Istituto nazionale della previdenza sociale) PhD scholarship program Action III, Sustainable Developmentand and LTER (Long Term Ecological Research) network.

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Coastal-marine habitats of Conservation Concern in the Molise N2K network: detection of Sandbanks with Cymodocea nodosa, and Estuaries in the Campomarino coast and Biferno mouth

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Introduction

Coastal-marine areas are important transitional ecosystems hosting a great level of biodiversity. Being dynamic environments, coastal areas need of continuous ecological monitoring. It is well known that coastal-marine strips and river mouths are biodiversity hot spots supporting several ecosystems services and blue economy activities, still, the knowledge of such ecosystems on the Molise region coast is partial and further research efforts are needed to better known them and support their sustainable management.

The present research presents preliminary results describing coastal-marine biodiversity in the Molise region and represents the first attempt of detection and mapping of habitats of European conservation concern in the coastal seabed and of assessment of the conservation status of the habitat of estuaries (Habitat Directive 92/43/EEC).

Materials and methods

The research was carried out during the spring and summer of the year 2022 in the coastal–marine zone of the Special Conservation Zone (ZSC) IT7222216 “Force Biferno – Campomarino coast” (Fig. 1).

For exploring the presence and to map the habitats of European conservation concerns occurring in the coastal seabed a dedicated diving campaign was implemented within 300 m of the seashore [1], in collaboration with the A.S.D. “Vivi il Mare”, boating association that helped with the logistics. Samples of marine phanerogams were collected in order to describe the seabed habitat.

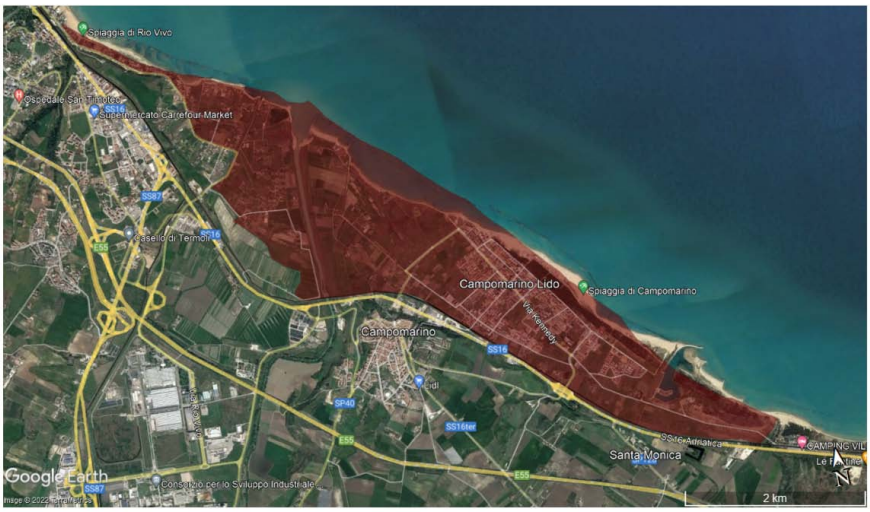

Fig. 1. Area of SCA IT222216 “Foce Biferno – Campomarino coast” in Google Earth.

Monitoring activities of the Habitat “Estuaries” (EC: 1130) [3] in the Biferno river mouth were implemented by macrozoobenthic [3] conenoses sampling and analysis according to ISPRA monitoring protocol (www.isprambiente.gov.it/files/icram/macrozoo.pdf). The sampling involved the collection from a boat, of macrobenthos samples of the estuary bottom and the measure of water salinity. Data were collected on 12 stations (with 3 replicates) distributed regularly (Fig. 2) from the sea towards the inner sector of the river mouth.

The analysis and taxonomic identification of floristic samples according with the “Flora d’Italia” [2], and of macro benthos with specialized guides [4] were carried out using a stereomicroscope in the S.A.S.I. SpA laboratories (Lanciano – Abruzzi).

Results

On coastal seabeds, the diving campaigns gave evidence of dense banks of marine phanerogams and areas where

Fig. 2. Macrozoobenthos sampling points collected in the Biferno river mouth.

Fig. 3. Identification of Cymodocea nodosa banks in the seabed of Rio Vivo beach Google Earth.

they are absent. The identification of *Cymodocea nodosa* confirm the presence of the habitat "Sandbanks which are slightly covered by sea water all the time" (EC: 1110) [5]. An example of the habitat map is reported in the figure (Fig. 3).

The results of the monitoring campaign in the Biferno river mouth showed low species richness values (e.g., 2 taxa/plot) across all plots and species composition referable to marine ecosystems [6] (e.g. *Pagurus bernhardus*, *Liocarcinus depurator* and *Echinocardium cordatum*) (Tab. 1), which is coherent with water salinity which resulted always close to 31‰ (average 34.08‰).

Tab. 1. Table 1 - Summary table of preliminary analysis on Macrozoobenthos. The number of individuals and species of each taxonomic category at each sampling point (numbers) and replicates (letters) are reported.

Replicates	Polychaeta	Bivalvia	Amphipoda	Isopoda	*Pagurus bernhardus*	*Liocarcinus depurator*	Arenicola	*Echinocardium cordatum*	Indet.	*Philocheras*	n indiv/plot	mean indiv/plot	total indiv/point	sp per plot	mean sp/plot	Total species
1A	3	2									5	26	78	2	4	12
1B	29	9	1								39			4		
1C	26	5	1		1	1					34			6		
2A	4	8			1						13	7.7	23	3	2.3	7
2B	3	4									7			2		
2C	3										3			2		
3A	8	5	1								14	9.7	29	4	3.3	10
3B	2	4	3								9			4		
3C	3	3									6			2		
4A	3									1	4	5	15	2	2.7	8
4B	1	1						1			3			3		
4C	1	6							1		8			3		
5A	2	1									3	4	12	3	2.3	7
5B	1	3									4			2		
5C	1	4									5			2		
6A							82				82	28.3	85	1	1.0	3
6B	2	1									3			2		
6C											0			0		
7A	1	1									2	1.7	5	2	1.7	5
7B											0			0		
7C		1	1		1						3			3		
8A		1	4		1						6	3.3	10	3	1.7	5
8B			3			1					4			2		
8C											0			0		
9A			1			1					2	2.7	8	2	2.0	6
9B				2		2					4			2		
9C			1			1					2			2		
10A			1								1	0.3	1	1	0.3	1
10B											0			0		
10C											0			0		
11A			2								2	1	3	1	0.7	2
11B											0			0		
11C		1									1			1		
12A		3	1								4	1.3	4	2	0.7	2
12B											0			0		
12C											0			0		

Discussions and conclusions

On this research we identified and mapped, for the first time in Molise region, two types of the marine habitat 1110 [3]. On shallow waters (not exceeding -5 m depth), of a 300 m strip facing the ZSC seashore, we identified the type IV: "Sandbanks or seabeds permanently submerged by marine waters with vegetation of the Zosterion marinae" and on seabed sectors free of phanerogams the type I: "Sandbanks permanently submerged by marine waters without vascular vegetation" (http://vnr.unipg.it/habitat/).

In the Biferno mouth, the EC habitat 1130: "Estuaries" characterized by a low number of macrobenthic species

all referable to marine habitat along with high of water salinity, resulted on bad conservation conditions. New studies on different seasons are needed to further assess the presence and the conservation status of habitat 1130 on the Biferno mouth.
The presence of directive marine habitats in the area facing the N2K site gives scientific evidence of the need of extending the perimeter of the site towards the sea of developing an integrated management plan combining, biodiversity conservation (e.g., *Cymodocea* habitat) and sustainable blue economy activities.

Aknowledgements
The research was partially funded by the Project INTERREG V-A IT-HR CBC Programme – 'Strategic' project: 'CoAStal and marine waters integrated monitoring systems for ecosystems proteCtion AnD management' (CASCADE – ID: 10255941), INPS (Istituto nazionale della previdenza sociale) PhD scholarship program Action III, Sustainable Development and LTER (Long Term Ecological Research) network.

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Manual for the propagation of native plant species of Adriatic coastal dunes

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Adriatic coastal dunes plant communities are often arranged along a compressed sea-inland vegetation zonation and this gives rise to high values of biodiversity in terms of habitat and native species richness [1]. However, they are now highly endangered and often degraded by human pressures (e.g. urbanisation, trampling, pollution) [2]. These anthropic pressures enhance the spread of generalist and invasive alien plants, causing the loss of native plant species abundance and cover [3, 4].

There are many projects that propose the restoration or recovery of degraded dune environments by realizing plantations with native dune plant species, in order to reinforce resident species populations and to enhance the biodiversity of coastal areas [5]. However, native dune species seedlings are often hard to find on the market of nursery sector, and therefore more and more projects encourage public and private nurseries to propagate local native plants. Indeed, the promotion of the use of native plant species of local origin in habitat restoration, landscape design and gardening, not only can contribute to stop the expansion of invasive exotic plants, but also to preserve conservation of genetic resources [6]. In addition, this ensures better adaptability of the planted species, contributing to the creation of stable habitats with a high naturalistic value and resilience to climate change. Projects financed by EU represent a good solution for obtaining funding resources to ecological restoration actions and for capitalizing good practices already identified in other contexts. In this context the "Manual for the propagation of native plant species of Adriatic coastal dunes" was conceived, thanks to the LIFE Program and the collaboration between the projects LIFE17 NAT/IT/000565 CALLIOPE and LIFE16 NAT/IT/000589 REDUNE [7, 8].

The objective of this manual is to provide guidelines for the propagation of the native species growing along Adriatic coastal dunes and that are suitable for restoration ecology and gardening in this biogeographic region. The guidelines comply with national and international procedures and standards [9, 10] and are addressed to nurseries owners and workers, public administrations, architects, engineers, seed bank technicians and researchers. The manual includes a descriptive introduction of the dune habitats and vegetation along the northern and central Adriatic coast (Veneto, Abruzzo e Molise) [11-13] followed by a section dedicated to the collection of seeds from wild plants [9] and technical sheets for 9 species of shifting dunes and 10 species for backdunes, reporting the applied methods of propagation and coltivation for each target species.

We hope that this experience of cooperation between scientists and technicians can lead to promote the use of native plants for restoration and gardening interventions, contributing to landscape protection and enhancement of our natural and cultural heritage.

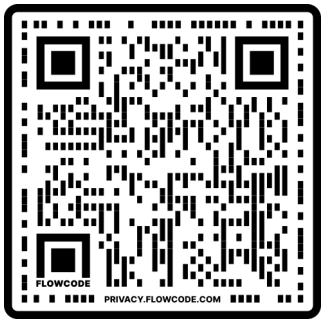

Fig. 1. Qr code for downloading a copy of the "Manual for the propagation of native plant species of Adriatic coastal dunes".

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Fig. 2. Front page of the "Manual for the propagation of native plant species of Adriatic coastal dunes".

The role of coastal defense structures and decreased fluvial sediment inputs in coastal erosion dynamics: The case of southern Molise Coast

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Coastal erosion has complex reasons and depends on both natural and anthropogenic factor. Marine climate conditions, sea level changes due to ongoing global climate change and soil subsidence are among the most studied natural control factors. Nevertheless, anthropogenic causes are probably the most common ones. In fact, the coastal equilibrium is strongly conditioned by the reduction of sediment supply, whose distribution and availability not only depends on natural dynamics, but also from human interventions on river channels and basins, and the presence and distribution of coastal defense and maritime structures.
In the present study, we have investigated the Southern Molise coast, which is located along the central Adriatic coast and extends from the Termoli promontory to the Saccione River mouth (Fig. 1). Major aim of this study is to contribute to a better understanding of the possible role played by defence structures in coastal erosion, by comparing data about local erosion rates, erosion susceptibility and types and distribution of defence structures.

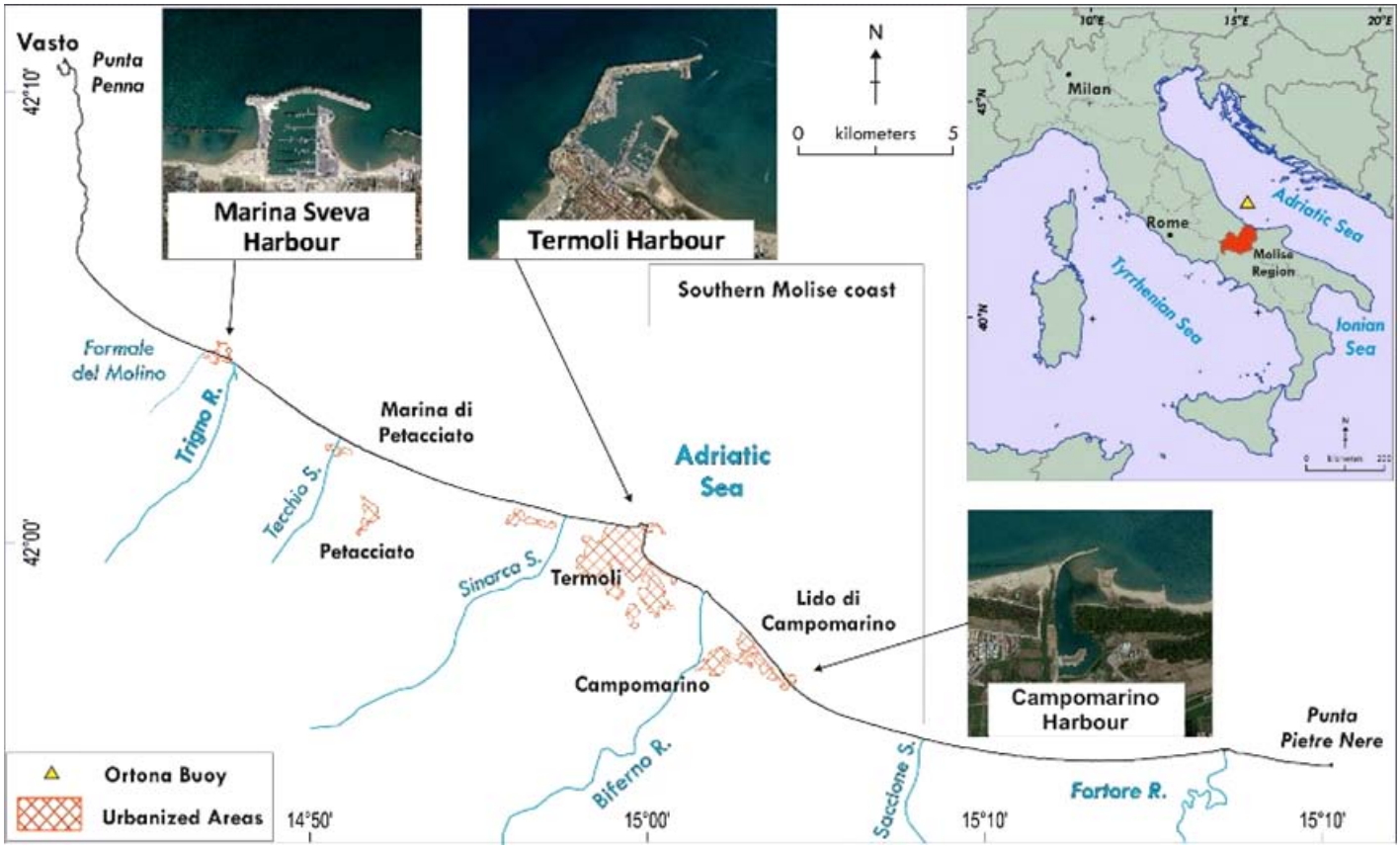

Fig. 1. The Molise coastline with of its three harbours. The southern Molise coast extends from the Termoli promontory to the Saccione Stream mouth.

The analysed coastline is mainly fed by the fluvial sediments transported by the Biferno River to the coast. Starting from the 1910s onwards, this coastline was affected by erosion that caused first the destruction of the Biferno delta then, from the mid-1950s onwards, a more extensive shoreline retreat that produced an elevated loss of land during approximately the last 65 years [1]. Shoreline retreat mainly affected the coastal segment including the Biferno mouth, reaching average annual rates of-2.90 m/y in the long-term [1]. Such a strong shoreline retreat

can be traced back to several control factors among which the decrease of sediment delivery to the coast by the Biferno River is among the major ones, if not the most important one [2]. Among these, the presence of the Liscione Dam, located 22 km upstream of the Biferno mouth, is retained one of the major causes. In fact, between 1965 and 2007, 4.4 x106 m^3 of sediments were trapped in the Liscione Lake, equal to an annual rate of 105x103 m^3/y and, therefore, did not contribute to the coastal sediment balance of the Southern Molise coast [3].

To contrast further erosion, hard coastal defence structures such as groins, adherent and detached breakwaters (both emerged and submerged) were realized over time. Nevertheless, erosion further accelerated during the last twenty years, with maximum annual rates recorded for periods 1998-2004 and 2011-2016, and involved increasingly the shoreline extending south of the Biferno mouth coastal segment [4, 5].

To verify the possible influence of hard defences along the Southern Molise coast on erosion rates, a "back analysis" was carried out by going ahead with the following steps:

- Analysis of the general longshore trend of the coast without any structure (Fig. 2A);
- Addition of the jetties armouring the Biferno mouth (Fig. 2B);
- Addition of the entire coastal defence system (Fig. 2C).

Comparing the predicted shoreline with the measured shoreline changes (see in black EPR and in red EPR_GENESIS, Figs. 2A, 2B and 2C) shows that they fit rather well in the "back analysis" in which the entire defence system along the Southern Molise coast was considered (Step 3, Fig. 2C). This result demonstrates that the presence and distribution of defence structures has conditioned both the location of erosion hot spot areas and the amount of shoreline change rates.

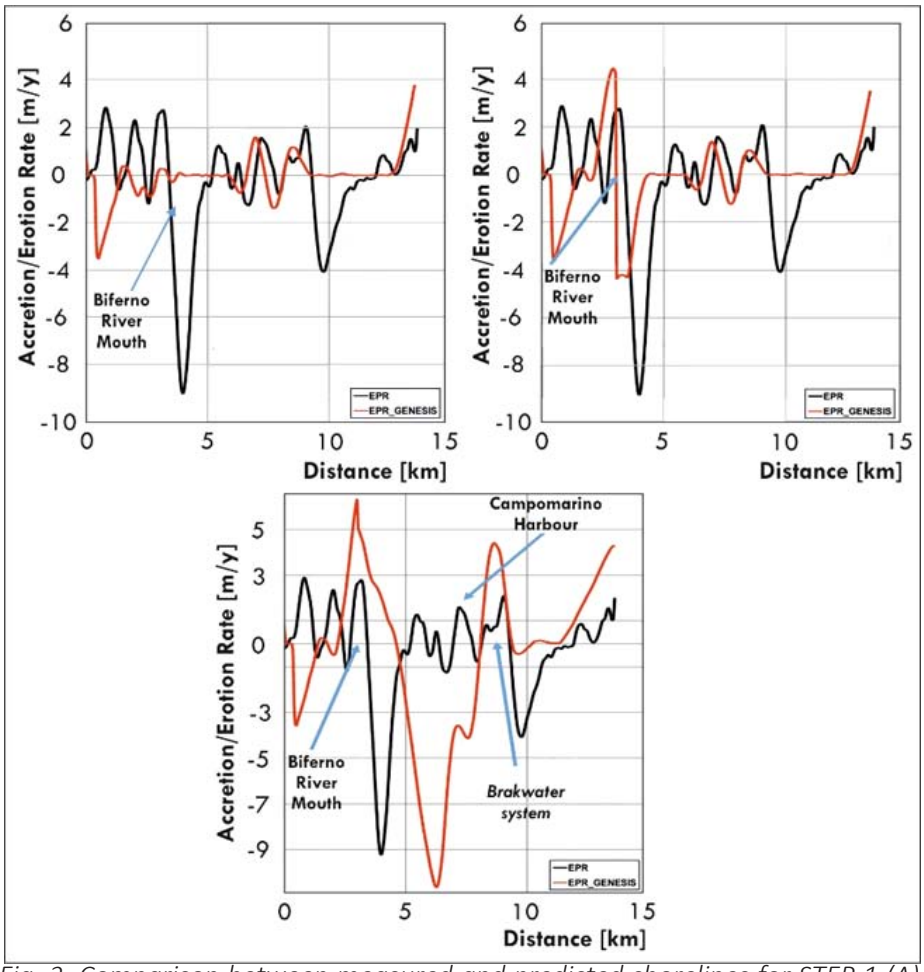

Fig. 2. Comparison between measured and predicted shorelines for STEP 1 (A), SPEP 2 (B) and STEP 3 (C) .

Furthermore, direct observations of shoreline variations over time, wave climate analysis and numerical simulations performed with the model GENESIS, showed that the coast was governed by the wave component 10°N [5, 6]. In fact, the consequent equivalent direction of solid transport, coupled with a net decrease in sediment inputs from the Biferno River, and its interference with coastal defences, has contributed to increase coastal erosion rates, particularly high around the Biferno River mouth. Moreover, obtained results show that the bimodality of the wave climate may has influenced the recent beach dynamics with the more inclined wave components responsible for the genesis of coastal instability that amplifies the erosion phenomena.

In conclusion, this study highlights that defence and maritime structures along with local marine climate conditions have deeply controlled shoreline erosion but also, most likely, increased its amounts, for which these controls have to be carefully considered in further erosion control interventions on the coast.

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The contribution of geodiversity to the valorisation of inner areas: the mountain karst landscape in the Molise Apennines

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The Mediterranean zone, and with it the Italian territory, is characterized by an extraordinary variety of landscapes. These landscapes and the related landforms are generated by a large range of geomorphological systems (e.g. glacial, fluvial, karst, coastal), sometimes in interaction and mostly still active, that have given origin to a great geological and geomorphological diversity.
The Italian Apennines and, in particular, the central-southern Apennines are characterized by a high geodiversity. This high geodiversity mostly depends on the geological-structural features of the Apennine chain, its long-term tectonic and geomorphological evolution, and present-day morphodynamics, with the last ones depending mostly on both relief features and climate conditions as well as related changes over time that have occurred since the emersion of the Apennine chain from Pliocene onwards.
Among the most ancient and typical landscapes and landforms that characterize the central- southern Apennines and, in particular, the Molise Apennines (Amato et al., 2017; Filocamo et al., 2019) are those originated by karst processes, which are widespread in Limestone (carbonate) mountainous areas. In these mountain areas, the karst landscape is characterized by an extensive vertical development and a high variability of karst landforms. The latter include both exokarst and endokarst features, which are generated respectively by the surface solution of soluble, especially carbonate rocks, or by underground karst corrosion.
Aim of the present study is to illustrate the richness and diversity of karst landforms that characterize the Molise Apennines.
The present study starts from the general consideration that geological features and especially geological elements to which a value in relation to geodiversity can be attributed or that can be recognized as part of the geological heritage, can play an important role in the sustainable development and valorisation of inner areas and especially of mountain areas (e.g. Filocamo et al. 2015; Filocamo & Rosskopf, 2019; Filocamo et al. 2022).
Based on this consideration, karst geosites and geodiversity sites not only are important testimonials of the geological and geomorphological evolution of the Apennines, but most of all give evidence of the high geodiversity related to karst environments, about long-term karst processes and (karst) landscape evolution, as well as active karst dynamics, offering all their potential for their cultural fruition.
For our purposes, we have as study areas the Matese and La Montagnola Mountains, two major mountain areas in Molise region, because of their particular richness in karst features.
Among the numerous karst features present in these two mountain areas (Fig. 1), we selected 32 sites, as representative of the main karst processes that occur in Mediterranean mountain areas and excellent examples to explain the complex long-term evolution of the Apennine karst landscape.
The karst landscapes of the Matese and La Montagnola areas are characterized by several types of karst landforms (Fig. 1) starting from very ancient features witnessing long-term karst corrosion, such as karst pavements (Fig. 2) and remnants of paleosurfaces.
The latter in particular may be also of complex origin, for example of fluviokarst or glaciokarst origin, giving evidence about the interplay of different geomorphological systems. Furthermore, a high variability of exokarst and endokarst features, mostly still active, include tectono-karst depressions (polje), dolines, karren fields, resurgences, karst springs and tufa terraces originated by karst springs, as well as endokarst landforms such as ponors and cave systems. The latter, in particular, are inventoried in the cave cadastres of the Campania and Molise regions that have provided important data and details.

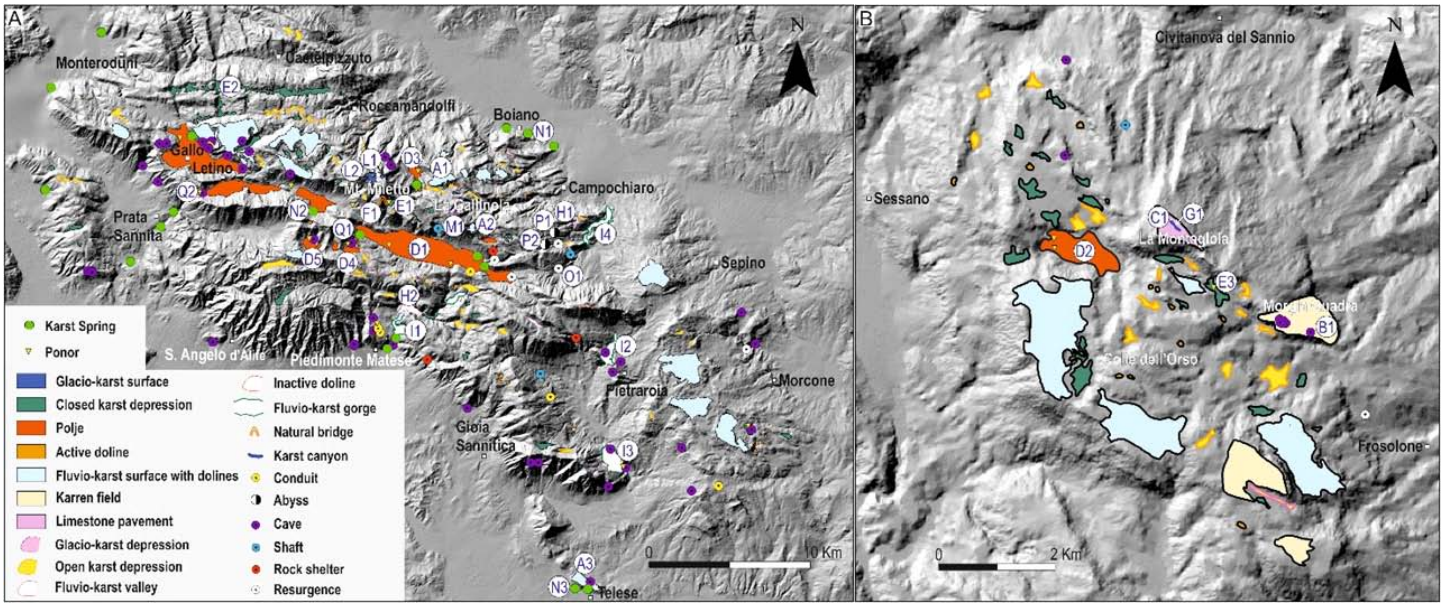

Fig. 1. Map showing the location and types of main karst landforms found in the Matese and La Montagnola Mountain areas. The sites selected for this study are indicated by a letter and a number (for example A1).

Site selection was mainly based on the criteria of representativeness and exemplarity, and aimed at representing all main categories of identified karst landforms. information about the typical landform elements that characterize each single site, the type of geomorphic evidence, its origin and significance in landscape evolution, were added to best characterize these sites.

Some of the selected sites are included in the national inventory of geosites managed by ISPRA and/or in the Molise region or Campania region geosite inventories.

Overall data collected not only allowed a detailed characterization of the selected karst sites, but also an overall view about their distribution and possible spatial or thematic relations. As such, these data can be usefully employed for proposals/interventions aimed at the valorisation of these mountain areas with respect for their high degree of naturalness and need of sustainable development, possibly also in combination with other tourism uses and potentials.

Fig. 2. View of the limestone pavement with flutes and grikes that characterizes La Montagnola Surface.

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Characterization of seed oil from six in-situ collected wild *Amaranthus* species

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The genus *Amaranthus* L. (Amaranthaceae Juss.) comprises 65–70 species, of which approximately half are native to the Americas [1]. Some taxa are used as ornamentals, food, and medicinals, and they are able to spread out of cultivation, negatively impacting agricultural and natural ecosystems [1-3]. Taxonomically this genus is complex due to its high phenotypic variability, which has resulted in the current nomenclatural disorders and misapplication of several names [4-9]. *Amaranthus* species have the C4 photosynthetic pathway, which allows them to proliferate at high temperatures and light levels, tolerate drought, and aggressively compete with warm-season plants for light, moisture, and nutritive substances.
The interest in Amaranth seeds, both for their use in the food field (human and animal nutrition) and their application in the industrial field, is increasing mainly because its seeds are a source of minerals and vegetable oil rich in essential fatty acids, vitamins, and unsaponifiable substances and particularly squalene [10]. Squalene is a biochemical antecedent of sterols, occurs naturally as a triterpenoid. It has had a significant role in medicine, cosmetics, and therapeutic applications. Recently, it has a significant role in drug design as an antioxidant and anticarcinogen [11]. Additionally, it is also used to reduce cholesterol levels among human beings. It also has a promising role as an excellent oxidation-resistant lubricant in the industrial sector [12].
In the past, the primary source of squalene was whale and shark liver, containing 40-80% squalene by weight. Due to marine animal protection concerns, researchers have turned their attention to exploring and identifying affordable and sustainable sources of squalene, such as plants [13, 14]. Among the different sources of squalene (yeast, olive oil, rice, corn, soy, peanuts, etc.) identified so far [12], Amaranth seeds are the most important and reliable source for their high squalene concentration (6-8%) compared to other sources [15, 16].
Accelerated solvent extraction (ASE) is a helpful extraction technique that gives an excellent yield of extracted materials, involving the use of different organic solvents. This technique is time-saving and reduces solvent use [3, 11, 17]. To increase the investigations on Amaranth seeds and broaden knowledge from a geographical point of view, it was decided to study *Amaranthus* species that grow spontaneously in Italy in different environments. Environmental factors such as climate, altitude, and soil pH should affect the chemical composition and squalene content [18]. In detail, the present paper aims to evaluate the chemical composition of seed oil extracted from six *Amaranthus* species collected *in situ* using the ASE apparatus. In particular, the squalene content, free fatty acid content, tocopherol, and sterol composition and content were investigated. This research aims to understand the value of seed oils of wild species belonging to the genus *Amaranthus* providing guidelines for future studies on food chemistry and industrial applications.

Plant Material
Plants of Six Amaranth species [*Amaranthus cruentus* L., *Amaranthus hybridus* L., *Amaranthus hypochondriacs* L., *Amaranthus muricatus* (Gillies ex Moq.) Hieron., *Amaranthus tuberculatus* (Moq.) J.D. Sauer., *Amaranthus. viridis* L.] were collected in the wild fields in Italy. In the fruiting stage, 40–50 individuals per site were collected. Synflorescences were then air-dried in the shade, and the fruits were removed and put in covered containers. The voucher specimens were deposited in the Herbarium of the University of Molise Italy (IS).

Methodologies
In the wild fields in Italy, six different types of Amaranth species were collected. The *Amaranthus* seeds were

cleaned, dried in an oven, and then ground into powder. An ASE350 apparatus (Dionex) (Fig. 1A) was used for oil extraction. When the extraction was finished, a rotary evaporator (Büchi) (Fig. 1B) was used to evaporate any extra solvent. Using gas chromatography with a flame ionization detector (GC-FID), the amount of squalene in the oil was measured. The results of the analysis were assessed with the help of Chromeleon 6 software.

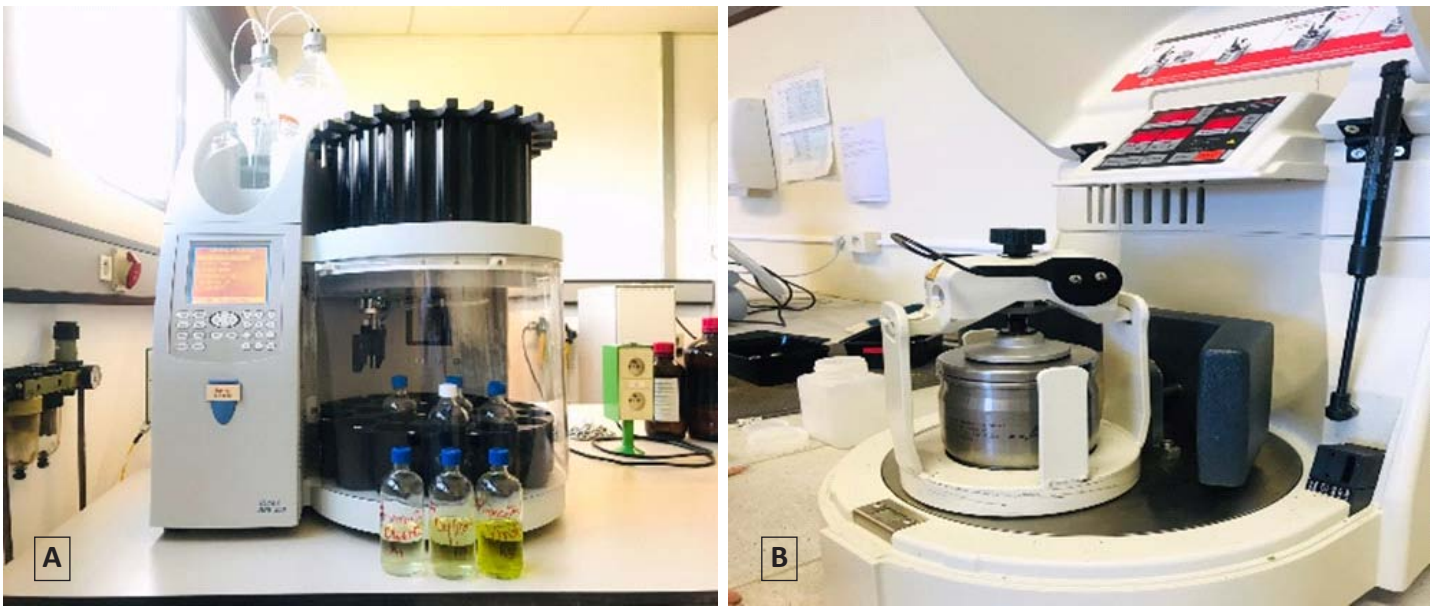

Fig. 1. A: ASE350 apparatus (Dionex); B: Rotary evaporator (Büchi).

Results

Seed features

For the six *Amaranthus* species that were examined, the color of seeds is very consistent, ranging from black to dark brown. However, there was a considerable variation in seed size.

Oil and squalene content

According to our findings, there were significant differences in the oil and squalene content in the seeds of the six *Amaranthus* species collected in the wild fields in Italy (Fig. 2). The seeds of *Amaranthus tuberculatus* showed the highest oil content, while *Amaranthus hypochondriacus* showed the highest squalene content. *Amaranthus muricatus* showed the lowest oil and squalene content.

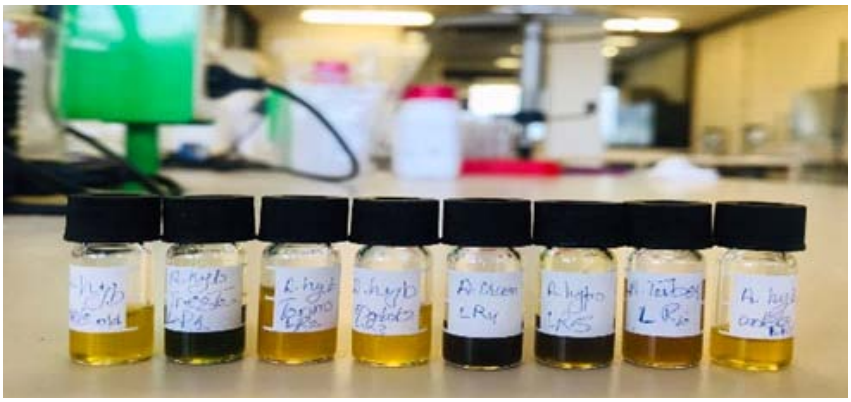

Fig. 2. Oil extracted from the six Amaranthus species collected from the wild habitat.

Free fatty acid, sterol, and tocopherol content

Our finding demonstrates that the free fatty acids in *Amaranthus* oil are Linoleic acid and Palmitic acid. On the other hand, the majority of phytosterols are beta-sitosterol, brassicasterol, campesterol, and stigmasterol. The total sterol content is much higher than that of the other studied plants like Olive, Peanut, Palm, Coconut, Walnut, Cashew, and Almond [19]. Based on our studies, the primary tocopherol (TSO) in *Amaranthus* oil is alfa-tocopherol, beta-tocopherol, and delta-tocopherol.

Conclusion

For the first time, this study provides data on six *Amaranthus* species that grow in wild habitats, including their composition, squalene content, free fatty acid, tocopherol, and sterol content. In terms of oil and squalene content,

these species showed traits comparable to those of commercial species. This study demonstrates that the collection site affects the oil and squalene content of the *Amaranthus* species. Particularly, a remarkable amount of squalene was found at low altitudes. Expanding the range of Amaranth species that may be grown as a good source of squalene and the other component is important due to the increasing interest in the seed of the genus *Amaranthus* around the world for applications in industries like nutraceutical, industrial, and medicinal.

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Combining multi-temporal active remote sensing and landscape analysis for measuring urban expansion: towards new indicators of urban sustainability

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Introduction

Urban built areas in the world have registered, since the 50', uncontrolled and fast expansion processes that have occurred at higher rates than cities' population growth [1]. The ongoing land take, causes the irreversible replacement of natural and semi-natural ecosystems by impermeable surfaces [1]. In this context, the development agenda 2030 of the United Nations (UN-2030 goals) and specifically the 11th UN goal states the need of effective tools for monitoring urban changes [2] able to support sustainable management strategies. Among monitoring tools, Remote Sensing (RS) offers a cost-effective, spatially contiguous, and timely manner support to register urban cover and could effectively depict urban growth pattern (infill, edge-expansion, and outlying) [3]. RS data registered by Synthetic Aperture Radar (SAR) sensors provide images free of clouds or sun illumination variability and, at the same time, the new SAR platforms also assure (e.g., Cosmo-SkyMed) very high spatial resolution (VHR). SAR data can also effectively support accurate urban mapping as built-up structures present a characteristic double bounce backscatter response, which is stable over time [4]. However, the use of only multitemporal VHR SAR data to analyze urban expansion and land take is still limited, in particular in south American countries where most of the population lives in urban areas and peri-urban zones are facing an uncontrolled land use change and land take [1]. Accordingly, this work aims at identifying built-up expansion and urbanization processes occurred in the city of Cordoba (Argentina) during the last decade (2010-2021) by coupling SAR multi-temporal satellite classification and landscape pattern analysis. In particular, based on purposely produced multi-temporal urban maps of the metropolitan landscape, the research aims at: (i) depicting urban cover over time and detecting the new built-up zones; (ii) quantifying the intensity and extension of land take; (iii) describing, through trajectory analyses, changes on urban cover and spatial configuration and (iv) differentiating ongoing processes of infill, edge-expansion, and outlying.

Materials and methods

The study was carried out on the metropolitan area of Cordoba (Central Argentina) and suburbs land mosaic with agricultural, natural and seminatural areas. Cordoba is the second most populous city in Argentina (1.3 million inhabitants) with an average annual population increase of 1% since 2001 [5]. We downloaded 12 Geocoded Terrain Corrected images from the Cosmo-SkyMed portal (https://portal.cosmo-skymed.it). These images were collected in ascending orbit and HH polarization in the period 2010-2021 in Stripmap Himage mode with 3-meter spatial resolution [6]. The Cosmo SkyMed First (CSK) and Second Generation (CSG) images were grouped in four time intervals (T_0: 2010, T_1: 2011-2012, T_2: 2018-19, and T_3: 2020-21, computing for each period the mean backscatter values. Built-up (here equivalent to "urban") areas were extracted for each time step (T_0, T_2, T_3) by using the Urban EXTent extraction (UEXT) algorithm applied to CSK and CSG data [7]. The accuracy of the extracted urban maps at each time step was assessed through comparison with the World Settlement Footprint (WSF) layers for 2015 and 2019, considering three independent sets of 590 random points [8, 9]. The corresponding confusion matrices were used to compute the Overall Accuracy (OA), the Cohen's Kappa statistic (K), the Producer Accuracy (PA), and the User Accuracy (UA) metrics [10]. To detect urban expansion at each time step ($UrbExp_{Tn}$), we used a time filtering method based on the comparison of the series of three successive urban maps [11]. We considered as a true urban expansion only those areas wider than 0.25 km^2 and classified as "not urban" in a given time step (NU_{Tn}) and as "urban" in the successive two (U_{Tn+1} and U_{Tn+2}) [11]. We estimated the accuracy of detected urban expansion maps through confusion matrices and calculating the OA, K, PA, UA metrics as follows: we defined a

buffer of 100 meters around each urban expansion area and analyzed 200 random checkpoints inside them. For each point a multi-temporal visual inspection was performed exploiting Google Earth aerial orthophotos referable to the analyzed period. Each point was eventually classified as $UrbExp_{Tn}$ (i.e., referring to a real urban expansion) and $NUrbExp_{Tn}$ (no urban expansion found). Urban areas changes were analyzed at landscape scale by trajectory analysis, an approach effectively used to depict the non-linear relation between landscape composition (proportion of land-use types) and configuration (spatial arrangement of land-use types) on heterogeneous landscapes [12]. For trajectory analysis we implemented three steps: i) identification of urban expansion cells: the metropolitan area of Cordoba city was divided into a regular square grid of 1 km^2, classified on soil sealing classes (very low, low, medium and high) extraction of urban expansion cells for further statistical analysis; ii) landscape metrics calculation: four landscape metrics were selected to estimate landscape composition and configuration: the percentage of landscape covered by built-up structures (PLAND), the patch density (PD), the edge density (ED), and the mean patch area (AREA_MN) [13,14]. Landscape metrics were computed by a moving window (200 m radius) for each urban expansion cell and soil sealing class. Differences in urban composition and configuration (PLAND, AREA_MN, PD, and ED) over time were estimated using a nonparametric bootstrapping test of the mean cell values [13]; iii) trajectory space: changes in the relation between composition (PLAND) and configuration (PD, ED, AREA_MN) were explored by projecting the mean values of each time step (T_0, T_1, T_2) into specific Cartesian spaces of composition *vs.* configuration metric values.

Results and discussion

The UEXT algorithm implemented on SAR images with VHR resolution allowed to map urban areas of Cordoba city with very high accuracy with overall effectiveness of urban extraction greater than 90% and almost perfect reliability ($K \geq 0.82$). The city of Cordoba has undergone an intense process of urban expansion, with new built-up structures reaching almost 9 km^2 (Figure 1). The confusion matrices of urban expansion maps revealed high accuracy values as well with OA greater than 90% and $K \geq 0.73$. The number of cells of very low and low soil sealing categories consistently decline over time as those of medium and high increased (Figure 2). The landscape composition and configuration of the different sealing classes defined at T_0 (Very Low, Low, Medium, High) significantly changed (Figure 3). PLAND and AREA_MN significantly increased evidencing a steady growth of urban cover on all the soil sealing classes. ED and PD presented comparable mean values due to their parabolic behavior in relation with the ongoing urban cover increase. Furthermore, trajectory analysis of urban areas referable to different soil sealing classes at T_0 (mean landscape metric values of Very Low, Low, Medium, High urban classes), evidenced urban sprawl, expansion and filling processes occurring on all the analyzed contexts. Areas with very low and low soil sealing faced edge-expansion and outlying processes while medium and high ones were affected by infilling urbanization. Cordoba city has undergone during the last decade a consistent process of urban expansion along with steep variations on landscape composition and configuration. Such changes are most likely related with population growth and migratory movements towards large cities [15]. The spread of Cordoba city mainly occurred by edge-expansion and outlying processes occurring in peri-urban areas. The CSK and CSG SAR images effectively supported urban mapping with very high accuracy values, similar to what has been achieved in other cities and different environmental conditions [4, 7]. SAR derived multi-temporal urban maps effectively describe urban landscape dynamics and the relation between urban expansion and changes

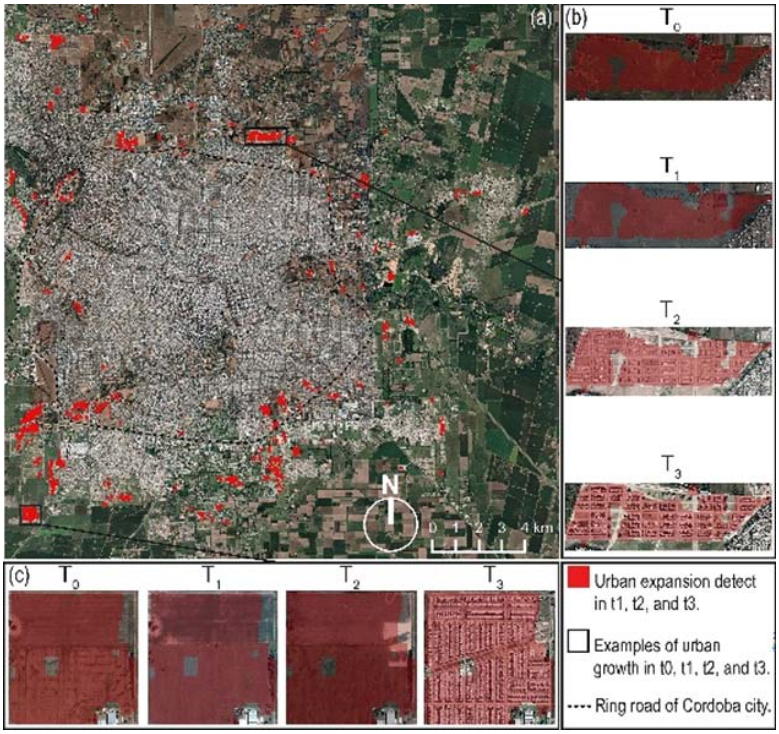

Fig. 1. Urban expansion map in timeframe 2010-2021 (a) and on two examples of expansion processes. (b) UrbExpt2 (c) UrbExpt3.

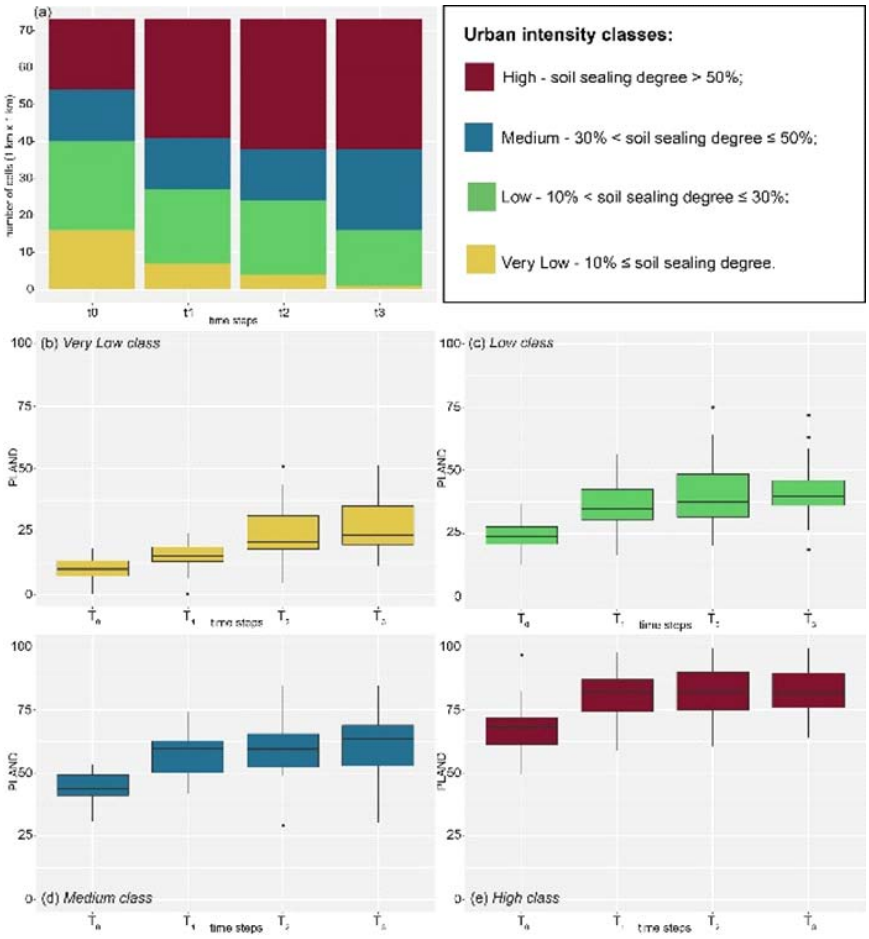

Fig. 2. (a) Number of urban expansion cells categorized by urban intensity classes. The boxplots report the percentage of landscape covered by built-up structures (PLAND) over time on cells labelled a T0 on urban intensity classes. (b) Very Low, (c) Low, (d) Medium, and (e) High.

in composition and configuration. Our results underline the irreversible expansion of built-up cover in all soil sealing classes over the 2010-2021 timeframe. Edge- and outlying expansions, mainly due to new low-density residential zones in the suburbs with scattered development or new built-up agglomerations in the peri-urban areas, were identified for lower soil sealing classes (Very Low, Low classes). In these classes, the sprawling development may reduce urban sustainability due to the increased reliance on the private transportation, high energy use, increment in pollution and traffic congestion and the decline in the sense of community and cohesiveness [19]. In parallel, the infilling process, mainly occurred inside Cordoba city ring road on areas with high soil sealing degree (Medium and High classes), may reduce the ecological connectivity of the city, promoting aggregated and compact urban pattern in which the built up matrix constitutes a barrier for a large number of species.

Understanding urban transformation processes offers key information for defining sustainable planning and management strategies. For instance it may support sustainable planning at different scales (from single settlements to the entire city and peri-urban areas), aiding decisions concerning where and when new urban areas can be built up preserving ecosystems quality, and social benefits [21]. The sustainable management of urban areas could be aided by the integration of effective tools of landscape analysis with easily reproducible mapping approaches like RS analysis.

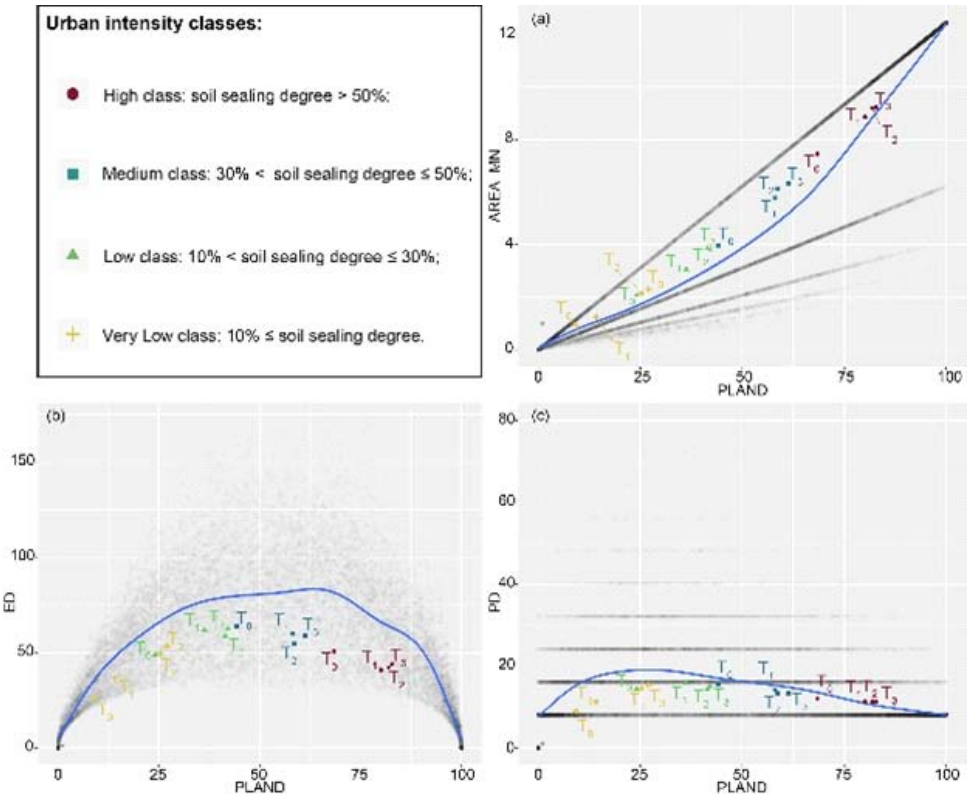

Fig. 3. Trajectory analysis for the different urban intensity classes defined at T_0. Cartesian relationship spaces report measured configuration metrics (grey dots) in relation to urban cover, along with the relative fitted curves (blue line) and the mean metric values (colored dots) for each time step (T_0, T_1, T_2, T_3). PLAND: percent of urban area, AREA_MN: Mean patch area, ED: Edge density, PD: patch density.

Acknowledgements
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Chloroplast DNA Variation and Differentiation across European taxa of white oaks (*Quercus* L.)

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Chloroplast DNA (cpDNA) variation was studied in European white oaks (*Quercus*, subgen. *Quercus*, sect. *Quercus*) from a vast area (11 countries) of central and southern Europe (including one north African population) to reconstruct phylogenetic relationships between maternal lineages and clarify the biogeography and the history of populations and taxa.
Roughly 2000 trees from 90 populations representing 13 taxa (*Q. banatus* P.Kucera, *Q. congesta* C.Presl, *Q. dalechampii* Ten., *Q. faginea* Lam., *Q. frainetto* Ten., *Q. ichnusae* Mossa, Bacch. & Brullo, *Q. leptobalanos* Guss., *Q. petraea* (Matt.) Liebl., *Q. petraea* subsp. a*ustrotyrrhenica* Brullo, Guarino & Siracusa, *Q. pubescens* Willd., *Q. pyrenaica* Willd., *Q. robur* L., *Q. virgiliana* (Ten.) Ten.) (Fig. 1) were sampled, and a total of 270 individuals (3 trees for each stand) were selected and investigated for the chloroplast genome analyses.
To date, all sections of *Quercus* genus have been studied using the maternally inherited chloroplast genome [1]

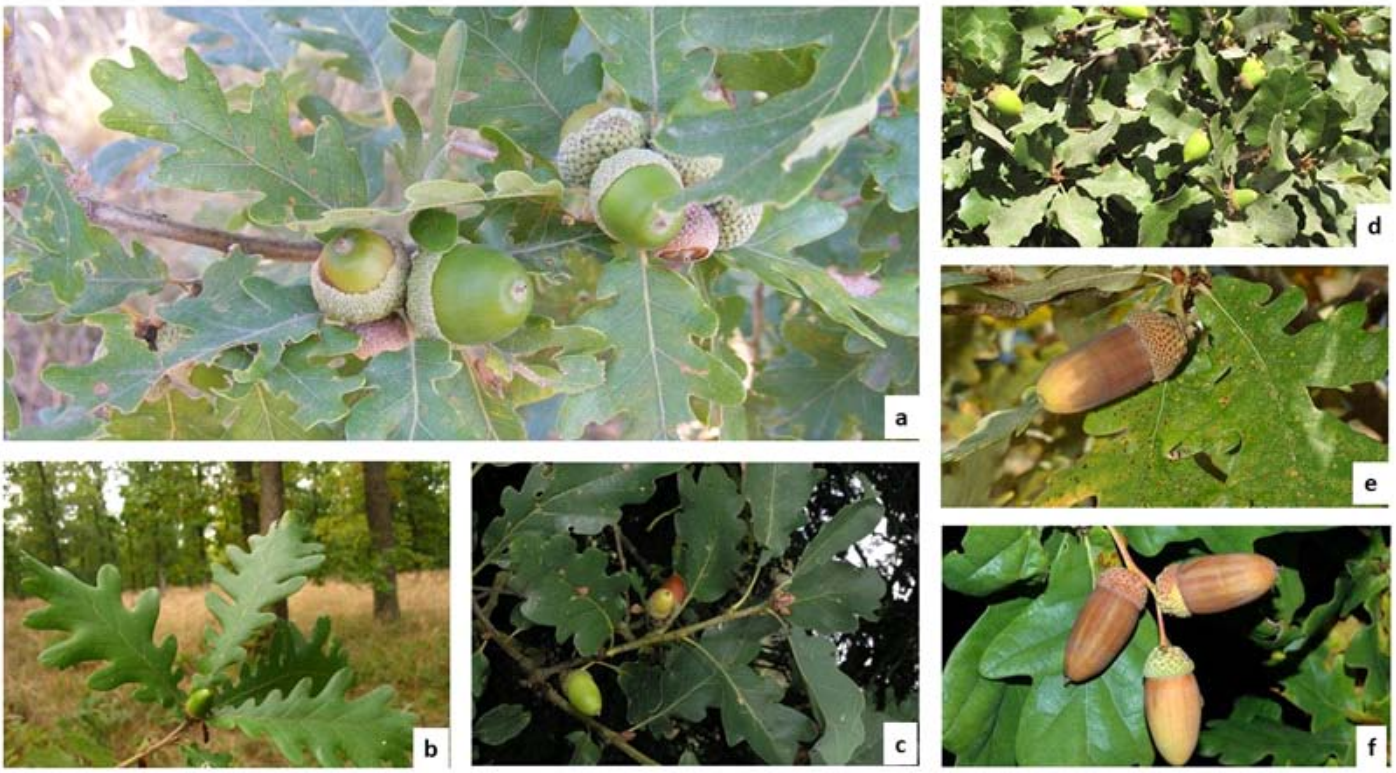

Fig. 1. Species of Quercus genus (subgen. Quercus, sect. Quercus) studied. a) Q. pubescens Willd. b) Q. frainetto Ten. c) Q. petraea (Matt.) Liebl. d) Q. faginea Lam. e) Q. pyrenaica Willd. f) Q. robur L.

to investigate their evolutionary histories. However, in the Euro-Mediterranean region, recent and detailed reconstructions are available only for section *Ilex* and section *Cerris*, while for section *Quercus*, no updated data are now available.
Therefore, this study was carried out to:

- reconstruct phylogenetic relationships between plastid DNA variants in the European white oaks

- improve our knowledge on the genetic structure and the diversity of populations
- capture rare genetic variants that are important because they could subtend divergent evolutionary lineages

To achieve these goals, the genomic DNA of 270 samples was analysed by means of DNA sequence polymorphism at two plastid loci: the trnH-psbA intergenic spacer and a portion of the trnK-matK region (3' intron and partial gene).

These markers were selected because of their high number of accessible sequences on GenBank, and the variability displayed in previous studies [2-5]. DNA sequences were amplified using PuReTaq Ready-To-Go PCR Beads (Cytiva) and purified PCR products were sent to Macrogen Europe [6] for sequencing. Electropherograms were edited with CHROMAS 2.6.6 [7] and checked visually. Multiple alignments and pairwise uncorrected p-genetic distances (p) were produced using MEGA 10.2.6 [8]. DNAsp 6.12.03 [9] was used to generate haplotype lists and to analyse and calculate the number of haplotypes (H), the haplotype diversity (Hd), the number of polymorphic sites (S), the nucleotide polymorphism (θw), the nucleotide diversity (π), and to check the number of Parsimony Informative Characters (PICs).

NETWORK 10.2.0.0 [10] was used to reconstruct phylogenetic relationships between cpDNA haplotypes and to make inferences about biogeography and history of populations. In addition, a planar (equal angle, parameters set to default) phylogenetic network was generated using Neighbour-Net (NNet) algorithm implemented in SPLITSTREE 4.18.3 [11].

Both marker loci were successfully amplified and sequenced in the 270 investigated samples. Consequently, the final dataset included 540 plastid DNA sequences (270 samples, two markers each).

The main features and the diversity values of the two investigated markers were analysed comparing all the obtained sequences (section *Quercus*) with all the sequences of the same loci retrieved from GenBank, belonging to section *Cerris* and section *Ilex* (subgen. *Cerris*). As expected, the trnH-psbA intergenic spacer was the most variable marker region in comparison with trnK-matK in all the three *Quercus* sections investigated. Although the number of species and samples compared was different, section *Cerris* and section *Ilex*, which were the less numerous in terms of samples, showed the highest values of diversity. Haplotypic diversity measures within the samples of this study followed the same profile of the two sections of the subgenus *Cerris*. More in detail, respectively in trnH-psbA and trnK-matK, the values of the uncorrected p-distance ranged between 0.00 and 0.0058 and between 0.00 and 0.0032, the number of haplotypes (gaps included) (H) was 9 and 5, the haplotype diversity (Hd) varied between 0.7713 and 0.5557, the number of polymorphic sites (S) was 9 and 4, the number of Parsimony Informative Characters (PICs) was 4 and 2, the nucleotide polymorphism (θw) ranged between 0.00157 and 0.00052, and the nucleotide diversity (π) had values of 0.00128 and 0.00084.

Site variation observed in the 270 analysed samples resulted in fourteen total haplotypes (H01-H14). Nine haplotypes (T01-T09) were observed with trnH-psbA, and five (K01-K05) were identified with trnK-matK. No species-specific haplotypes were detected, consequently different haplotypes were shared by several species of *Quercus* genus.

Considering the total chlorotypes, haplotypes H01-H04 were the most common across the area of study and the species analysed. Chlorotype H01 was the most prevalent with a frequency of 26.67% in total. The second most frequent chlorotype was the haplotype H02 with a frequency of 22.59%. Chlorotype H03 and H04 followed with a frequency of 16.3% and 11.48%, respectively. The remaining chlorotypes presented lower frequencies. Six haplotypes were rare (H05; H10-H14) with frequencies less than 1.12%.

Most populations were fixed for one haplotype, although fourteen out of ninety populations contained two or three different haplotypes. Furthermore, while it was not found a relation between species and haplotypes, a more significative and direct association was recognized with the geographic distribution, especially for some chlorotypes. More in detail, haplotypes H01 and H02 were the more abundant and they were found in central eastern Europe, in Austria, Croatia, Czech Republic, France, Italy, Romania the first one, and in Bulgaria, Croatia, Greece, Italy, Serbia the second one. Haplotype H03 was found in the Italian Peninsula, including Sicily and Sardinia Islands, and in Spain. Haplotype H04 showed a discontinuous distribution, and it was found across the Italian and Iberian Peninsulas, in Morocco and in Bulgaria; a single Bulgarian sample showed this haplotype, so it was re-sequenced four times, always confirming the same results. Haplotype H05 was exclusive of Croatia. Haplotype H06 was distributed across all the Italian Peninsula, as well as in Sicily Island. Haplotypes H07, H08, and H09 were

found only in Italy: in Sardinia and in Calabria, in Calabria and Sicily, and in Sardinia, respectively. Haplotypes H10, H11, H12, H13, H14 were exclusive of one single sample represented respectively, by a sample of *Q. ichnusae* from Sardinia, a sample of *Q. virgiliana* from Sicily, a sample of *Q. pubescens* from Apulia, two samples of *Q. faginea* from Spain. Focusing on the Italian Peninsula, 11 plastid haplotypes (H01-H04; H06-H12) were detected in the 60 populations sampled.

In the median joining network based on the combined plastid loci (trnH-psbA and trnK-matK) one or two mutations differentiated the 14 chlorotypes, providing evidence that the populations and the species of section *Quercus* were little differentiated, thus they probably diverged rather recently.

Although lineages (e.g., clusters of highly different haplotypes) cannot be identified, because of the low rate of mutations separating each haplotype, two major groups can be assumed on a geographic basis. The first includes haplotypes spread through Italian Peninsula, Austria, Czech Republic, France, and Balkan Peninsula, and the second is represented by the haplotypes from Italian (major islands included) and Iberian Peninsulas, and by one Bulgarian sample.

More evident and consistent is the separation in clusters observed in the median joining network built for the cpDNA sequences belonging to different sections of subgenus *Quercus* (*Quercus*, *Ponticae*, *Virentes*, *Lobatae*, *Protobalanus*) with the addition of one sample of *Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H.Oh as outgroup, all retrieved from GenBank. Seven main clusters attributable to the main sections and to the outgroup can be easily recognized, confirming that the variability present is subdivided according to major evolutive and geographic patterns.

In fact, the main differentiation appears between American and Eurasian sections in the complex. The highly differentiated plastomes within North American sections are highlighted by the high number of mutational changes between different haplotypes in comparison with the plastomes of the European sections.

The low differentiation observed within the Eurasian oaks of section *Quercus*, even between members from western Eurasia and East Asia is remarked in the Neighbor-Net analysis.

From the Splitstee graph is clearly recognizable the split between North American and Eurasian sections, *Quercus* and *Ponticae*. New World and Old World members of the same evolutionary lineage, i.e., sections *Quercus* and *Ponticae*, are always placed apart from each other in recent plastome trees [12, 13], indicating that there must have been a geographic differentiation in the primordial members predating divergence and manifestation of modern taxa.

All the members of the outgroup set are clearly separated and well differentiated in the Neighbor-Net.

This study allowed to confirm that the modern Eurasian section *Quercus* species diverged rather recently [14]. Supposedly their progenitors have only relatively recently distributed across Eurasia, hence, their little differentiated plastome, which was probably highly exchanged across the ancestral forms. Moreover, the large population sizes and the initial climate and ecological stability may have contributed to reduce the genetic diversity in the plastome of the original stock. In more recent times, human pressure (selection, coppicing) may still have contributed to drift. In addition, recent interspecific chloroplast capture cannot be excluded, so in future perspective of this study, nuclear data may be deepened to shed light on this.

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Lepidoptera Diversity in Mediterranean Coasts: Preliminary Results in the Central Adriatic Dunes

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Introduction

Human pressure with habitat destruction, biological invasions and climate change [1-3] are drastically altering entomological biodiversity [4] and pioneering studies have evidenced a relation between habitat alterations and butterfly and moth diversity [5-7]. Among natural ecosystems characterized by a highly specialized entomofauna, coastal dunes, conformed by a complex mosaic of unique habitats, can host numerous species of Lepidoptera [8-10]. Despite the known link between insects and natural vegetation, studies dealing with Lepidoptera communities and coastal dune vegetation are still partial, and on the central Adriatic Sea such studies are still absent. This research aims to explore the biodiversity of diurnal and nocturnal Lepidoptera communities in relation with coastal dune zonation in the Central Adriatic coast (N2K IT7228221 site: Foce Trigno-Marina di Petacciato).

Materials and methods

Vegetation and Lepidoptera communities were sampled according with Long Term Ecological Research (LTER) protocol, which determined data collection across the environmental gradient between seashore and backdune [11] with three replicates. Vegetation sampling was carried out following a random stratified protocol on 4x4m plots distributed on the main habitats across the dune zonation. On each plot, the complete list of vascular plants was recorded and their relative abundance estimated. Plants were identified at species level following the Flora d'Italia [12]. For Lepidoptera specimens, moths were collected using light traps equipped with UV LEDs [13], while butterflies were sampled by entomological net during day-time following Butterfly Monitoring Schemes (BMSs) [14]. Lepidoptera data was collected every 15 days (from December 2021 to November 2022), on shifting/transition dunes) and backdunes (Fig. 1-2). The preparation of specimens was carried out in the Envix-Lab Termoli (UniMol). The identification was done by comparing specimens with the collection of the Department of Agriculture, Environment and Food (UniMol) in Campobasso and with scientific illustrations. For the identification of some moths the extraction and detailed analysis of genitalia were needed.
Vegetation data was analyzed by multivariate classification techniques followed by the interpretation and assignment of each group to relative Habitat of European concern (http://vnr.unipg.it/habitat/).

Preliminary results and discussion

Vegetation analysis evidenced the presence of several habitats (*sensu* 92/43/EC Directive) of dune zonation: 2110-Embryonic shifting dunes; 2120-Shifting dunes along the shoreline with *Ammophila arenaria*; 2230-*Malcolmietalia* dune grassland; 2260-Cisto-Lavanduletalia dune sclerophyllous scrubs and 2270-Dunes with forests of *Pinus pinea* partially invaded by *Acacia saligna.* We found 100 species of vascular plants belonging to 35 families, among which the most abundant are Poaceae, Asteraceae and Fabaceae.
Concerning Lepidoptera, preliminary results showed a consistent number of individuals with a higher number of specimens on backdune with Mediterranean maquis (841) in comparison with those recorded in shifting and transition dunes with beachgrasses (466). This difference could be due to the presence of perennial vegetation, structurally more complex and with greater biomass that supports a greater diversity of Lepidoptera.
The taxonomic identification work is in progress, and the preliminary results obtained on the 50% of the specimens (collected on twenty-four samplings of nocturnal Lepidoptera and ten of diurnal) are attributable to 20 families (14 nocturnal and 6 diurnal) and 127 species (107 nocturnal and 20 diurnal).
It is interesting that the family Noctuidae has the largest number of species (52) followed by the family

Fig. 1. Lepidoptera sampling design in Petacciato Marina using UV-LEDs light traps and entomological nets in 3 sites in the shifting and backdunes.

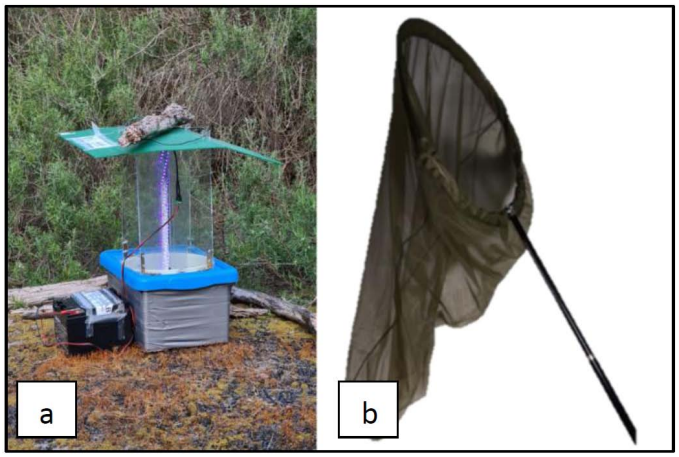

Fig. 2. Lepidoptera sampling methods. (a) UV-LEDs light trap for nocturnal moths consists of a 2.5 m long ultraviolet strip (12 volts and ~15 W) positioned on a cylindrical PVC support framed by transparent Plexiglas plates. The structure is mounted on top of a container covered by a funnel. The traps are powered by a portable battery. The adult moths attracted and flying towards the light, knock on the Plexiglas surface and fall through the funnel into the underlying container equipped with egg cartons and a bottle containing tetrachloroethane to narcotize insects; (b) The entomological net used consists of a handle about 1 m long and a circular steel support with a diameter of 35 cm on which a tulle bag is installed.

Geometridae (25) (Tab. 1) (Fig. 3).

The high number of Lepidoptera species recorded in correspondence of dune vegetation may be likely related with the well preserved complex dune zonation which provides a wide variety of niches for the different life stages. Knowing the relation among Lepidoptera and dune vegetation is essential to establish which habitats and sites deserve of conservation actions. Moreover, Lepidoptera species associated with coastal habitats might be used as indicator taxa to monitor the conservation status of coastal dunes.

Tab. 1. Number of nocturnal and diurnal Lepidoptera species identified so far (out of 50% of specimens caught).

NOCTURNAL LEPIDOPTERA		DIURNAL LEPIDOPTERA	
Family	Number species	Family	Number species
Noctuidae	52	Nymphalidae	7
Geometridae	25	Lycaenidae	5
Erebidae	6	Pieridae	4
Pyralidae	6	Hesperiidae	2
Sphingidae	4	Papilionidae	1
Tortricidae	4	Zygaenidae	1
Cossidae	2		
Lasiocampidae	2		
Drepanoidea	1		
Notodontidae	1		
Alucitidae	1		
Pterophoridae	1		
Yponomeutidae	1		
Tineidae	1		
N families = 14	N tot species = 107	N families = 6	N tot species = 20

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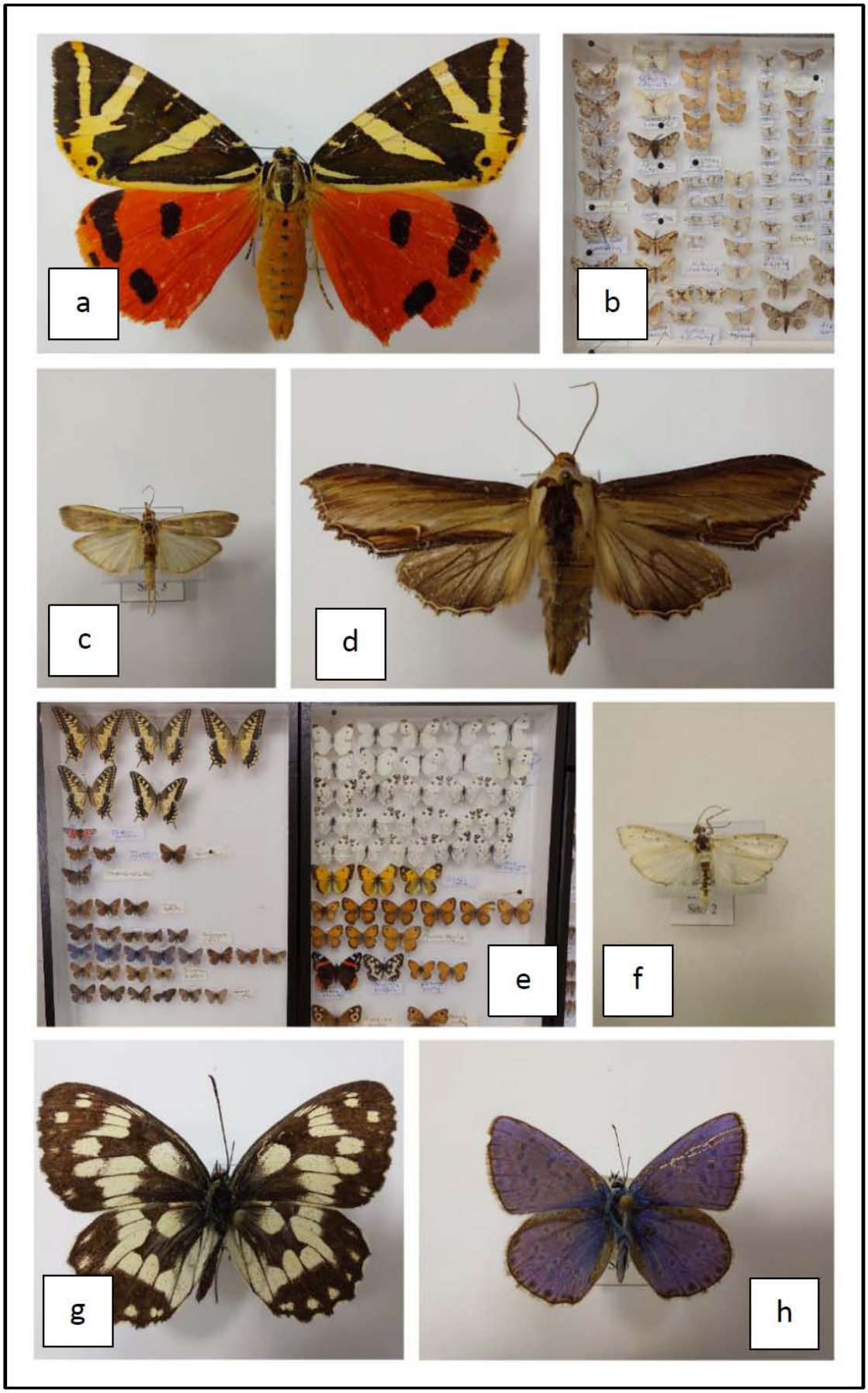

Fig. 3. Some specimens of Lepidoptera sampled in the study area. (a) Euplagia quadripunctaria *Poda, 1761 [Erebidae]; (b) Entomological boxes containing specimens of the Geometridae family; (c)* Etiella zinckenella *Treitschke, 1832 [Pyralidae]; (d)* Cucullia verbasci *Linnaeus, 1758 [Noctuidae]; (e) Entomological boxes containing diurnal specimens; (f)* Myelois circumvoluta *Fourcroy, 1785 [Pyralidae]; (g)* Melanargia galathea *Linnaeus, 1758 [Nymphalidae]; (h)* Polyommatus bellargus *Rottemburg, 1775 [Lycanidae].*

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Invasive Alien Plants (IAPs) in Mediterranean mountains: the first MIREN survey in Central Apennines

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Introduction

High mountain ecosystems are characterized by a highly specialized biodiversity which provides crucial ecosystem services. These environments are threatened by ongoing global changes, such as climate and land use changes [1]. Plant communities inhabiting mountain areas are dynamic and both, native and exotic species, are shifting their distribution range upwards due to global warming [2]. The Invasive Alien Plants (IAPs) are species whose introduction and/or spread outside their natural distribution threaten biological diversity [3].

Some evidences suggest that mountain roads are important dispersal corridors towards higher mountains and propagules' reservoirs of alien species coming from the surrounding lowland and more inhabited areas. Thereby, mountain roads allow studying patterns of distribution of alien species and their potential for invasion into high-elevation habitats [4].

Recent research evidenced the rising concern of IAPs impact on mountain ecosystems [5-7] and among them, it is noticeable the great contribution of the Mountain Invasion Research Network (MIREN; http://www.mountaininvasions.org/). MIREN, founded in 2005, constitutes the first global attempt to implement a standardized research framework of plant invasion ecology in mountain environments. The core of the MIREN protocol, focused on vegetation monitoring along mountain roads, is easy to implement across the world and can be repeated over time [4].

The Mediterranean region, characterized by high levels of plant diversity with a relevant endemism rate, constitutes one "biodiversity hotspot" [8]. Across the Mediterranean region, several mountain ranges represent "hotspots within the hotspot" due to their geological, biogeographic and climatic history. Yet, these mountains are experiencing significant changes in biodiversity for their sensitivity to land-use changes and are threatened by the ongoing climate change [9]. Although there is a large number of published research that deals with IAPs occurrence and spread in different ecosystems, only few experimental studies analyze their elevation range and ecological impact in mountain environments [10].

In this context, our work aims to analyze the distribution of IAPs in mountain ecosystems of Central Apennines, establishing the first MIREN monitoring site in the Mediterranean basin and in Italy.

Methodology

We selected two roads that extend over a wide elevation range (around 500-2000 m a.s.l.) in Maiella National Park (MNP), and in Gran Sasso- Monti della Laga National Park (GSMLNP). Vegetation sampling was carried out into 20 sites evenly spaced from the lowest to the highest altitude along each road (Fig. 1).

At each site, we sampled 2 plots of 2m x 50m, arranged in a "T" shape (Fig. 2) during the period between mid-May 2022 to mid-July 2022, for a total of 80 plots. All the vascular plant species were recorded, and their abundance and cover percentage were estimated. Species identification was carried out in EnvixLab (UniMol), at the "Centro Ricerche Floristiche dell'Appennino" of GSMLNP, and at "Majella Seed Bank".

In the last week of September 2022 we came back to the sampled sites looking for species with autumn bloom. Nomenclature follows Flora d'Italia [11]. The status of alien or native plant species has been defined according to Galasso et al. 2018 [12].

Results

We recorded more than 600 plant taxa of which 22 are alien plant species.

32 % of alien plant species belong to Poaceae taxonomic family, 23 % to Asteraceae and 9 % to Fabaceae.

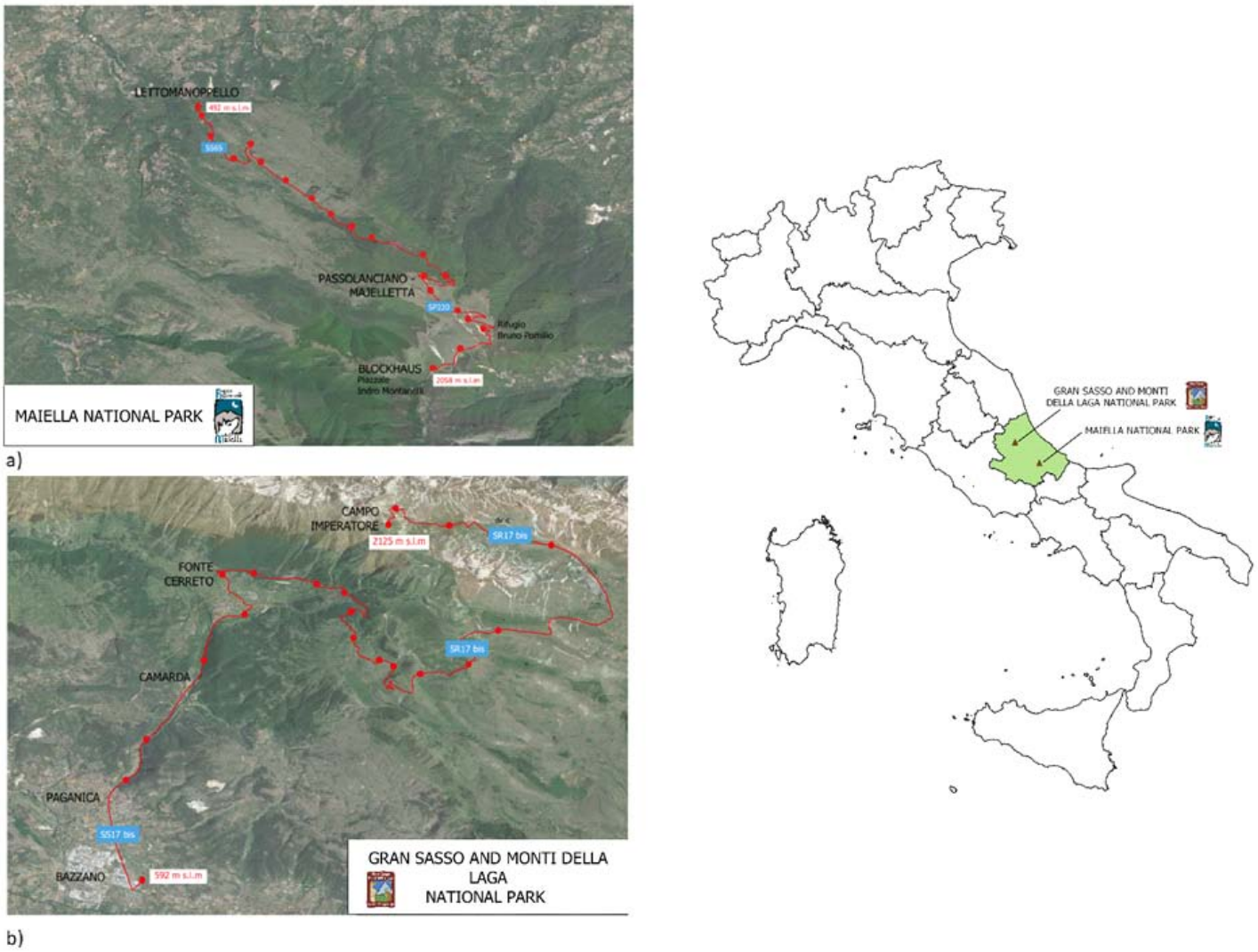

Fig. 1. Roads in the Maiella National Park (a) and in the Gran Sasso and Monti della Laga National Park (b) along with 20 sampling sites are located (red circles).

As for the geographical area of origin, 27% are of Euro-Asian origin, 23% come from the Asian continent, 23% from the American continent of which 3 species from North America and 1 from South America, and 10 % have African origin.

The preliminary results indicate as the most frequent and abundant alien plant species: *Ailanthus altissima, Erigeron canadensis, Senecio inaequidens* and *Robinia pseudoacacia*.

Ailanthus altissima reaches the highest altitude at 1195 m a.s.l. and this insight is consistent with what was recorded in Croatia [13] and Spain [14]. On the other hand, *Erigeron canadensis* doesn't exceed 870 m a.s.l in the study area, even if it was recorded at highest elevation in other mountains of the Mediterranean basin, reaching 1831 m a.s.l. in Croatia up to 902 m a.s.l in Slovenia [13, 15, 16]. *Senecio inaequidens* reaches 978 m a.s.l. in Gran Sasso and this data is consistent with what was recorded in Spain [17]. The maximum altitude recorded for *Robinia pseudoacacia* in Central Apennines is 1140 m a.s.l., as it was observed in Croatia [13].

Conclusions and future prospects

Our results evidenced that Central Apennines are already facing processes of introduction and dispersal of IAPs, and pointed out that mountain roads up to 1200 m a.s.l. are important sources and corridors for alien species propagules.

The obtained results may offer several research and applied insights. It offers the baseline for monitoring and better exploring invasion processes on Mediterranean mountain ecosystems and gives new information needed for implementing efficient management strategies able to mitigate IAPs spread in mountain environments.

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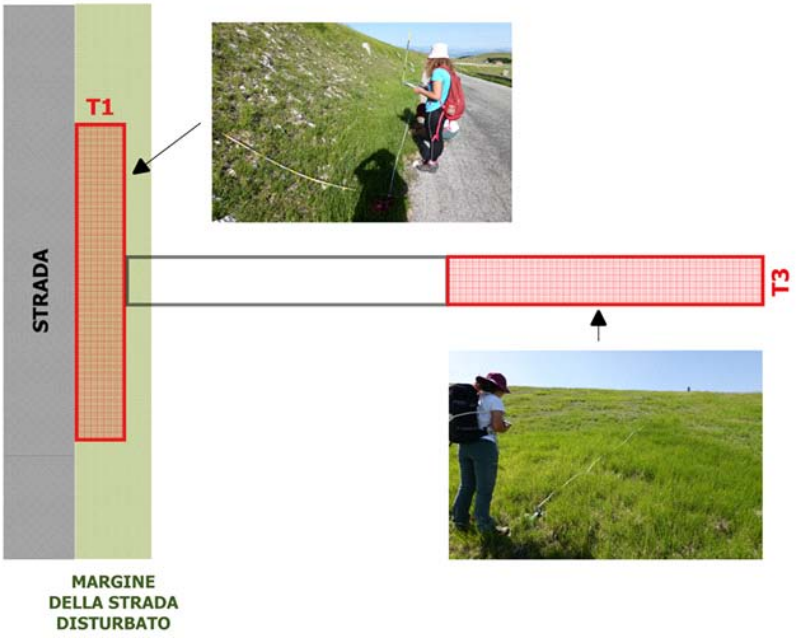

Fig. 2. Each sample site consists of 2 plots of 2 m x 50 m (in red): T1 - parallel to the roadway (including the first vegetation strip on the roadside); T3 - perpendicular and centered 50 m far from T1.

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What happened in Molise coastal wetlands after ecological restoration actions?

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Introduction

Coastal wetlands, distributed in the transitional areas between fresh and marine waters, are complex ecosystems hosting stress tolerant and well adapted plant species [1, 2]. However, coastal marshes are among the most threatened by global change [3]. For such outstanding threatened biodiversity, wetlands are protected by the intergovernmental Convention of Ramsar [4] and in Europe, most of the salt marsh plant communities are Habitats of Community Concern (hereafter in the text: EU Habitats) and listed in the European Directive 92/43/EEC, for which restoration actions are claimed to keep them in a good "conservation status". In this contest, the present study aims to analyze temporal changes on plant species composition in a salt marsh mosaic in Central Italy after the implementation of restoration interventions.

Methodology

The study area is located in a Natura 2000 site (S.A.C. IT7222216 Foce Biferno-Litorale di Campomarino) in the Central Adriatic coast and it is characterized by brackish marshes, where a mosaic of EU habitats grows (Fig. 1). In 2016, this area was part of an ecological restoration program, funded by LIFE10 NAT/IT/00262 project, aimed at recovering the wetland ecosystem. Specifically, the water flow pattern was re-established by opening the artificial wetland drainages, the banks were reconstructed and a boardwalk and a set of picket fences were put in place to protect salt marshes from human trampling [5] (Fig. 1).

Fig. 1. Re-visitation of a vegetation plot (left) close to the boardwalk and the study area with the positive effects of restoration interventions: water flow pattern re-established and banks reconstructed (right).

The climate of the study area is Mediterranean and in the last 50 years has changed, with increasing temperatures and decreasing annual precipitations.

Ex-post monitoring was implemented by a re-visitation approach on 33 georeferenced historical vegetation plots collected in the years 2010 (T1, before restoration actions) and 2021 (T2, after restoration actions) (Fig. 1).

Vegetation plots are representative of the most important salt marshes EU Habitats: 1410: Mediterranean salt

meadows - *Juncetalia maritime*; 1420: Mediterranean and thermo-Atlantic halophilous scrubs - *Sarcocornietea fruticose* and 1510*: Mediterranean salt steppes – *Limonietalia*. In each relevé of 16 m^2 (4×4 m) we registered the complete list of vascular plants and their cover values in compliance with Braun-Blanquet scale [6], using the classical phytosociological approach (Fig. 1).
We investigated changes on each EU Habitat studied, supported by the bio indication value of three plant groups: diagnostic (typical species of native community), ruderal (opportunistic species favored by human disturbance) and alien species (non-native and invasive species) [7, 8].
We analyzed the temporal variation in richness and cover of the ecological groups by a Mann-Whitney post hoc test.

Results

We recorded 71 vascular plant species and subspecies in T1 and 54 in T2, 24 diagnostic species in T1 and 22 in T2, 21 ruderal plants in T1 and 10 in T2, finally 5 alien species in T1 and 4 in T2.
The temporal analysis of ecological groups revealed significant gains of diagnostic species cover in the salt meadows (P_{same} = 0.046) and steppes (P_{same} = 0.011) and a significant decrease of their richness per plot in the halophilous scrubs (P_{same} = 0.007) (Fig. 2).

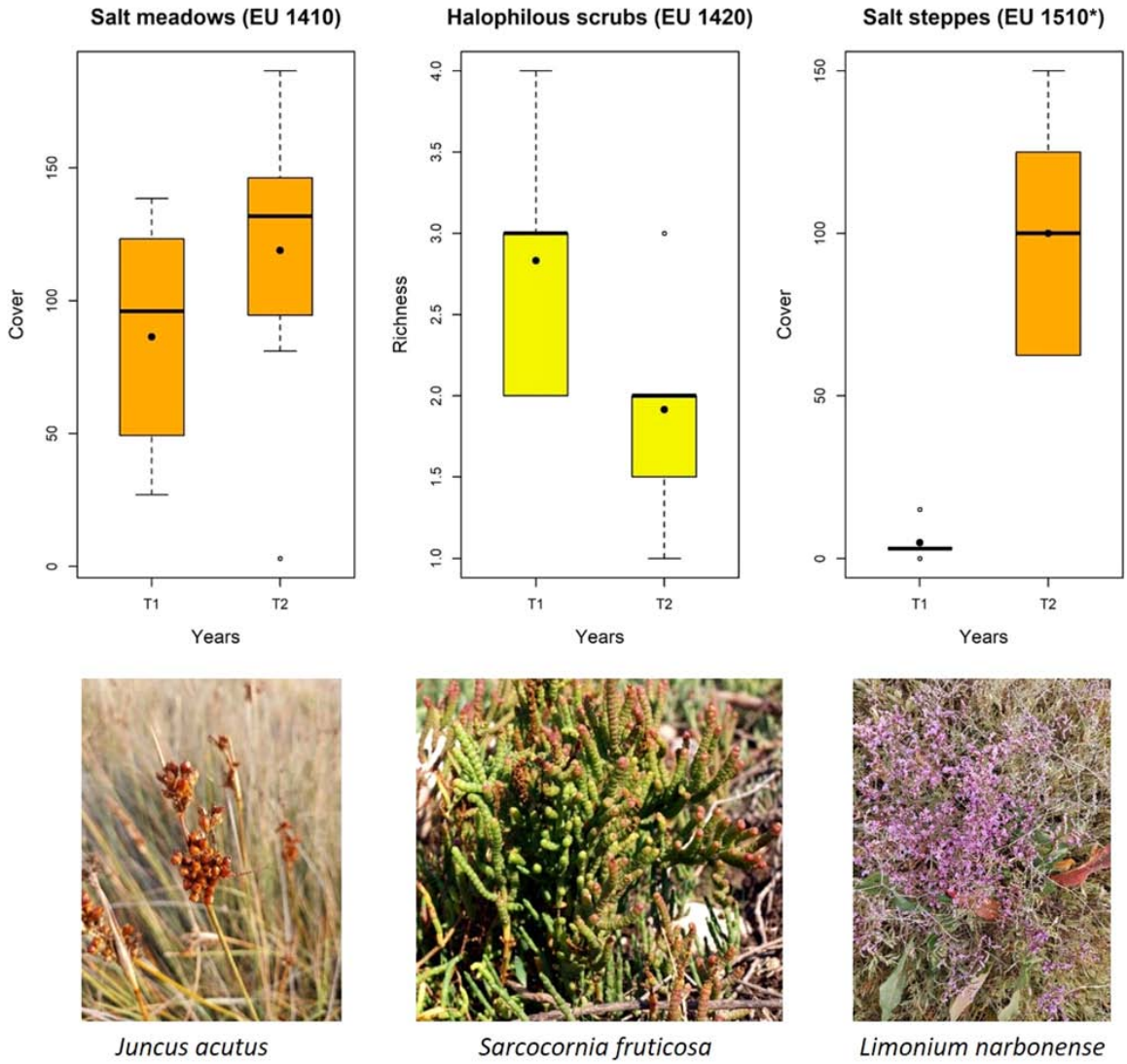

Fig. 2. Boxplot comparing cover (orange) and richness (yellow) for the diagnostic species in the two-time steps (T1: 2010 and T2: 2020/21) for the Salt meadows (EU Habitat 1410); Halophilous scrubs (EU Habitat 1420) and Salt steppes (EU Habitat 1510).*

As regards the ruderal species, we observed a significant decrease in cover and richness in halophilous scrubs (respectively P_{same} < 0.001 and P_{same} < 0.001) and salt steppes (respectively P_{same} = 0.025 and P_{same} = 0.025).

Concerning alien species, we found a significant decrease in cover in salt steppes (P_{same} = 0.038).

Discussion and conclusion

The significant increment of diagnostic species cover, along with the significant decrease of ruderal plants, could be related to the improvement of the environmental and ecological conditions after the restoration actions. Specifically, the gain in cover of the salt tolerant diagnostic species (*Juncus acutus*, *J. littoralis* and *Schoenus nigricans* in EU habitat 1410, *Halimione portulacoides* and *Limonium narbonense* in EU 1510* and *Sarcocornia fruticosa* in EU 1420), which have morphological and physiological adaptations to live on saline environments [9], is probably linked to the reconstruction of ponds and wetland. While, the rarefaction of ruderal and alien species (e.g. *Arundo plinii*, *Melilotus albus* and *Vicia sativa*) could be likely due to the decrease of human trampling disturbance prevented by dedicated path (boardwalk) for tourists and visitors of the area [10].

As observed in other wetlands of America [11-13] and Europe [14], also in the Adriatic coast, after adequate restoration interventions and the reduction of human pressure, species composition of each habitat type quickly tends to recover the typical halophytic species assemblage [15]. Moreover, the outcomes of our study suggest that vegetation dynamics monitoring could be used to study coastal restoration trajectory in the mid- and long-term timespan. The applied re-visitation approach, based on historical plots, represents a cost-effective monitoring procedure that matches the need of periodical reporting, requested by the European Directive roadmap. Finally, coastal wetlands play a key supporting role for animal biodiversity as they provide critical habitats for resident and migratory fauna [16] and a wide range of ecosystem services as nutrient cycling, flood control, carbon sequestration and climate regulation [17], consequently their protection is considered fundamental at global scale.

Acknowledgements

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Functional diversity in high mountain vegetation. What happened in Central Apennines during the last 20 years?

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Introduction

During the last few decades, there was an increased considerable interest in functional ecological research to analyze the distribution and fitness of natural communities across environmental gradients and to better understand community assembly rules, a key factor in assessing the effects of global change on biodiversity [1, 2]. Plant communities with high functional diversity values are considered better adaptable to environmental changes, whereas, in community with lower functional diversity, species coexistence may be ruled by harsh environmental factors that promote species convergence towards a few well-adapted and redundant functional traits [3].
The present work explores temporal changes in functional diversity on high mountain vegetation occurred during the last 20 years in Central Apennines, based on a Vegetative Plant Height (H), commonly used for depicting processes of competition [4, 5].
In particular, we focused on the following questions: (i) Does functional diversity based on plant height vary across the different plant communities? (ii) How that functional diversity vary over time?

Materials and methods

We analyzed four high mountain vegetation types, widespread in the Italian Apennine's alpine belt and referable to two Habitats of Conservation Concern in Europe (EU8120: Calcareous and calcshist screes of the montane to alpine levels, and EU6170: alpine and subalpine calcareous grasslands): *Galium magellense* community growing on screes, *Trifolium thalii* community of snowbeds, *Sesleria juncifolia* community of steep slopes and *Carex myosuroides* community of windy edges.
The study was carried out in the Maiella National Park (MNP) by a re-visitation study of a set of 55 georeferenced vegetation plots firstly collected in 2003 (hereafter T1) and resampled in 2020/2021 (hereafter T2).
Plant height was measured on the most abundant species (e.g. taxa whose cumulative cover ordered from the most to the least abundant reach the 80% of vegetation cover). The set of measured taxa included 67 species and subspecies. Species trait values were partially retrieved from recent measurements performed in the study area [6] and by dedicated field measurements, conforming the standardized protocol [7, 8]. During the 2020 and 2021 summer seasons, we measured Vegetative Plant Height (H, cm) for at least 10 different individuals of each species (Fig. 1).
We calculated the community-level functional diversity (FD) for this trait across the four plant communities with the Rao's quadratic entropy [9]. Then, to quantify how much the observed pattern deviated from a random distribution, we calculated the standardized effect size (SES) for FD. Finally,

Fig. 1. Field measurements for measuring H: Vegetative Plant Height.

we explored the variations in the SES-FD for H across the considered plant communities in the two time steps with a one-way ANOVA followed by Tukey's post hoc test.

Results

Our study highlighted that functional diversity for plant height vary across the different plant communities and a change in the pattern of functional diversity among the high mountain communities in Central Apennines occurred over time.

In particular, in T1 three of the four plant communities were characterized by a low functional diversity values of plant height (functional convergence), with the exception of the *Carex myosuroides* community which had a significant functional divergence (Fig. 2). After 20 years, the functional diversity for this trait has been maintained in *Carex myosuroides* community, but has been increased in *Sesleria juncifolia* and *Trifolium thalii* communities. *Galium magellense* community (scree vegetation), instead, has retained its low functional diversity over time.

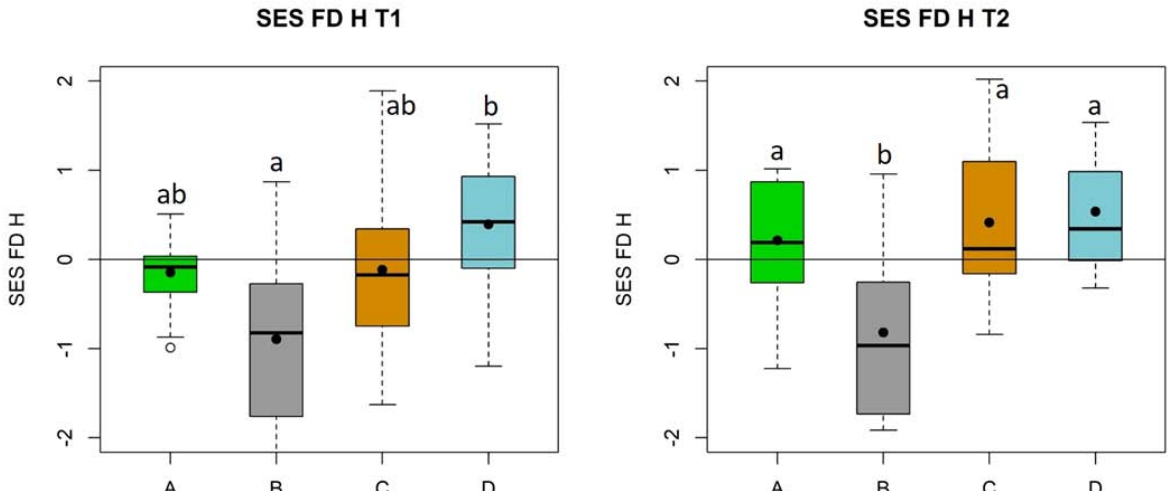

Fig. 2. Boxplots comparing the analyzed plant communities in terms of standardized effect size functional diversity for plant height (SES-FD-H) in two time steps (T1: 2003, T2: 2020/21). A: Trifolium thalii community (snowbeds), B: Galium magellense community (screes), C: Sesleria juncifolia community (steep slopes), D: Carex myosuroides community (windy edges). Boxplot letters indicate statistically significant differences among communities after Tukey's post hoc test ($p < 0.05$).

Discussion and conclusions

Carex myosuroides community showed a significant functional divergence both in T1 and T2. This vegetation type grows on windy ridges, where the growing season is short and functional diversity in terms of plant height and leaf traits allows to better exploit resources in the short time, as recorded in previous research [2, 6].

The functional diversity of *Trifolium thalii* community (snowbed vegetation) increased in the last 20 years and this is likely due to the general vegetation cover increase recorded in this habitat overtime [10]. Snowbed plant species with different size (e.g. *Plantago atrata subsp. atrata*, *Taraxacum apenninum*, *Anthyllis vulneraria subsp. pulchella*, *Trifolium pratense subsp. semipurpureum*, *Phleum rhaeticum*) experienced that cover increase in the last decades. Moreover, an expansion of thermophilic herbaceous species (e.g. *Helictochloa praetutiana subsp. praetutiana* and *Astragalus depressus subsp. depressus*) was recorded [11].

Surprisingly, functional diversity also increased in *Sesleria juncifolia* community, even if this stripped grassland has undergone a significant decrease in species richness in the last decades [12]. We can hypothesize that there was a high functional redundancy for plant height in T1 and the current species loss was offset by the cover increase of several remnant species, different in size such as *Sesleria juncifolia subsp. juncifolia*, *Aster alpinus subsp. alpinus* and *Salix retusa*.

Conversely, *Galium magellense* community, growing on screes, has kept low functional diversity over time. The stable low functional diversity values in the scree vegetation should be related to the constraints exerted by the limiting environmental conditions that characterize this habitat (incoherent substrate, abundant limestone debris and undeveloped, dry, and poor in organic matter soils) with sparsely vegetation distributed and intermingled with bare surfaces [13].

In conclusion, we observed that most of investigated high mountain plant communities is facing global change effects increasing the functional diversity in term of plant height, as the functional divergence likely allows to take advantage of longer vegetative period but with warmer and drier summer seasons, that have been recently recorded for Mediterranean mountains [14].

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Study on the air pollutants removal capacity of Monte Sant'Antonio (CB) urban forest using i-Tree Eco v.6

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In 2050 about 70% of human population will live in cities [1]. The urban areas are characterized by high levels of air pollutions caused by anthropogenic sources [2]. According to the World Health Organization [3], air pollution killed seven millions of people in the year 2016 and 91% of world population breathes air contained high levels of pollutants. The urban forests are components of the urban areas and representants the major provider of multiple ecosystems services able to improve and maintenance the human well-being through the improvement of local climate conditions, removal of air pollutants and recreation [4-8]. The ecosystem services affected by the assessment are those related to the regulation such as the removal of air pollution and carbon storage [9].
The aim of this work is to assess the capacity of the Monte Sant'Antonio urban forest in Campobasso to remove air pollutant and carbon atmospheric by pollutant uptake modelling tool called i-Tree Eco (v.6).
The study was the Monte Sant'Antonio urban forest located in central of Campobasso, in Molise, Italy. This area was affected by numerous reforestation activities: in 1961, 1975, 1981 and before the 1961 could not be identified the year [10-13]. These four reforestation activities have created four different stands that covered about 12.87 ha. The stands (called '61, '75, '81 and *Adulte* respectively) have been studied. The field data was collected by four circulars plots with surface area of about 530 m^2, one for each stand and representative of the average conditions of each of them.
i-Tree is a suite software for pc created by USDA with the aim to assess the forest structure parameters, the ecosystem services, their value and the risk for the forest and people. The software i-Tree eco (version 6) uses the field data collected by sample plots or total stand survey together with local air pollution data and weather information to assess the forest characteristics but also forest health and vitality, the risks, the ecosystem services, and their economic value. The analysis of the data revealed differences between the stands regarding density, specific composition and dendrometric parameters. The stand with highest number of tree species is '81, with ten different species, while the one with the lowest number of tree species is '75, with only six species. The *Adulte* and '75 stands shows a similar specific composition as most of the trees present belong to *Fraxinus ornus* L. and *Pinus nigra* JF Arnold.
The study of distribution in Dbh classes of 5cm shows that *P. nigra*, excepted the '81, covers the higher diametrical classes of the stands, while in the lower classes found the *F. ornus*, *Ulmus minor* Mill. and *Ailanthus altissima* (Mill.) Swingle. In the '81 is present the *Cupressus arizonica* E. Greene and *Cedrus deodara* (Rob. Ex (D.Don)) in the larger Dbh classes, *P. nigra* covers the 20cm class and *F. ornus* occupies the lower classes.
The dendrometric parameters of the *Adulte* stand are influenced by the abundance of small *F. ornus* trees and a few large trees belonging to *P. nigra*. The density (number of trees per hectare, Nt/ha) for the *Adulte* stand is the highest at 1509 Nt/ha, while the value of Mean Dbh (Dg) is the lowest (approx. 14.9 cm); this indicates a very dense stand with predominant numerical component of small trees that settle in the layers below the large *P. nigra* trees. The highest value of Dg (equal to 22.1 cm) and of basal area per hectare (G) equal to 28.10 m^2/ha is found in the '81 with one of the lowest numbers of Nt/ha (735 Nt/ha). These data describe the structure of a sparse stand with a high number of large trees. The latter statement is supported by the average height, volume, and biomass data, which are the highest of the four stands.
The data collected in the field were used for processing with the i-Tree Eco v.6 software to calculate the annual subtraction capacity of atmospheric carbon (Gross C sequestration), carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), particulate matter smaller than 2.5 micrometers ($PM_{2.5}$) and sulphur dioxide (SO_2) of each stand.
The analysis of the values per hectare shows that the stands with the highest sequestration capacity, except for the CO values, is '81. This result is probably due to specific biodiversity present in the '81 stand, which is also

characterized by large broadleaf trees. About CO values, the stand with the highest capacity of removal this pollutant is *Adulte*, due to the high value of Nt/ha of this stand. The results of the air pollution removal capacity of the stands and the entire Monte Sant'Antonio urban forest are shown below (Table 1).

The results obtained by i-Tree Eco are related to allometric formulas produced to North America [14-17]. This leads to deviation between the results produced by i-Tree Eco, using the North America allometric formulas, and those obtained by using allometric equations constructed for the Italian environment such as those of Tabacchi *et al*. [18]. The comparison took place following the definition of linear regression models that contained the total tree biomass, expressed as dry weight, as an independent variable, each time replacing the biomass calculated by i-Tree (take as the actual reference values) with those calculated by Tabacchi *et al.* [18]. The models were constructed by stepwise analysis. They are species specific and calculated for each air pollutant (Fig. 1). The results, obtained by application of linear regression models for each individual tree recording in the sample plots, shows that for all air pollutants considered, the trend for linear regression is the same, falling below the bisector (y=x), except for CO and Gross C sequestration calculate by biomass of i-Tree Eco. For the latter, the regression trend is flawed by the significantly high CO values estimated with the application of the i-Tree biomass model for *C. arizonica*. In general, it can be said that all models have found a very good correlation (R^2 ranging from 0.65 to 0.92) and that the trend, although different, is very similar, indicating that the estimation of pollutants with the use of the i-Tree biomass is always higher than the estimation of the same pollutant using the equations of Tabacchi *et al*. [18]. Furthermore, the sums of the respective values obtained from the estimates made with the use of the models created tend to converge and do not differ much from each other except for Gross C sequestration.

Tab. 1. The values by i-Tree Eco v.6. In blue the highest value for each air pollutant. The right column shows the values for the entire study area.

	/ha				
	Adulte	*61*	*75*	*81*	*Monte Sant'Antonio*
Gross C sequestration (kg/yr)	12494.34	13779.25	12609.43	**16590.57**	174652.15
CO (kg/yr)	**1.34**	1.14	1.20	1.21	15.56
O_3 (kg/yr)	64.81	49.34	50.11	**64.94**	706.31
NO_2 (kg/yr)	25.56	19.31	20.25	**26.29**	281.52
SO_2 (kg/yr)	4.68	3.68	3.85	**4.78**	52.72
PM2.5	3.43	2.58	2.60	**3.93**	38.03

$$y = a_0 + a_1\ Ca_{sup} + a_2\ F$$
$$y = a_0 + a_1\ Ce + a_2\ F$$

Fig. 1. The two most commons models used in this work. "Ce" is crown light espose. "F" is tree biomass (in kg). "Ca_{sup}" is the crown surface (in m^2).

The last phase of study analyses the variation in the air pollutant removal capacity over time by *Pinus nigra* and *Fraxinus ornus*. These two species were chosen because they are present in sufficient numbers in all four stands. This comparison was made, within the same species, per individual air pollutant investigation. The comparison showed that for *Pinus nigra* there is a slight tendency for the air pollutant removal capacity to increase with increasing age. This trend is less pronounced in Gross C sequestration. For *Fraxinus ornus* the variation in the capacity to subtract pollutants is even more pronounced than for *P. nigra*, while the values of gross carbon sequestration remain similar over the years. This variation in air pollutant removal capacity may be due to the increase of the plants in both woody volume and crown size over the years.

The study and the results obtained were compared with other work reported in the literature. An initial comparison can be made with the work of Rossi et al. [19] carried out in Perugia using i-Tree Eco v.6. A total of 373 plants were investigated compared to our study in which 209 were investigated. The comparison was made by setting up a proportion to compare the data for the same number of plants. We thus have 25.9 kg/yr of pollutants removed from Perugia and 1475.8 kg/yr for gross carbon sequestration. The Monte Sant'Antonio urban forest remove, according to i-Tree Eco v.6, about 18.82 kg/yr of air pollutants with a gross carbon sequestration of 2768.10 kg/yr. A further comparison is possible with the work of Alpaidze & Pace [20]. In this case, the study scenario is in the city of Tbilisi, Georgia, with RED and EXPO city parks as study areas. In these parks, there is a specific composition and structure of the tree stand like stand 81. The differences found in the pollutant subtraction values are due to a different number of plants per hectare, which is much higher in stand 81 respet to RED and EXPO city parks. Finally, the last comparison was with the work of Russo et al. [15] this study was conducted in Bolzano where the survey was conducted on 475 trees in the city sequestering approximately 5.82 tons per year of carbon. On the

other hand, the 209 trees on Monte Sant'Antonio have a gross carbon removal capacity of approximately 2.71 tonnes per year estimated through linear regression models. Considering that there are twice as many trees investigated in Monte Sant'Antonio as in Bolzano then the values are proportional.

According to the data reported on the ISPRA [21] website for the indicators relating to urban areas, a comparison can be made between the SO2 and NO2 subtraction capacity of the Monte Sant'Antonio urban forest and the atmospheric emission of these gases by the anthropic activities of the municipality of Campobasso. ISPRA data tell us that the city of Campobasso in the year 2015 was responsible for the emission of 7.82 tons of sulphur dioxide and 369.85 tons of NO_2. The entire urban forest of Monte Sant'Antonio is capable of subtracting about 0.07% of NO_2 emitted and 0.62% of SO_2. It should be noted that these values are relative to the 12 ha of Monte Sant'Antonio urban forest, therefore relative to a small portion of the whole urban "green infrastructure" of the city.

The future research focus will be on investigating and highlighting the possible relationships between the capacity of urban forests to remove atmospheric carbon and the main air pollutants (CO, NO2, SO2, O3 and PM2.5), in relation to the forest structure and the degree of tree-specific biodiversity of the latter, aimed at defining suitable management models for urban forests aimed at maximizing these services and achievable through the application of proper urban forestry. This objective will be achieved using user-friendly technologies, such as i-Tree Eco, supported by dendrochemical surveys aimed at defining stable isotope ratios in wood ($\delta\,^{13}C$, $\delta\,^{18}O$, $\delta\,^{15}N$) and the results of the national ICOS network.

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A spatially explicit approach to strengthen the implementation of national policy agenda and maximize the co-benefit of Nature Based Solutions in Italy

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Today societies are facing the long-standing global environmental crises of climate change and biodiversity loss. Currently, 73% of Europe's population lives in urban areas and this percentage is projected to increase to 82% by 2050, posing a range of challenges for urban contexts, including resource availability, equitable economic growth, and population health [1, 2]. Multiple environmental stressors threaten human health in urban areas and their mitigation represents a key environmental challenge. Air pollution and the extreme events related to Climate Change (e.g., heatwaves and floods) are the environmental stressors of main concern in the European Union [3], and the policies to face them might be coupled in the political agenda [4]. Furthermore, the health condition of populations is strongly affected by complex interactions among factors (e.g., air pollutants and heatwaves), which are interconnected and evolve through time [5]. The extension of legally binding policies and actions (mitigation and adaptation) to multiple stressors could help to reduce risks and vulnerability for exposed populations and to improve the resilience capacity of urban areas [1]. Recent studies showed that the link between urban green spaces and public health is robust and economically significant [6]. Accordingly, the maintenance and enhancement of ecosystem services (ES) are promoted by science and policy as a valid approach to face environmental challenges and ameliorate human health and well-being. Specifically, Nature-Based Solutions (NBS) are recognized for their multifunctionality and capacity to provide co-benefits. In this context, several policies and strategies were implemented at the European and national levels to improve environmental quality in urban areas and tree-planting initiatives were encouraged so far (e.g., "Trillion Tree Campaign" [7], the "Great Green Wall" [8], "3 billion trees" under the European Green Deal [9]). Several approaches to explore priority areas for interventions were proposed in literature, and the ES framework was broadly recognized for its role in supporting urban planning [10]. However, these methodologies are generally conceived at the municipal scale and focused to mitigate single stressors, thus revealing their intrinsic limitation given by the use of administrative boundaries in ES assessments as well as the poor attention on NBS multifunctionality. Therefore, urban policies that spatially allocate funds for NBS implementation based on administrative boundaries might not necessarily guarantee their spatial match with environmental challenges throughout the Country [11].

This study aims to support national urban sustainability policies in (i) the identification and quantification of areas considered under health hazard for the population; (ii) the identification of interventions priority based on the intensity of environmental challenges; (iii) the proposal of a more efficient funds allocation framework for NBS interventions to guide their implementation on a local scale, to improve in turn their multifunctionality [11, 12]. For this scope, Italy was adopted as a case study, since several policies were recently envisaged, allocating funds from the national government to the municipalities (e.g., Decree on Climate, Resilience and Recovery Plan [13]). We identified and mapped with a spatial resolution of 1 Km^2 areas harmful to human health, based on three environmental stressors defined by different factors, air pollution (PM_{10}, NO_2, O_3), thermal stress (heatwave), and hydraulic vulnerability (flooding return period) that exceed specific Environmental Quality Standards (EQS). The greater the exceedance (the difference between the current value and EQS value for each factor) the greater the challenges the population is exposed to in that portion of territory. A spatial multi-criteria analysis assessed the cumulative occurrence of stressors by combining them into a single Aggregate Index of Challenge (AIC). Then, a hotspot analysis identified the spatial aggregation of AIC through the territory, and the product of the AIC and the population density provided the risk index AICpop. Hence, AICpop increases as AIC and population density increase. Finally, 24 NBS were proposed, ranked, and mapped according to their performance to mitigate stressors

throughout the national territory [14].
Results evidenced that just 6% of the country does not exceed the respective EQS for any stressors. Concerning the three factors related to air pollution, O_3 exceeds its EQS for about 89% of the national territory with a maximum value of 176.08 µg/m^3, PM_{10} for about 8% with a maximum value of 90.91 µg/ m^3 while the annual NO_2 exceeds EQS for less than 1% with a maximum value of 54.39 µg/m^3. The highest frequency of heatwaves occurred in the time span considered (2005–2012) ranges from 0 to 36 days. About 45% of the national surface exceeds the EQS of 4 days of heatwaves/year. Regarding hydraulic vulnerability, about 10% of the national territory is in exceedance, being the flood hazard expected in the future. Seven different spatial combinations of stressors in exceedances were identified. Three for the individual stressors, 47% air pollution, 0.2% flood hazard, and 4.3% thermal stress, 7.9% of the territory shows all three stressors combined simultaneously while three different combinations covering 35% of the territory shows the coexistence of two stressors in EQS exceedance. Although policies and directives are already in place to mitigate single stressors, the interaction among different factors is still usually neglected. Our findings highlight that detailed knowledge of the single factors (distribution, concentrations, exposed population) and their spatial interaction can play a crucial role to identify intervention priorities. AIC allowed to establish the relative priority of intervention for each pixel (1 km^2), representing an effective knowledge synthesis tool to inform decision-makers about the relative magnitude of challenges that a given portion of territory is exposed to (e.g., [15]) and thus need to address. Moreover, using AICpop instead – or combined to – AIC, allows to include the population density in this evaluation, helping to (i) consider the variation of risk exposure [16]; (ii) evaluate funds allocation concerning the potential number of beneficiaries involved in the interventions. Indeed, especially in urban contexts, the positive value of enhancing ES provision is strictly related to the number of their beneficiaries and not limited to their physical characteristics [17]. Despite the urgencies evidenced by AIC values and even more by AICpop, several constraints might hamper the feasibility of targeted interventions (e.g., limited space in historic centers, dense fabrics, ownership, landscape, or archeological restraints). In these cases, the possibility to enlarge the potential area of interventions is supported by the hotspot/coldspot approach proposed in this work. Indeed, the aggregation of high AIC values reveals a hotspot area of 54,000 km^2 (18% of the national territory), where diffuse interventions on the territorial matrix can enhance the overall environmental quality [11].
For each of the seven combinations of stressors identified, we provided a ranking of 24 NBS based on their Performance Score (PS) to mitigate the stressors [14], both individually and aggregated, ii) and implementable in both impervious and permeable surfaces. In this way, suitable and multifunctional NBS were selected for each combination based on the land cover available. Vertical green (i.e., green façades, green wall systems, vegetated pergolas, vertical mobile gardens) shows high PS for the mitigation of both air pollutants and heatwaves while low PS for the mitigation of flood hazards. We found the opposite PS trend for rain gardens, swales, and vegetated grid paves, which are particularly useful to mitigate flood hazards. Seven NBS have high PS simultaneously in all groups: infiltration basins, green corridors, urban forests, large urban parks, heritage gardens, intensive green roofs, and semi-intensive green roofs.
Hence, thanks to these co-benefits, these seven NBS can be potentially implemented throughout 94% of the Italian territory, thus ensuring good performances employing less than one-third of the here proposed NBS. The environmental stressors assessed in this work can adversely affect human health and well-being, with associated mitigation costs. Accordingly, our work contains a novel framework that will help both the national government and the municipalities to identify the intervention priority to allocate NBS able to maximize the ES supply while addressing multiple challenges. However, the relevance of our framework is not just focused on the NBS application at the local scale but shows a great impact on a wider scale too (e.g., national and regional), where it helps to tackle the recurring problem of planning silos. Therefore, an evidence-based approach seems promising for enhancing the cost-effectiveness of funds allocation as well as their return in terms of human health and wellbeing. On a national scale, the framework proposed here can reliably (i) identify the areas showing a simultaneous demand for the achievement of multiple national targets (i.e., health hazard); (ii) spatially orient the new investment needed to mitigate the stressors and in turn address the environmental challenges (e.g., Green Deal); and (iii) support the NBS selection that provides more co-benefits, playing a crucial role in enhancing budget allocations efficiency. On a municipal scale, the NBS ranking can be used as a guideline for further specific planning and design activities based on local issues, barriers, and peculiarities, while remaining consistent with national

targets. The multi-scale approach here proposed can help in considering different stakeholders as well as social, economic, and biophysical characteristics playing a crucial role in the benefit provision and thus claiming a better inclusion in decision-making contexts related to national, regional, city/site-scale spatial plans.

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The hidden biodiversity of managed forests

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Forests are the principal sinks of terrestrial biodiversity and provide many ecosystem services to society. Climate change and human activities have a strong impact on forest ecosystem functions, contributing to the loss of biodiversity [1, 2]. The establishment of protected areas was one of the most common approaches to limiting forest biodiversity loss [3]. This approach allowed the protection of biodiversity in small, circumscribed areas, fostering the maximization of exploitation in the remaining forest areas [4]. A new forest management approach is reaching increasing attention in the last years to balance the provision of ecosystem services and enhance biodiversity conservation within commercial forests.

Integrated forest management is an innovative forest management approach that aims to maximize the manifold objective at the same time and place [5]. The main objective of the integrative approach is to balance the ecosystem services trade-offs, particularly between biodiversity conservation and timber production [6, 7]. Releasing biodiversity-relevant structures, so-called Tree-related microhabitats (TreMs) such as cavities, dead branches, and epiphytes, typical of unmanaged forests, represents a suitable strategy to promote biodiversity conservation even in managed forests [8, 9].

TreMs are defined as “distinct, well-delineated structures occurring on living or standing dead trees that constitute a particular and essential substrate or life site for species or species communities during at least a part of their life cycle to develop, feed, shelter or breed” [10]. TreMs represent the potential habitat of a variety of species of different taxonomic groups and, particularly in managed forests, are becoming increasingly important as an ecological indicator to assess the value of forest habitats [8, 11]. Among the taxa that TreMs host, beetles are the most frequent.

Beetles, especially saproxylic ones, are the most important biodiversity indicators of the degree of conservation of natural habitats [12]. They have some important ecological roles and contribute to fundamental ecosystem services such as decomposition and nutrient cycling in forest ecosystems [13]. Saproxylic beetles, because of their strong relationship with the deadwood component, are often threatened by human activities, so much so that several international and national lists (e.g., the Italian red-list of saproxylic beetles) have been created to monitor threatened species [14].

Although studies about the relationships between saproxylic beetles, forests stand variables, and deadwood increased significantly, the interrelationships among saproxylic beetles and TreMs are still poorly explored.

This study was realized in 60 circular plots within a beech forest belonging to the Apennine mountains of central Italy (Fig. 1). We hypothesized that: (i) TreMs are a proxy for beetles’ abundance and diversity; (ii) the abundance and diversity of both TreMs and beetle communities vary with environmental characteristics.

Four Generalized Linear Models (GLMs) were run to analyze the relationships between structural, environmental, and biodiversity parameters on beetles’ community abundance and diversity. Two correlation matrices were realized to observe how and how much the single TreMs influence beetles’ abundance and diversity.

A total of 19 different TreMs groups and 48 TreMs types were counted within plots. The most frequent TreMs types were the *small root buttress cavities* (*GR11*). These and *small rot holes* (CV31) were also the TreMs types observed in all 60 plots. We collected 4022 specimens of beetles, belonging to 165 species, within which 65 species for a total of 2335 out of 4022 specimens, were in the Italian red list of saproxylic beetles. Based on the risk category defined by Carpaneto et al. [14] the threatened Italian red-list saproxylic species captured were classified as Near Threatened (8) and Vulnerable (2).

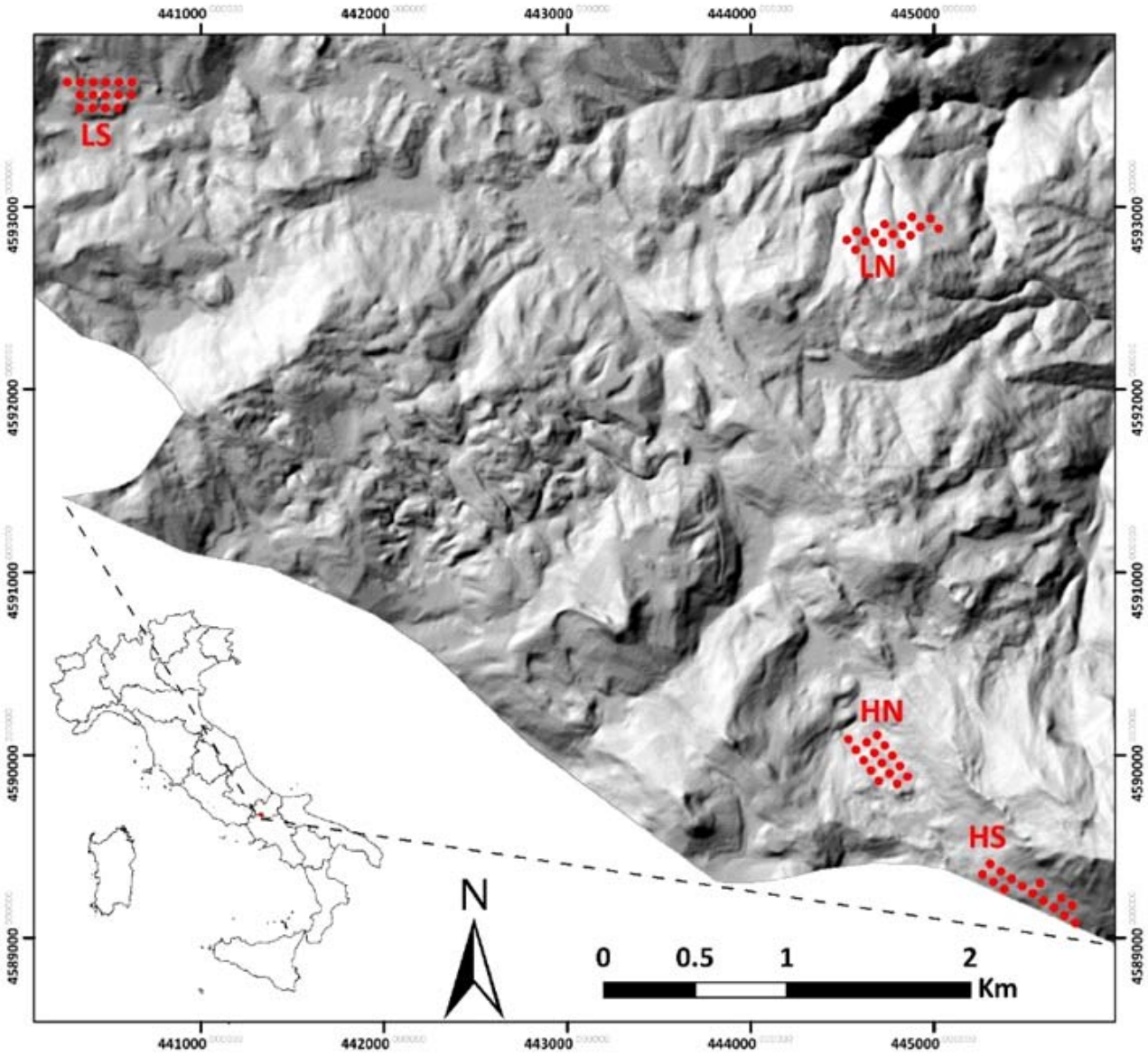

Fig. 1. Location of the study area for assessment of forest stand structure, beetles sampling, deadwood, and TreMs. Red points represent the 60 plots according to the 4 sectors: Low North (LN), Low South (LS), High North (HN), and High South (HS).

Results showed that TreMs greatly influence both the abundance and diversity of beetle communities, including saproxylic species. Most of the significant relationships with Italian red list saproxylic beetles were positive (Fig. 2). Specifically, *root buttress cavities* (GR1) were positively related and statistically significant for saproxylic beetle abundance and diversity. On the other hand, *dendrotelms and water-filled holes* (CV4), *dead branches and limbs* (DE1), and *bark pockets* (BA1) were significantly negatively related to the abundance and diversity of beetles and saproxylic beetles. The TreMs type that significantly interacted with the greatest number of saproxylic beetle species was the CV44 (*large dendrotelms in the crown*).

The study demonstrated that TreMs can be considered a proxy for enhancing forest biodiversity in managed forests. Retention of trees-bearing TreMs during forestry interventions can promote the conservation of beetles' biodiversity and, more in general, promote the conservation of biodiversity within forests managed for commercial purposes.

In addition, the specific relationships between individual saproxylic species, particularly for those belonging to the red list, and TreMs facilitate forest managers to integrate biodiversity conservation with timber production.

Results encourage verifying the relationships with other saproxylic beetles' species, as well as carrying out multitaxonomic studies improving the knowledge about TreMs and sustainable management of forest ecosystems.

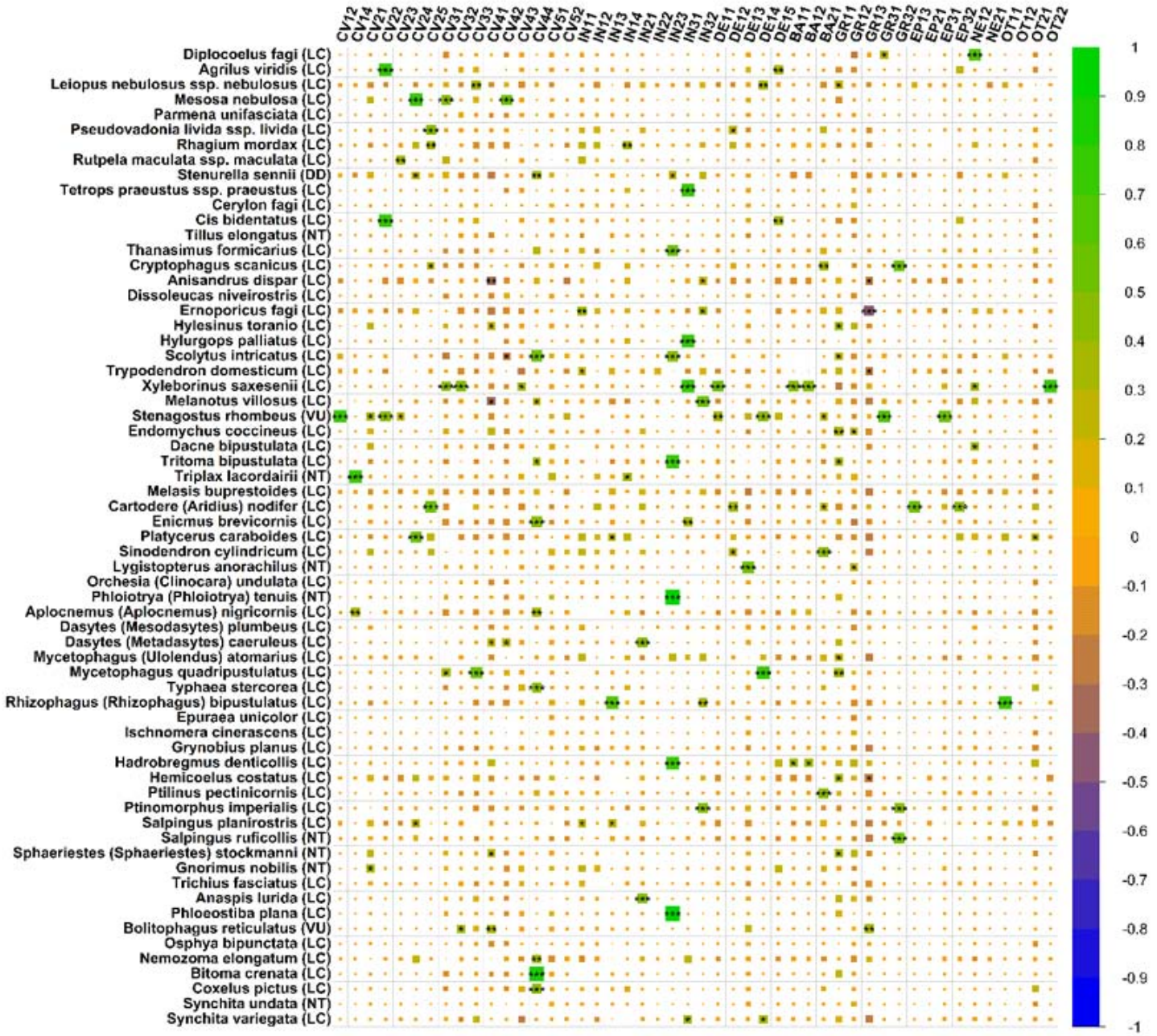

*Fig. 2. Correlation plot between the number of each Italian red-list saproxylic species and abundance of TreMs types. Significance value: 0 *** 0.001 ** 0.01 * 0.05.*

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Assessing Resilience Components in Maritime Pine Provenances Grown in Common Gardens

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Forest ecosystems and their management play an important role to reducing the negative impacts of climate crisis [1]. With increasing temperature, warming-related disturbances are also becoming more frequent and sever [2]. In the last decades the droughts and heat waves increased significantly in the Mediterranean area [3] indeed it is considered as hotspot of climate change. Thus, knowledge acquisition on the response of Mediterranean tree species to drought is an important step to guide adaptation strategies to climate change impacts, e.g., assisted migration. *Pinus pinaster* Ait. (maritime pine) is native to the western Mediterranean basin, and it's an important ecological and economical species. Its range is strongly fragmented, so it grows under different environmental conditions, which may trigger the development of different intraspecific strategies [4]. The common gardens experiments on this species are limited in the Italian Peninsula and in Sardinia Island in comparison with those established in Iberian Peninsula and southwestern France. Since this gap, in this study we assessed the resilience components (based on dendrochronology data), i.e., resistance, resilience and recovery [5], to drought in five provenances of maritime pine, and analysed the possible influence of climate variables on these indices. The provenances studied – i.e one from Corsica, one from Tuscany, one from Portugal and two from Sardinia (Telti and Limbara) – were planted in 1981 in four common gardens in Sardinia (Fig. 1). All sites, except for Montarbu, were thinned and previously surveyed through dendrometric measures at 7, 16 and 20 years since planting [6-8]. In 2018 diameter at breast height (DBH) and tree height were collected. At the same time, two wood cores were taken from 8-10 healthy trees per provenances.

The elaboration and analysis of data were done with Rstudio software used its packages. The resilience components were calculated and analysed only for the drought years common to all experimental sites. The drought years are identified when the value of SPEI (Standardised Precipitation Evapotranspiration Index) calculated in six-month window ($SPEI_6$), in August ($SPEI_{6AUG}$) were less than -1. To characterize the climate conditions of experimental sites was calculated the De Martonne Index. To test the differences in climate conditions among the sites, tree growth rates and in resilience components among the provenances was done the Kruskal-Wallis' test. When this was rejected, the comparison between groups was done with Conover test. To determine what and how climate variables might influence the resilience components were built some Generalized Linear Models (GLM) for each index. The minimum and maximum temperatures, precipitation, $SPEI_{6AUG}$, diameter at breast height (DBH), and the height were used as predictors, and those with low multicollinearity were used to elaborate several models.

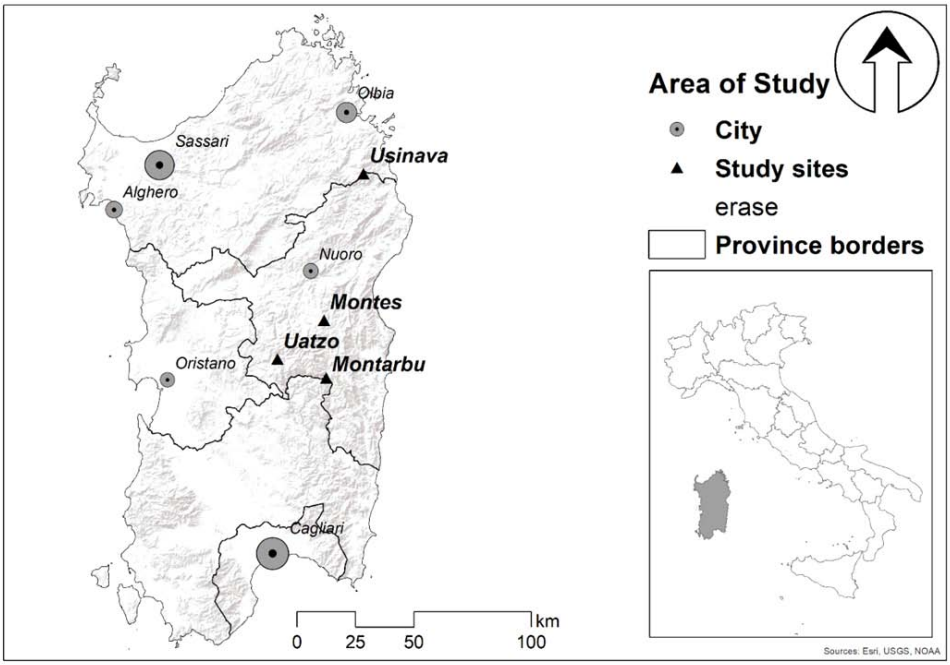

Fig. 1. Location of the four common gardens in Sardinia.

Afterwards, the best model was selected with the lowest Akaike's Information Criterion (AIC) and for each model was calculated the R-squared. For all analyses was used as a significant threshold a *p-value* ≤ 0.05.
From elaboration of climate data, it was found that only Usinavà was drier than others and the common drought year for all sites was 2003. The provenances differed in growth rates. Overall, the Tuscany recorded the lowest tree growth rates, while the Corsica was the most productive. However, one of local provenance (Limbara) showed the good performance in terms of tree growth. The resilience components were influenced only by the climate conditions at the common gardens. All provenances recorded the lowest values of resistance and the higher recovery values in drier sites. Concerning the resilience index, it was found only one difference for Portugal provenance, which showed higher resilience in Uatzo than in Usinavà. However, during the drought event the opposite trend was found in the two sites with lowest stand density, probably due to thinning operation. Multicollinearity issues prevented precipitation to be included in the model construction. The structure of models was different for each index. The DBH was not included in any models, while the height might have a negative influence on all indices. The models' results showed the important role of the temperature during drought stress because the minimum temperature was included in the best models for all resilience components. However, the models highlighted the noticeable adaptation of this species to drought conditions of Sardinia. Evidence of adjustments was somewhat underlined in the comparison between resilience and recovery models. The influence of $SPEI_{6AUG}$ on these indices was similar, though less pronounced on resilience than on recovery.
Despite the provenances in maritime pine affected only tree growth and not the resilience components, the present study provides important insights for both productive tree plantations and assisted migration measures regarding this species. Furthermore, it highlighted the importance of establishing common gardens and long-term experiments to tackle future climate change. This study contributed to expand the knowledge of the behaviour of maritime pine provenances planted in Mediterranean environments, in particularly in the Italian Peninsula and in Sardinia Island where the studies are limited. It would be appropriate to use the same indices tested in this study across subsequent stages of the tree life span and compare a wider collection of provenances. Nonetheless, the information obtained by this study may help to improve the Climate-Smart Forestry strategies suitable for this species in Mediterranean environments. This study suggests that the provenance of Corsica may provide appropriate material for forest plantations in Mediterranean conditions with
mitigation purposes.

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Pointing Limitations in Current Evapotranspiration Model schemes for their Application over The Mediterranean region

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Mediterranean ecosystems in specific arid and semi-arid production systems are more vulnerable to precipitation decline causing hydrological imbalances in the basin [1]. Our limited understanding of Mediterranean ecosystem's reactions to stressed hydro-climatologic conditions is a great challenge [2].

This information gap demands increasing our monitoring and modeling capabilities in terrestrial water Flux quantification (Evapotranspiration, runoff, and groundwater storage) [3]. Especially of Evapotranspiration (ET) which is a principal water-extracting component in the arid/semi-arid part of the Mediterranean region. Its accurate modeling is necessary to manage growing water demand and food insecurities in the stress-prone regions [4, 5]. This research aims to highlight the limitations of the existing modeling schemes and bring forth some important constraints required in the physical schemes to make them suitable for Mediterranean basin.

In this objective, an inter-model comparison study was conducted to compare daily and annual Evapotranspiration (ET) patterns over the Mediterranean region. For a reasonable comparison among ET products, a common meteorological and physical boundary condition is defined to simulate ET using energy balance (TSEB), eco-hydrological (STEPS, GLEAM), and process-based schemes (SCOPE). In this experiment, all models provided different ET estimates in their particular set of constraints and structured algorithms. The modeled ET validation with ground datasets revealed that process-based models function well over crop and forest ecosystems, but their parameterization requires the inclusion of soil moisture constraints for their application in arid and semi-arid ecosystems. Besides that, improved land and soil representation is necessary for use of ensemble product of modeled ET.

The study highlights the need for revising parameterization scheme of these models in order to achieve their successful application in dry ecosystems. One example is connecting stress factor and soil moisture profile with dynamic vegetation modeling. The lack of uniformity in the current model schemes makes ET estimates more uncertain which is observed in a thermal-based energy balance scheme without soil water constraints where ET is overestimated over irrigated agroecosystems.

The study also noticed that these models require adjustments in their parameterization criteria for evergreen boreal forests because they are also vulnerable to heat and water stress in fact more prone to wildfires. The comparisons suggest that new research should focus on ensemble ET products targeting a particular climatic zone in the Mediterranean region whether arid or semi-arid.

We can make these models seasonally adapted as well while considering a dominant vegetation cover for a certain period. Such improvements can better predict increased water stress and irrigation needs of the Mediterranean region.

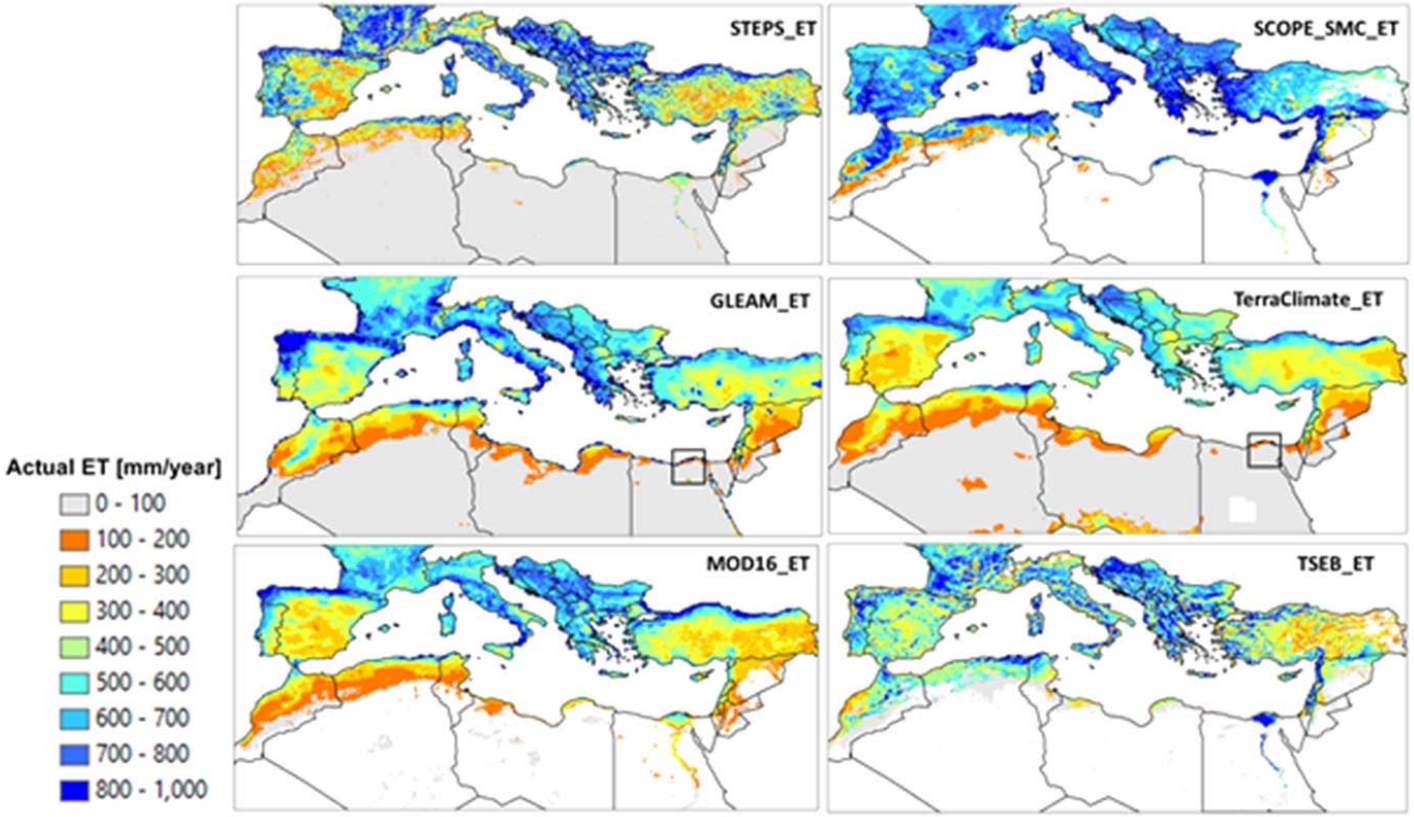

Fig. 1. Total ET estimated from remote sensing energy flux models [TSEB, GLEAM, MOD16, SCOPE] and process based model [STEPS] compared with TerraClimate [Hydrological model] over Mediterranean Region.

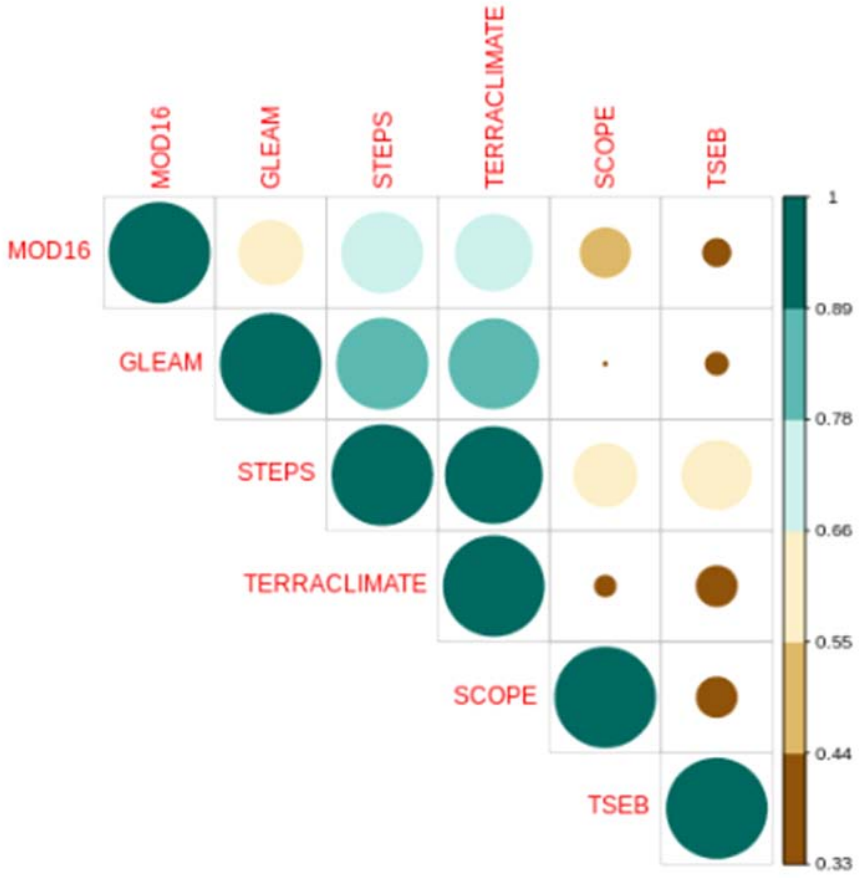

Fig. 2. The correlation matrix of modeled ET. The highest correlation found among STEPS, GLEAM and TerraClimate in annual estimates, TSEB and SCOPE showed varied response to others.

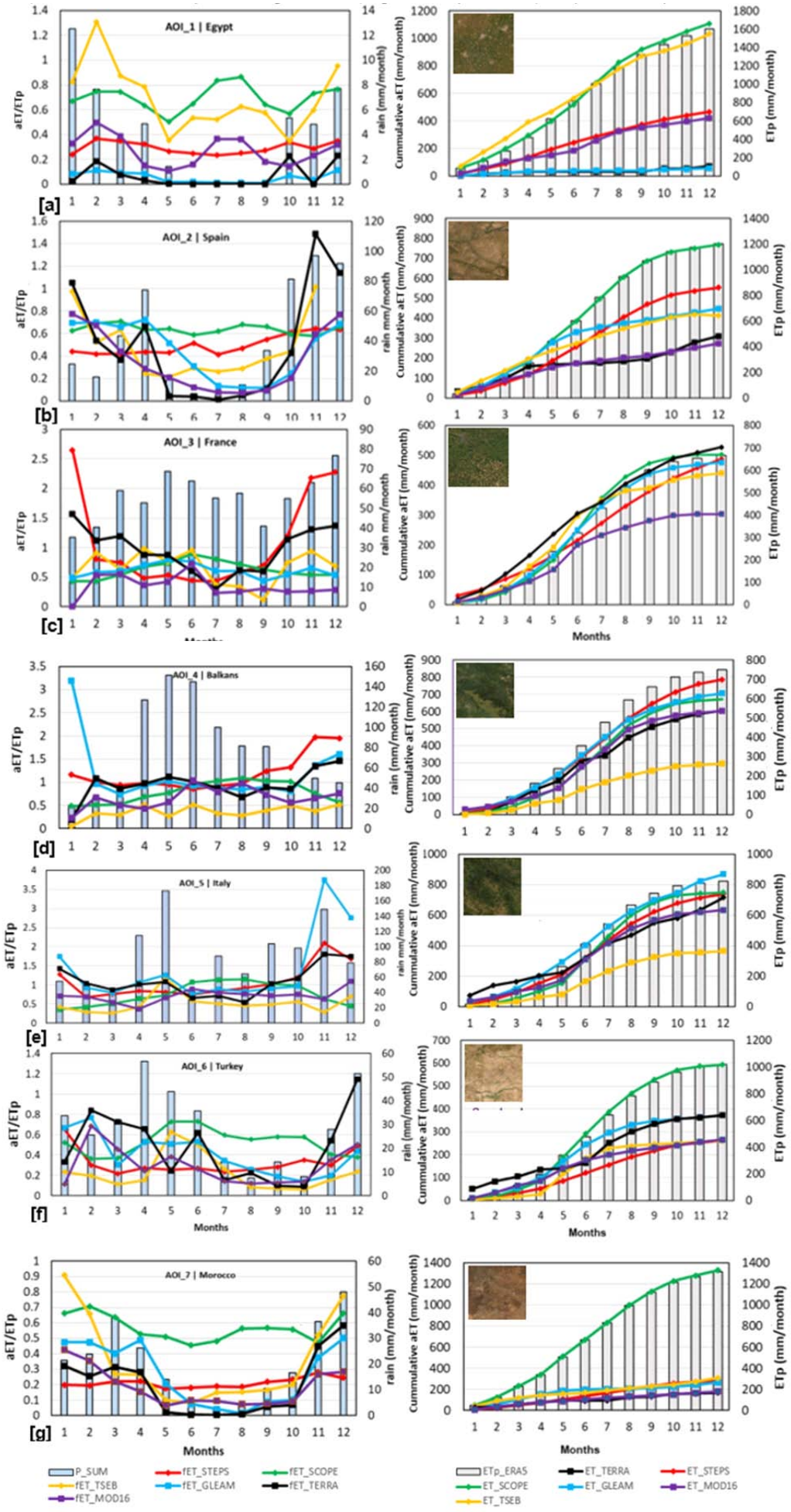

Fig. 3. Presents comparison of modeled cumulative actual ET from potential ET derived from ERA5 Gridded hourly datasets in which ratio of monthly actual and potential ET is compared with monthly precipitation to find their temporal relation and inter-annual variability over climatically different parts of the region i.e. Egypt [a], Spain [b], France [c], Bosnia Balkans [d], Italy [e], Turkey [f], Morocco [g].

Fig. 4. Displays r-square coefficient of determination [a] root mean square error RMSE [b] and mean bias error (MBE) of all models [STEPS, SCOPE, TSEB and GLEAM] that depicts their good performance in specific ecosystems.

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Local environment prevails over population variations in growth-climate relationships of *Pinus pinaster* provenances

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Continuous climate change, i.e. increasing temperatures and decreasing precipitation, are leading to an alteration of the start and duration of the growing season [1], with negative impacts on tree growth and stand productivity across European forests [2]. One of the areas where climate change is most pronounced is the Mediterranean basin. The Mediterranean Basin is a climate change hotspot, in which the occurrence of intense and prolonged drought episodes may compromise the ecological resilience of tree species [3]. However, in environments with complex and heterogeneous climatology, such as the Mediterranean region, tree responses to climate patterns vary across species populations [4] and lead to divergent intra-specific strategies for coping with climate stressors [5]. This make it difficult to predict how trees respond to climate change and natural disturbances in different regions. Therefore, understanding how a species can be threatened or privileged by changing environmental conditions is a prerequisite to select genotypes for climate-smart forestry programs [6] aimed at promoting forest resilience and adaptive capacity [7;8]. Considering forest management perspectives, common garden experiments may help unravel intra-specific variation in growth responses to climate conditions [9]. Traditionally, in common gardens, seeds were collected from provenances, referred to the geographic and climatic different origin populations, and planted at common sites for the conservation ex situ of seed sources or for the genetic comparison of forest species provenances [10]. These experiment trials allow investigating local adaptation in tree species and represent a novel opportunity for studying functional traits (e.g., tree growth) to be used as proxies for fitness [11]. In this study, by means of four replicated common gardens in the unique environment of Sardinia Island (Italy), we assessed population variations in growth traits (i.e., stem diameter, tree height, and stem radial growth) and analysed climate-growth relationships in five maritime pine provenances. Maritime pine (*Pinus pinaster* Ait.) is a native tree species from the western Mediterranean Basin of great ecological importance for conservation purposes and the protection of soil from erosion [12]. Our aims were to identify: (i) provenance-related differences in dendrometric traits and their relationships with environmental conditions at the common garden sites and (ii) site-provenance variations in growth-climate relationships.

Our results highlighted the main climatic factors influencing stem radial growth dynamics in 40-year-old maritime pine growing in four common garden sites in the harsh environmental conditions of Sardinia. Positive precipitation, especially in late winter and early spring, indicated a good water supply, that favored the stem radial growth of trees during the growing season. Conversely, excessive temperature and low precipitation in mid-summer, had negative impacts on stem radial growth in maritime pine.

Furthermore, the variations in dendrometric traits and in climate responses showed significant differences mainly between sites. In particular, our results showed significant differences regarding stem DBH and tree height among common garden sites and among provenances within each site, pointing to minor differences in stem radial growth among provenances. Furthermore, although environmental conditions in the four common garden sites were relatively similar, notable differences between sites were observed in maritime pine growth and survival. Therefore, this study highlighted that, at age 40, dendrometric parameters of maritime pine were conditioned mainly by the site characteristics (phenotypic plasticity) and that none of the considered provenances showed a substantial advantage in more than one common garden site. The climate responses, as well as sensitivity to summer drought, on the other hand, highlighted significant differences only among the four common garden sites, suggesting how site characteristics and environmental conditions influence on growth sensitivity to climate.

Although tree growth responses to climate change may differ across the heterogeneous distribution range of

maritime pine [e.g., 13], convergent intra-specific growth-climate relationships suggest that seed sources with a divergent biogeographical origin may have comparable functional responses to environmental changes. Nonetheless, further research is needed to explore whether differences among seed sources in climate responses occur by extending the geographic range of the provenances and including other common gardens.

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Human ecologies and climate vulnerability of the landscape

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This contribution illustrates the (partial) results of a research work that the authors are developing starting from the activities carried out so far in the context of the Erasmus+ KA203 strategic partnership project e-CREHA, "education for Climate Resilient European Heritage Architecture", in which the University of Molise is involved together with Eindhoven University of Technology (Netherlands, coordinator), INSA-Strasbourg (France), Sofia University (Bulgaria), NIKU Research Institute (Norway), Tobb Ekonomi ve Teknoloji Universitesi (Turkey).
The objectives of the project are: i) the definition of an innovative learning methodology (which also includes an e-learning course) around the general theme of resilience to climate change of built cultural heritage in Europe; ii) the increase in the quality of training and design research applied to the reduction of the negative effects induced by climate change on the built cultural heritage; iii) the development of multidisciplinary knowledge aimed at the progressive construction of a design culture oriented towards the prevention and mitigation of the effects of climate variability.
Unimol's contribution to the research work addresses the issue of vulnerability, and in particular it deals with planning approaches and tools capable to reduce the vulnerability of the built cultural heritage to the hazards induced or increased by the effects of climate change, but also to simultaneously trigger processes of continuous regeneration of the heritage itself, as well as self-sustainable development of the settlements [1].
The research fits into the general framework of studies on the effects of climate change on human activities and focuses on the need to (re)conceive forms of human settlement that are more adaptable to the changing environmental contexts, thus reducing their vulnerability to pre-existing or new hazard factors, and the consequent negative effects on the built cultural heritage. The latter understood in an extensive and territorial sense, thus overcoming the limits of both the approach centred on the scale of the single artefact of particular value, and of that one focussed only on landscape assets or landscapes of outstanding universal value; considering rather the whole landscape as the "result of the action and interaction of natural and/or human factors", which expresses the different civilizations which have contributed over time to shape it, including the contemporary one, .
In the context of the research work, these processes of "action and interaction" are considered according to an ecosystem approach that goes beyond the simple "interconnection" of "social, economic and ecological systems" [2], and also beyond the interpretation of the Social-Ecological System (SES) as "an integrated system that includes human societies and ecosystems, in which humans are part of nature" [2], which however still implies a clear distinction between "human societies" and "ecosystems".
Moreover, the research refers to that concept of SES which intends it as an ecological system closely connected and influenced by one or more social systems [3], in which the ecological system, in the broadest sense, is considered as an interdependent system of organisms or biological units, and "social systems" are all systems in which organisms of the same species tend to form cooperative and interdependent relationships relationships.
The specific purpose of the research is precisely to contribute to the development of this approach to SESs in a planning sense, i.e. identifying those settlement and production practices that make it possible to reactivate local and networked processes of co-evolutionary interactions between human species and environment, aimed at reducing the vulnerability of the territory, through interventions that are not purely conservative.
Starting from the definition of human ecologies as a complex of "interactions between humans and their environments" [4] "at all scales, times, and places" [5], the research considers the multiform and multiple relational plots through which spread out the above interactions between human species and non-human natural elements producing evolutionary changes. It is therefore considered necessary that any planning intervention is aimed at (re)enabling the aforementioned ecologies, identified as the only ones capable of responding to the new and changing needs induced by the climatic variability affecting territories, both on a global and local scale.

An interpretation of human ecologies looking at anthropic interactions in the environment as a complex of retroactive processes (feedback) also allows to overcome the persistent idea of opposition between vulnerability and resilience- still existing in the definitions of IPCC [2]- consistently with a more innovative approach, defined as "resilientiary vulnerability" [6], which instead places them within a relational continuum, in which the vulnerability factors themselves also can constitute resilience factors.
A further progress proposed by the research, always based on the notion of SES provided above, is the overcoming of the concept of "Built Environment" as anthropic polarity opposed to the natural polarity of the global environment- in turn still present in the IPCC contributions, although oriented towards a greater integration of the two terms- but rather as a field of synthesis and symbiotic integration between the human and non-human (abiotic and biotic) components of the environment.
The research also recognizes the substantial coincidence between this conception of built environment and the European Convention definition of landscape, further interpreted as "continuing landscape"[1]- i.e. as a landscape whose evolutionary process is still in progress and that at the same time exhibits significant material evidence of its evolution over time - provided that this interpretation would be referred not only to the landscapes of "outstanding universal value" but to all landscapes [7], considered as a whole as the 'Built Cultural Environment'.
The reactivation of human ecologies is therefore identified as the most effective approach for reducing the 'climate vulnerability' of landscapes. In fact, "in human ecology, [since the beginning, ed] the concept of adaptation did not refer to the survival and reproduction of genetically heritable traits, but rather the continual process of choosing among and refining strategies of making a living (reproducing a way of life) in a changing world" [8]. It is also recognized in the literature that local and autonomous settlement practices have often allowed greater ability to adapt to the changing environmental contexts compared to institutional planning and interventions [9].
In the research framework, such an approach has been experimented in four areas of Molise region[2], characterised by the widespread presence of environmental hazards (potentially accentuated by climate change) and by heritage elements already recognized (archaeological, architectural and landscape heritage) or recognizable (continuing landscapes). The four areas were selected for the purposes of the planning exercise foreseen in the programme of the e-CREHA workshop which took place at the Termoli branch of the University of Molise[3], with the participation of tutors and students from all the partner institutions. The preliminary study for the exercise consisted in the implementation of a geographic information system to integrate data on the hazards and values of the areas under investigation.
The data were used to create planning proposals aimed at reducing the vulnerability of the built cultural environment. The proposals followed the approach described above, and therefore they also contemplated the use of Nature-based Solutions (NbS). Regarding in particular these latter, within the broader range proposed by the European Commission[4] [10], the typologies were identified which seem to be able to reduce the climatic vulnerability of landscapes, while at the same time outlining management systems of agricultural land, water and forest resources and ecosystem connections, as suggested by the European Environmental Agency [11]; or in the terms or our research approach, which are not purely conservative, but rather capable of re-constituting human ecologies, that in turn can re-generate landscapes, aka built cultural environments.
In conclusion, the ongoing research is demonstrating that the use of NbS, also possibly in integration with 'grey infrastructures' (i.e. "traditional" civil engineering interventions), can produce significant and long-lasting effects in reducing the climate vulnerability of landscapes only if it is also conceived as a trigger tool for the reactivation of continuous processes of co-evolutionary adaptation between humans and their environments.

[1] UNESCO, "Operational Guidelines for the Implementation of the World Heritage Convention" (1994).
[2] Guardialfiera Lake area (CB); Pietrabbondante area (IS); Agnone area (IS); Petacciato area (CB).
[3] From 13/09/2022 to 22/09/2022.
[4] The general areas identified by the CE are: i) urban regeneration through NbS; ii) NbS for improving well-being in urban areas; iii) establishing NbS for coastal resilience; iv) multi-functional nature-based watershed management and ecosystem restoration; v) NbS for increasing the sustainable use of matter and energy; vi) NbS for enhancing the insurance value of ecosystems; vii) increasing carbon sequestration through NbS.

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Experimental tests on cement pastes

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Structural monitoring and OMA techniques are effective for extracting the modal properties (natural frequencies, damping, mode shapes) of the structure as damage-sensitive characteristics, but also offer an evaluation on the effectiveness of reinforcement systems and retrofitting interventions thanks to the variations of dynamic properties before and after the intervention. The cement mortar plays a primary role in determining the effectiveness of the reinforcement interventions, and it is essential to know the effects of curing on the stiffness properties and to know the evolution of the Young's modulus.
The evolution of the mechanical properties of a cementitious material is very important from a structural point of view, Knowledge of the evolution of the E modulus from the initial casting phase and during the subsequent setting and hardening phases is of fundamental importance for many fields of materials science. Various destructive and non-destructive methods have been proposed in the literature to characterize the mechanical properties. E modulus is characterized by a rapid rate of change at early age and it is necessary to use non-destructive techniques to monitor its evolution.
There are several non-destructive techniques: electrical measurements, acoustic methods, reflection of ultrasonic waves, acoustic emissions, impact echo, nuclear magnetic resonance, calorimetric tests, resonance frequency.
In this study, a non-destructive method based on resonance frequency was used for the evaluation and measurement of the evolution of the modulus E in cementitious pastes.
The EMM-ARM (E-modulus Measurement through Ambient Response Method) is a non-destructive technique which is based on the identification of the first vibration frequency. In literature there are applications on cement pastes, mortars and concrete. The applicability of the EMM-ARM method has been extensively discussed in the literature by comparing the results with destructive and non-destructive techniques.
The experimental test procedure was introduced by Azenha [1] for measurement of concrete E-modulus evolution since casting and the results were compared with compression tests. Subsequently this method was used for the evaluation of cement pastes [2]. The influence of the composition of the cement pastes on the evolution of the E modulus was investigated using the EMM-ARM method [3]. Some studies have been done for the problem of mould geometry and sampling methodologies [4, 5]. The results obtained with the EMM-ARM method has been compared with other non-destructive and destructive techniques at different temperatures with different materials [6]. The evolution of the mechanical properties of the cement pastes was studied through the simulation of the microstructure of the paste, a 3D lattice model and a FEM model were created by comparing the results with the EMM-ARM technique [7] The applicability of the method was studied for mixed lime-cement mortars [8-10]. Recent studies have combined EMM-ARM and NMR (nuclear magnetic resonance) results to compare E modulus capillary water and C-S-H evolution for the cement pastes [11].
The novelty in this study is represented by the choice of test material pozzolanic cement, in fact most of the studies considers CEM I and CEM II materials. A CEM IV/B 32.5R pozzolanic cement was chosen because in Italy is among the most used as it develops a high initial resistance and provides good protection against chemical attacks, making it suitable in aggressive environments. In this study the effects of storage and curing temperature were investigated.
The exposition of concrete to moisture can cause premature hydration, this phenomenon is called prehydration and negatively affect the performance of the materials. Prehydration leads to a considerable delay in hydration reactions with an increase in setting time and reduced compressive strength.
In literature there are works [12] on portland cements which from a chemical point of view describe the processes of the prehydration phenomenon. In this study, the effects of storage and temperature on a pozzolanic cement

were evaluated through a phenomenological approach.
The method is based on the identification of the first resonant frequency of a composite beam with with a cantilever scheme.
In this study the mould used for the preparation of the specimens is an acrylic tube with a length of 500 mm and with an internal and external diameter Φi = 16 mm and Φe = 20 mm, respectively. Two types of cement with different mechanical strength values were used: R325 and R425. An accelerometer has been glued to the free end of the beam.
After casting operations, the beam was placed in a horizontal position in a clamping device. The experimental setup is shown in Figure 1.

Fig. 1. Experimental setup.

The resonant frequency is related to the modulus E through the free oscillation equation of the composite beam (Eq. 1).

$$EI\frac{\partial^4 v(x,t)}{\partial x^4} + \bar{m}\frac{\partial^2 v(x,t)}{\partial t^2} = 0 \quad (1)$$

where E is the elastic modulus, I is the second moment of area of the composite cross-section and is the uniformly distributed mass. Considering the boundary conditions of the cantilever it is possible to solve the differential equation.
During the hydration phase it was possible to monitor the evolution of the stiffening contribution of the cement by increasing the natural vibration frequency of the beam.
Figure 2 shows the results for CEM IV/B stored under normal conditions for several months. Tests were carried out with a new material and stored at 1, 8 and 12 months. It is possible to observe a progressive reduction of the E modulus and an increase in the setting and hardening time with increasing storage time.
A CEM II/A material with a higher clinker content was also tested. Tests were carried out with new material and stored after 8 months. In this case

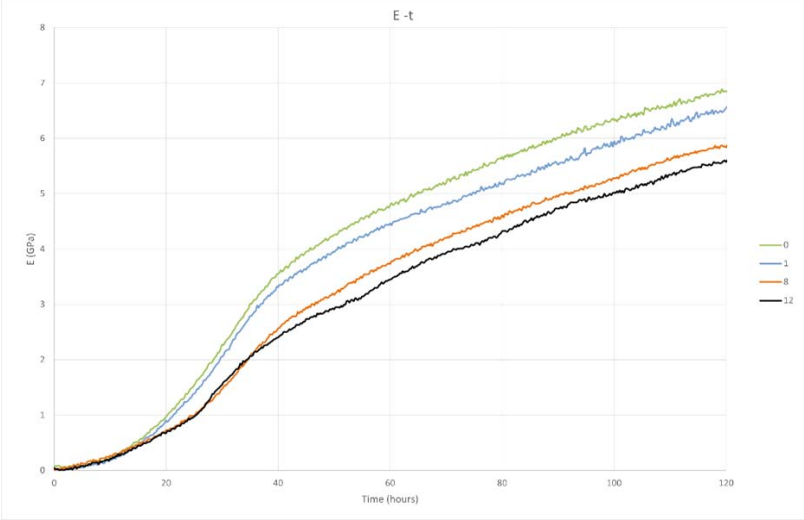

Fig. 2. Results CEM IV/B.

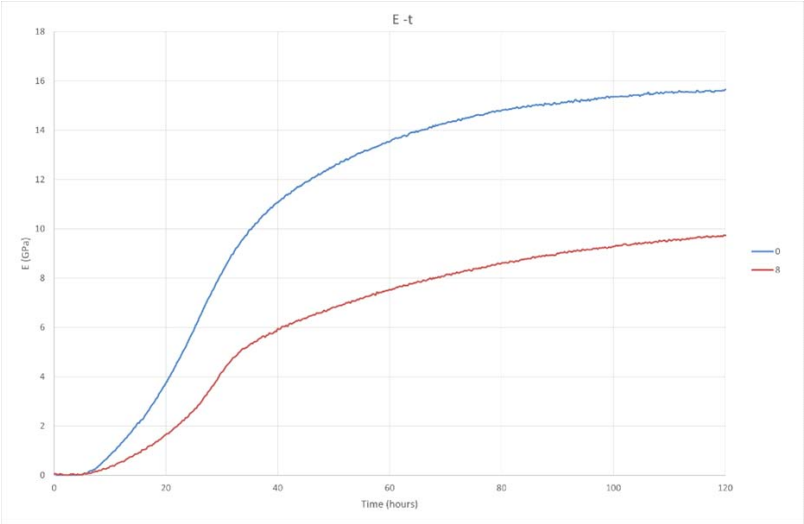

Fig. 3. Results CEM II/A.

the reduction of the E modulus is more significant due to the higher clinker content which is more sensitive to storage conditions (Fig. 3).

Tests were carried out with different curing temperatures for CEM IV/B. Figure 4 shows the results at 15, 21 e 24 ° C. Above 24°C the E modulus increases slightly, but at 15°C a strong reduction in setting and hardening time is noted. The goal is to be able to monitor different types of mixes even with innovative materials used for reinforcement systems such as FRCM (Fabric Reinforced Cementitous Matrix) and FRGM (Fabric Reinforced Geopolymer Mortar).

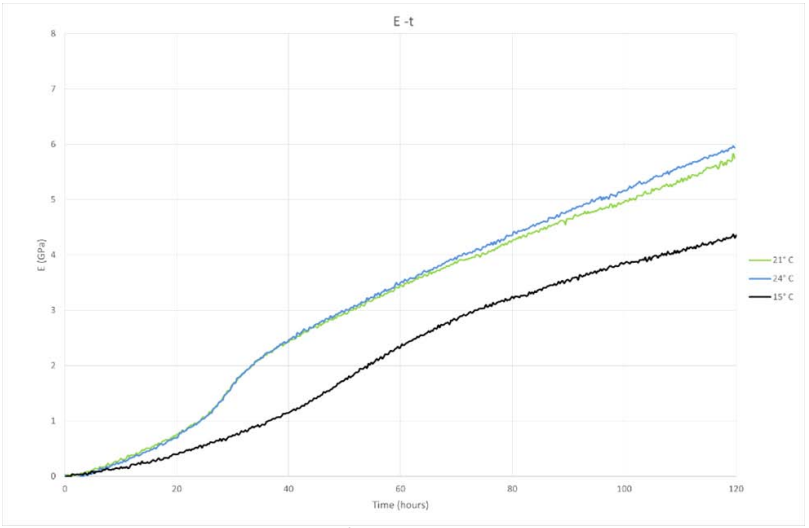

Fig. 4. Results temperature tests.

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Optimal management of the artificial reservoirs for the production of renewable energy and the hydraulic protection of the territory

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Artificial reservoirs are across the world, and their use spans from the traditional management of the water resource to flood reduction operations. The artificial reservoirs provide both direct and indirect benefits to the community. Direct benefits include supply of water for municipal, irrigation, industrial, and hydropower generation. Artificial reservoirs can also be used for recreational purposes and for flood control. Indirect benefits would lead to increased sustainability and natural habitat preservation downstream of the dam [1]. Usually, the presence of artificial reservoirs allowed the socio-economic development of the communities living downstream these infrastructures. But, in many cases, their presence had a significant impact on the surrounding environment, causing remarkable erosion phenomena in both rivers and shorelines downstream the dam. Moreover, it has been observed that the presence of artificial reservoirs caused alteration of the micro-climate of surrounding areas and a significant reduction of the environmental flow. However, the remarkable construction costs, the management issues, and the increased hydraulic risk for the community living downstream make the realization of new artificial reservoirs a complex task. Therefore, nowadays the realization of new artificial reservoirs must be justified only if great benefits are provided to nearby communities and the whole ecosystem. Artificial reservoirs could also be used to mitigate the effects of floods, thus reducing the damages to property as well as the loss of human lives. Nevertheless, this objective might seem in contrast with the traditional purposes of artificial reservoirs: namely, to preserve water volumes to satisfy the water demands by the surrounding communities. However, these contrasting objectives can be reconciled in some cases. Recently, a study commissioned by the Italian Dam Service (Servizio Dighe Italiano) to various Italian Universities (among which the University of Naples Federico II with working group lead by Prof. Domenico Pianese), showed the possibility of using big artificial reservoirs for both management of the water resources and flood attenuation was also demonstrated.
Therefore, in this framework, new rules for the efficient management of the water resource stored in existing artificial reservoirs are required, as their objectives might have changed over the time due to changes in their scopes (irrigation, industrial, hydropower, etc.) as well as in the volumes requested by users. These new management rules must be redesigned to account for the changes in users request, the climatic changes that have led to an increased variability of water resources over time, and to the increasing number of flood events caused by both climatic changes and anthropogenic activities. The main aim is to identify the optimal management rules for artificial reservoirs, given the storage capacity W*, and the time series of inflows. The optimal management could provide benefits to managing authorities and users, allowing an “economically convenient” and sustainable use of the water resource. Moreover, existing artificial reservoirs could considerably mitigate the detrimental consequences of floods. In the near future, especially in emerging countries, new artificial reservoirs will be built for irrigation and water supply for municipal and hydroelectric purposes. The design and optimal management of multi-purpose artificial reservoirs should be carried out taking into account both social and environmental impacts generated with their construction.
However, the optimal operation of artificial reservoirs is not an easy task as the combined management of both water resource supply and storage capacity can lead to conflicting objectives [2]. In the last 40 years, several methodologies have been developed for the optimal management of artificial reservoirs [3, 4], such as Dynamic Programming techniques [5, 6], Shuffled Complex Evolution [7], the Kidney Algorithm [8], the Grey Wolf Algorithm [9], etc. Recently, optimization techniques have been applied to balance the various users demand while ensuring environmental flows [10-12]. The reliability of the results obtained by optimization models is closely related to the robustness of the predictive models of water demands [13]. Genetic Algorithms (GAs) have been widely used

for the optimal management of artificial reservoirs. These algorithms resemble the evolution of living species aiming at finding the optimal candidate solution [14-25].

The aim of this study is to identify and standardize innovative procedure to allow for the optimal management of the water resource and the storage capacity within existing artificial reservoirs. In particular, a new methodology is proposed to identify the optimal management rules for multi-purpose artificial reservoirs. The methodology determines the "optimal management rules" for one or more reservoirs arranged in series/parallel returning the maximum benefit. The long-term variability of inflows and flood events are taken into account as well. This model also takes into account the reservoir operations, such as supplying high-quality water to the different users (municipal, agricultural, industrial, and hydropower), preserving the fluvial ecosystems and the health of the aquatic species living downstream. Moreover, this model is also account for the reservoir capacity to attenuate floods, with subsequent risk reduction for the communities living downstream, and the opportunity to exploiting the water volumes within the reservoir for recreational activities. In addition, the aforementioned model includes the progressive silting of the reservoirs due to the sediments from the upstream basin. These sediments can be dredged, in order to restore reservoir original storage capacity, as well as be reused for shores nourishment operations or for the production of innovative materials for "eco-sustainable architecture" [26]. To simulate the filling or emptying of the reservoir, the methodology is based on a consolidated approach developed by Italian researchers known as "Reservoir water balance method" ("Metodo degli scarti cumulati"). In particular, the proposed methodology is coupled with a stochastic forecast model of the inflows and a Genetic Algorithm (GA) optimizer. In order to maximize the benefits from the water supplied by a stored volume W* within the reservoir, accounting for the possibility of using a portion W* for flood attenuation, the following steps are considered:

1. generation, through the use of stochastic models, of S sets of time series, of length "n" years, of: inflows at the reservoir [25]; equally likely flood events, obtained through the combined use of a PRPN-S type model (Poisson Rectangular Pulses Neymann-Scott.) and a runoff model chosen to best fit the case study; daily volumes of solid material tributary to the reservoir [27];
2. evaluation of the low-flows regime that should be allowed guaranteed in the riverbed sections downstream of the dam in different periods of the year [28];
3. evaluation of the maximum benefit, B_s, achievable for a specific sequence of discharges and for a specific "utilization law" of the artificial reservoir (i.e., for the assigned sequence of values $C\{j,u_t\}$), for each of the S time series generated, within each step of the proposed optimization algorithm (namely a Genetic Algorithm; [25]), and for each "individual" uniquely defined by the choice of a N-tuple of values of the "utilization coefficient" $C\{j,u_t\}$ related to the generic sub-period "j" (j = 1,2,...,N) and to the generic user "u_t" (u_t = 1,2,...,m);
4. ordering, in ascending order, of the values thus obtained and estimation of the probability of exceeding the assigned value of B_s;
5. estimation of the maximum annual benefit "B", for the assigned exceedance probability and for the assigned method of distribution among the various users of the volume "E", in the various sub-periods of the year;
6. identification of the maximum benefit that can be obtained with the assigned exceedance probability, B_{max}, as the laws of use of the outflows vary (and, therefore, as the values of the "coefficients of use" $C\{j,u_t\}$ relative to the generic sub-period "j" (j = 1,2,...,N) and to the generic user "u_t" (u_t = 1,2,...,m) vary, and, therefore, as the "individuals" of the "Population" generated at each step of the GA;
7. iteration of the procedure outlined in points 4) to 7) using an optimization technique based on a GA, so as to progressively increase the value of the maximum benefit that can be obtained;
8. identification of the optimal utilization law for the correct management of the reservoir and the water resources that flow into it starting from the values of the utilization coefficients used to obtain B_{max}.

For each scenario of outflows and sediments arriving at the reservoir, a prefixed number P of so-called "adjustment possibility curves" (E-W) were been constructed. In particular, E is the annual supply guaranteed by the reservoir, and W the useful volume to be kept available inside the reservoir in order to allow the supply of the annual volume E. The E-W curves are able to solve both "design problems" and "verification problems". As a matter of fact, they provide the volume E annually deliverable from the reservoir, as a function of the useful volume W within the reservoir and for assigned values of the "utilization coefficient" $C\{j,u_t\}$ relative to the generic sub-period j (j = 1,2,..., N) and the generic user u_t (u_t = 1,2,...,m). In particular, for each step of the iterative optimization procedure, P

"outflow regulation possibility curves" were been created. Each curve is generated by creating P "individuals", characterized, in turn, by a specific set of values of the utilization coefficients C{j,u_t} relative to the different users to be served, and by a specific set of ratios (E{annual_user}/E{total_annual}) between the annual supply provided to each user and the total annual supply guaranteed by the reservoir, equal to the sum of the different values relative to the individual users. For the identification of the values of B_s and B_{max}, the benefits that can be derived from the supply of water for these uses are added to those related to the use of the volumes upstream of the dam, and to the reduction of the costs arising from any overflow from the stretches of riverbed downstream of the dam.

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NUMERICAL ASSESSMENT OF OUTPUT ONLY DYNAMIC IDENTIFICATION OF SIMPLY SUPPORTED BRIDGES UNDER TRAFFIC LOADS

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Bridges health is an important aspect which needs to be addressed especially in the context of strategical infrastructure management. Several theoretical and experimental frameworks could be followed to obtain a qualitative description of the bridges condition, ranging from visual inspections to more complete experimental campaign relying on several well-established techniques [1-3]. Among all the available methodologies, Operational Modal Analysis (OMA) is certainly one of the branch of Structural Health Monitoring (SHM) which over the last 30 years has worked its way due to the several benefits that brings with it. Although, the most explored framework is much more oriented to the environmental excitation, traffic induced vibrations can result in good modal estimation of the dynamic features of the manufact as well. As a matter of fact, these methodologies are also contemplated by several national codes [4] alongside some other solutions as the employment of Weigh In Motion (WIM) devices. However, it is possible to highlight how in the context of modal estimation of the bridges in their operative condition the adoption of WIM devices is not crucial. Indeed, it is possible to demonstrate, numerically, how the modal identification of the analyzed bridge structure excited by traffic, is not only feasible but also reliable. Here, the bridge structure is discretized and analyzed by means of Finite Element Method (FEM). Enriched Timoshenko beam element capable to represent even the non unform torsion phenomenon has been developed in order to describe the Vehicle Bridge Interaction (VBI) phenomenon (Fig. 1). The secondary systems, namely the vehicles, are all described introducing a simplified dynamical model, namely the Quarter-Car (QC) system. This system is capable of describe the dynamical behavior of single vehicle axles, hence each secondary system is described by a number of QCs equal to the number of the owned axles. Furthermore, the calibration of all the dynamic features associated with the QCs are retrieved by an algorithm based on literature consideration about the riding frequency range [5,6], which need to be ensured. Moreover, the numerical description of the VBI phenomenon cannot be restricted only to the traffic and the bridge because several environmental factors play a significant role especially in modal estimation. In this regards, several disturbing phenomena are introduced in the numerical analysis and described hereafter. The road roughness is a physical entity which can negatively impact the analysis as function of its severity. Road profiles can be generated by mean of the standard ISO 86096 [7] which divide the road roughness profiles in several categories and by choosing the corresponding Power Spectral Density (PSD) value it is possible to select the desired situation. Furthermore, one of the most critical phenomenon in the modal estimation context is the variation of the environmental temperature. This point can be introduced in the numerical model considering the elasticity moduli of the bridges' material. As a matter of fact, several mathematical relations can be found in the literature capable to describe the trend of the Young modulus of the concrete against the temperature. Subsequently, two different source of noise are considered in the simulations, as the environmental and the instrumental noise. The environmental noise is introduced in the simulations by means of stochastic equivalent generalized forces. These are calibrated considering Root Means Square (RMS) typical ranges [8] in term of accelerations and computing by trial and error procedures the forces which need to be applied to the structure in order to achieve equivalent

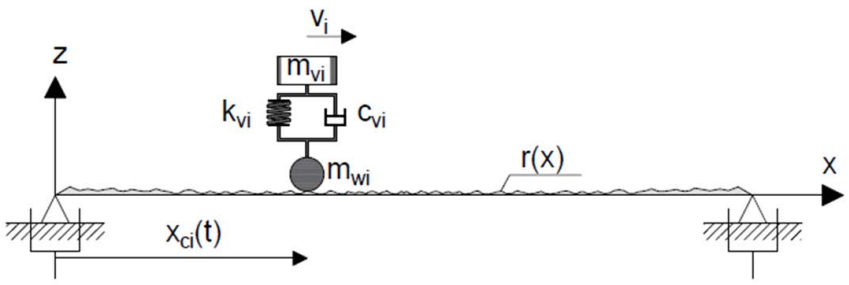

Fig. 1. i-th QC moving along the bridge.

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effects. On the other hand, the instrumental noise is implemented by some well-established techniques which are capable to contaminate the signals considering the desired Signal-to-Noise Ratio (SNR). According to the described numerical framework, it is possible to carry on several numerical simulations able to represent different real scenario. Indeed, it is possible to model classical OMA tests based on the environmental vibrations, OMA tests where the bridges are in operative conditions and simulate both continuous and periodic monitoring system on the investigated structures. In this work an experimental periodic campaign is illustrated. Different tests are assumed to be performed, in particular 4 experimental campaign are simulated with two different test layout which are reported in Table 1.

Tab. 1. Periodic tests description.

Test	Date	Start	End	T_1 [°C]	T_2 [°C]	T_3 [°C]	T_4 [°C]	Excitation
A1	15/01/2018	08:30	11:30	6.04	6.76	7.34	7.99	Traffic
A2	15/01/2018	16:00	18:00	12.32	12.51	12.72	12.73	Traffic
A3	15/01/2018	22:30	00:30	7.84	8.14	7.15	7.07	Environmental
B1	15/04/2018	08:30	11:30	12.13	13.23	14.71	15.44	Traffic
B2	15/04/2018	16:00	18:00	19.22	19.43	19.43	19.12	Traffic
B3	15/04/2018	22:30	00:30	8.21	8.55	8.17	8.00	Environmental
C1	15/07/2018	08:30	11:30	23.07	23.57	24.04	23.83	Traffic
C2	15/07/2018	16:00	18:00	26.49	26.45	26.39	25.83	Traffic
C3	15/07/2018	22:30	00:30	23.23	22.94	22.49	23.14	Environmental
D1	15/10/2018	08:30	11:30	20.66	22.23	22.66	22.17	Traffic
D2	15/10/2018	16:00	18:00	19.22	19.77	20.52	20.49	Traffic
D3	15/10/2018	22:30	00:30	16.46	15.93	15.63	16.46	Environmental

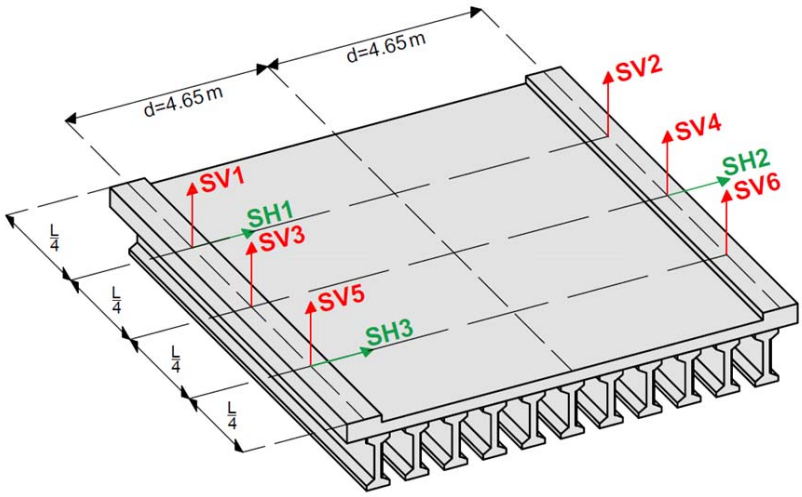

Fig. 2. Sketch of the sensor's layout.

A simplified model of the bridge cross-section is reported in Figure 2 together with the hypothesized sensor layout. Here, the traffic is described considering two lanes each one holds one direction of traffic flow. The QC mechanical parameters are calibrated by referring to a real WIM database [9]. The environmental temperature variations are retrieved considering the recording associated with to a real measurement station placed in Rome and named AL007. The environmental noise is calibrated by means of equivalent stochastic forces where the is equal to 0.0012 (Fig. 3) and the SNR associated with respect to the instrumental noise is set to 5%. Road roughness profile belongs to class A of the aforementioned ISO standard. The resulting time histories are finally processed considering some well testes algorithm in the OMA framework, such as the Frequency Domain Decomposition (FDD) (Fig. 4 holds the firsts singular values of the PSD matrix) and the Cov-SSI (Fig. 5 show the stabilization diagram for the test A).

Meanwhile the results achieved are reported in terms of relative errors in Tab. 2 for the two aforementioned techniques.

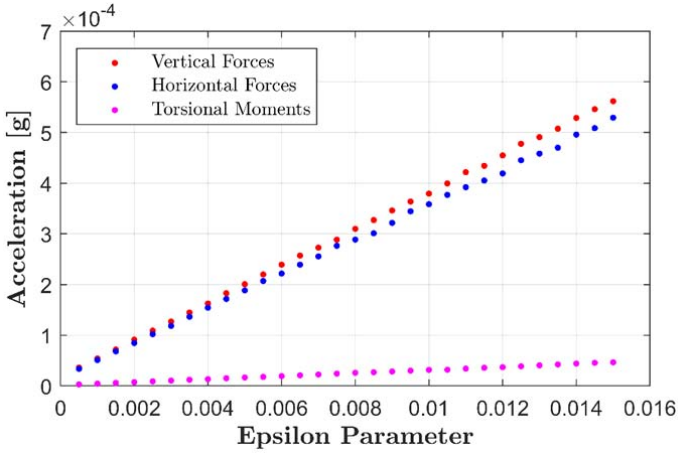

Fig. 3. Epsilon parameter calibration.

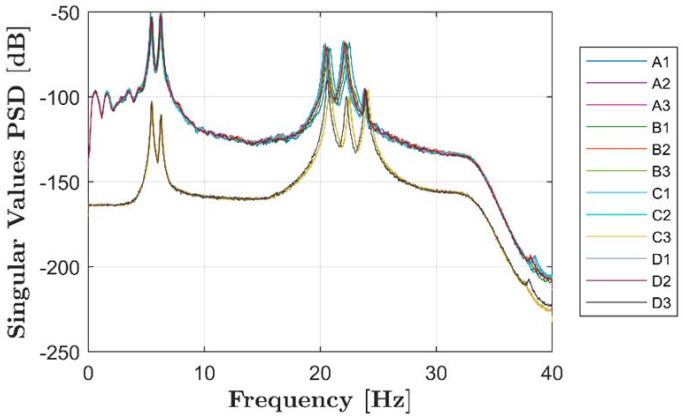

Fig. 4. First singular values of the PSD matrix FDD.

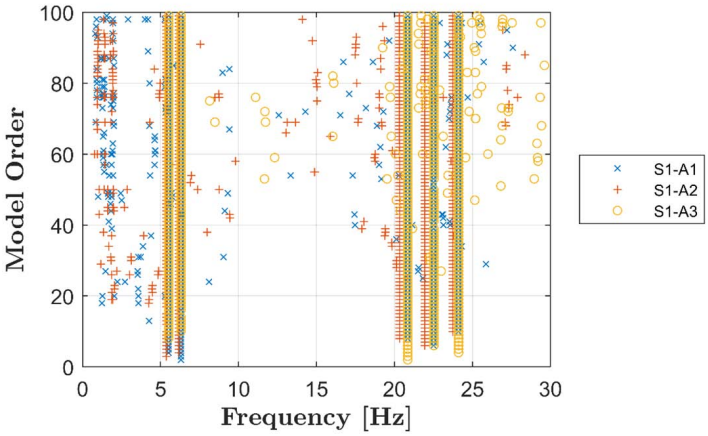

Fig. 5. Stable plot Cov-SSI, test A.

Tab. 2. Relative errors natural frequencies estimation.

	FDD					CoV-SSI				
	f1 [%]	f2 [%]	f3 [%]	f4 [%]	f5 [%]	f1 [%]	f2 [%]	f3 [%]	f4 [%]	f5 [%]
S1-A1	0.22	0.09	0.92	1.05	1.14	0.21	0.06	0.81	1.03	1.14
S1-A2	2.00	1.70	2.60	2.88	2.18	2.11	1.59	2.66	2.88	2.24
S1-A3	0.07	0.14	0.79	1.12	1.10	0.03	0.08	0.85	1.01	1.15
S1-B1	0.24	0.19	0.80	1.09	1.11	0.09	0.00	0.81	1.01	1.12
S1-B2	0.07	0.30	0.95	0.90	1.07	0.13	0.07	0.81	0.96	1.12
S1-B3	0.18	0.05	0.87	0.92	1.11	0.02	0.07	0.85	1.02	1.13
S1-C1	0.02	0.09	0.81	0.96	1.10	0.11	0.06	0.79	1.00	1.10
S1-C2	0.01	0.18	0.77	0.98	1.06	0.12	0.09	0.75	0.95	1.11
S1-C3	0.27	0.44	0.53	0.55	0.92	0.02	0.06	0.81	0.97	1.11
S1-D1	0.24	0.02	0.85	0.94	1.16	0.14	0.00	0.83	0.99	1.10
S1-D2	0.14	0.13	0.81	1.02	1.15	0.18	0.06	0.80	0.99	1.11
S1-D3	0.07	0.07	0.74	1.02	1.18	0.02	0.06	0.80	0.98	1.12

The presented results show the good accuracy of the investigation alongside the feasibility of the developed numerical framework which can be exploit in order to perform several kind of structural analysis in efficient manner.

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STRATEGICAL STRUCTURE ASSESSMENT BY BOUNDING SURFACE MATERIALS IN OPENSEES

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Strategical steel infrastructure assessment is a crucial task which needs to be performed in order to ensure reliability of several critical systems such as telecommunication and transportation. Furthermore, the interaction between the structures and the soil needs to be addressed. Indeed, is not uncommon the occurrence of site effects or the triggering of negative phenomena as the in the liquefaction case, that may jeopardize the structural stability. In this context the development of new tools aimed to the simulation of several scenarios is a fundamental step to their assessment. Indeed, over the last decades several software mainly based on the Finite Element (FE) framework were developed in order to exploit different techniques and the introduction of new material concepts, which led to increasing in the numerical accuracy and efficiency. Among, all the several FE software always more scholars are moving to the open source context which allows for much more flexibility. In this regard, OpenSees (OS) [1] is a FE framework, mainly based on c++, whose structure is highly customizable thanks to its strong object oriented structure. Furthermore, the versatility together with the capability of performing analysis characterized by large domain or parametric analysis by exploiting the parallel computing paradigm is certainly one of the key features which placed the software in a sweet spot especially for research purposes. In this work, OS is employed in order to assess implementation of a new steel material, namely SANISTEEL [2], which lies among the Bounding Surface (BS) concept [3]. The great success gained by the BS framework is essentially due to the possibility of describing different materials referring to the same simple theoretical concept. Here, uniaxial formulation of SANISTEEL is compared, in term of cyclic and monotonic response, against the Steel4 material, which is one of the many already present in the OS libraries. Although, Steel4 [4] is based on a complete different theoretical formulation, it allows to model the same response characteristics as the isotropic and kinematic hardening phenomena. The calibration of the two material is performed considering real experimental data on structural steel S275 [5] (Fig. 1 and Fig. 2). Once, that all the characteristic parameters are known, by mean of a trial and

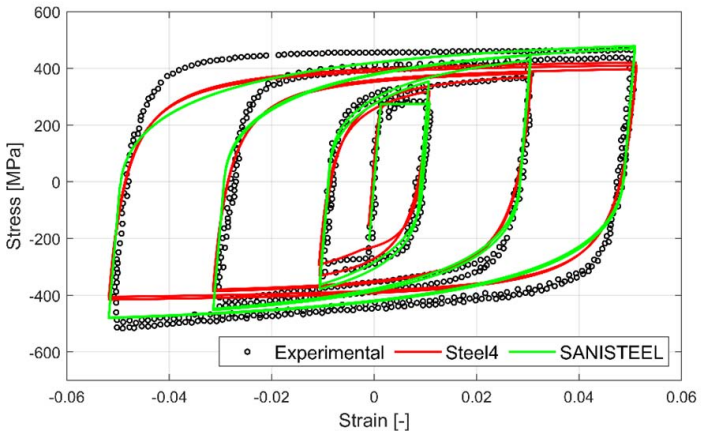

Fig. 1. Monotonic calibration of SANISTEEL and Steel4.

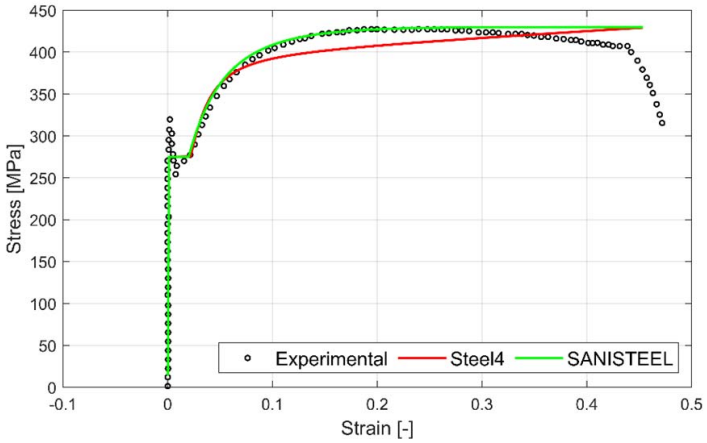

Fig. 2. Cyclic calibration of SANISTEEL and Steel4.

error procedure, some numerical analysis are introduced in order to model a simple structure and its interaction with the soil, in a simple manner considering the substructures approach. The structure is a simple Moment Resisting Frame (MRF) which is depicted in Fig. 3 where are reported the main geometrical properties together with the steel section employed in the analysis. From a numerical standpoint every structural element is modelled by referring to a force-based beam FE alongside the concept of distributed plasticity where the steel sections are modelled referring to a fiber method. Nodes at the same level are restrained introducing and equal-dof relation,

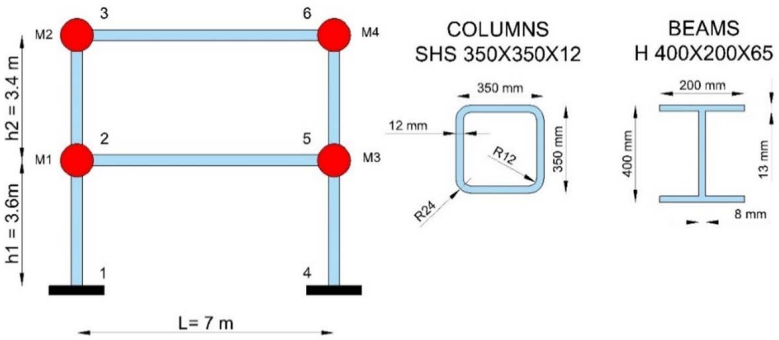

Fig. 3. MRF: layout and geometric description.

meanwhile the base of the MRF is considered fixed. From a soil perspective, it is assumed that the MRF is placed above 10 meters thick layer of Toyoura sand. To this end, a real accelerogram recorded during the 2016 Central Italy Earthquake from the Accumoli (ACC) station has been downloaded from the Engineering Strong Motion Database, whose features are reported in Table 1.

Tab. 1. Main features of the selected motion.

Date	Region	Location	Mw	PGA [g]
01/18/2017	Central Italy	Accumoli (ACC)	5.1	0.104

The Accumoli recording station lies on a Class A site as for the EC8. The site can be assimilated into a rock geological formation, meaning that it can be assumed as an outcropping bedrock. For this reason, a site response analysis is performed to consider the filtering effects exercised on the ground motion by the soil. The new acceleration time-series is then applied to the structure. Consequently, the ground motion recording is applied to a mono-dimensional 10 m-thick soil column discretized by 20 quadrilateral elements providing a coupled hydro-mechanical formulation and a single-stabilization point technique. The soil constitutive behavior has been modelled using the bounding surface material SANISAND [6].

To investigate the performance of the structural material considering different ground motion intensities, the same recording has been scaled achieving a Peak Ground Acceleration (PGA), equal to 0.3g, 0.1g and 0.05g. The motions at the ground level obtained after the site response analysis are plotted in Fig. 4 and are applied to the fixed nodes of the structure, which are the base nodes in this case, considering a uniform pattern excitation. The time step for the simulations is equal to 0.001 s, discretizing the 45.00 s duration of the motion into 45000 steps. The evaluation of the behavior of the two steel materials is carried out considering the time series of the absolute top floor displacement of the structure (Node 3 in Fig. 3). The evolution with respect to time of the top displacements are depicted in Figures 5-7, where it is possible to draw same considerations associated with

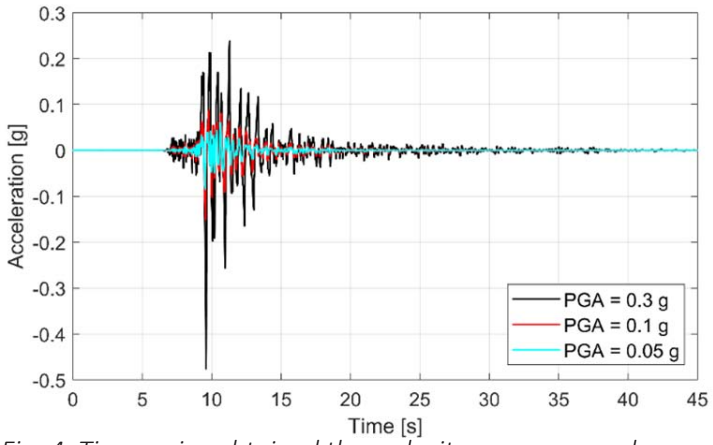

Fig. 4. Time series obtained through site response analyses.

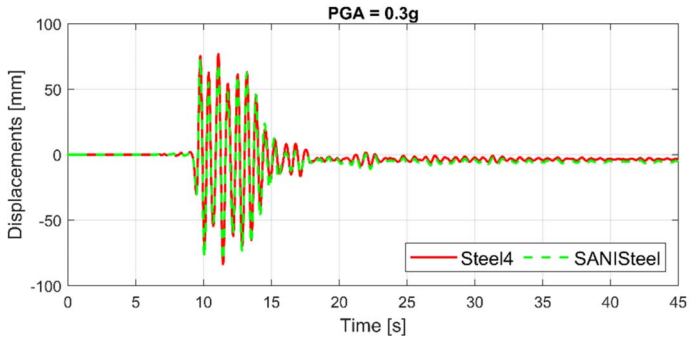

Fig. 5. Time series of the top structural displacement considering different input excitations: 0.3g.

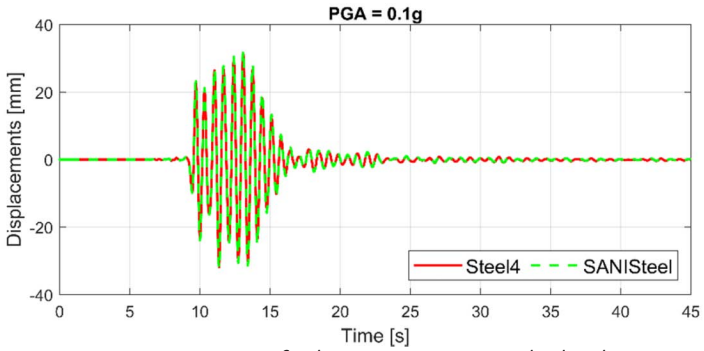

Fig. 6. Time series of the top structural displacement considering different input excitations: 0.1g.

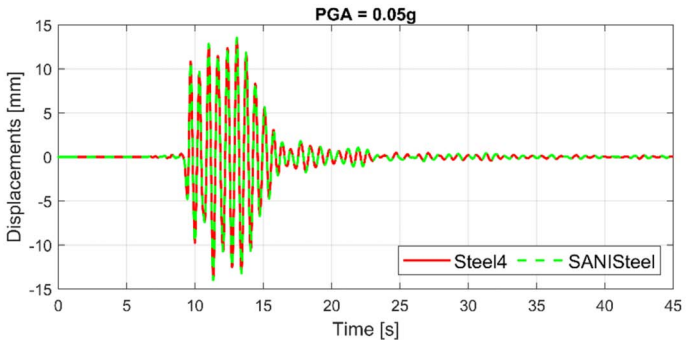

Fig. 7. Time series of the top structural displacement considering different input excitations: 0.05g.

the excitation levels assumed in the analysis. Indeed, for low level of excitation, (0.05g and 0.1g) responses associated with the two different materials are perfectly overlapping. This situation is essentially due to the elastic responses of manufact for the included levels of excitation. Meanwhile, in the 0.3g case scenario (Fig. 5), a slightly different response is observed, especially in terms of residual displacements.

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IMPLEMENTATION AND USE OF THE BOUNDING SURFACE PLASTICITY GEOMATERIAL NTUASAND02

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The modelling of the response of soil subjected to liquefaction represents one of the most challenging problems, from the computational point of view, in geotechnical earthquake engineering. Although reliable predictions can dramatically improve the effectiveness of the design procedures in areas subjected to liquefaction, the simulations properly describing this phenomenon and its evolution are quite complicated.
It is well-known that the elastic-perfectly plastic models are not able to reproduce the accumulation of high plastic-strain levels in granular soils, and, to overcome this limitation, specific constitutive platforms were developed accounting for different initial soil conditions through a single set of parameters (see [1]) using different theoretical approaches; in this sense, the bounding surface theory resulted successful. However, these models need to be adopted in numerical codes to simulate liquefaction-related phenomena. For this reason, a balance between analytical complexity and ease of implementation in numerical tools needs to be achieved.
Based on the above remarks, the present paper deals with the implementation of the constitutive model developed by Papadimitriou and Bouckovalas [2], referred to as NTUASand02, into the finite element framework OpenSees [3] to increase the set of available constitutive models for the solution of soil liquefaction problems. This also highlights the flexibility of the OpenSees numerical framework to support user-defined materials, and the ability of the constitutive platform to interact with finite element codes.
The Papadimitriou and Bouckovalas [2] constitutive model derives from Manzari and Dafalias [1] and represents the multiaxial formulation of the model developed by Papadimitriou et al. [4]. Due to its modularity and flexibility, the Papadimitriou and Bouckovalas [2] model was modified and built in the FDM code FLAC by Andrianopoulos et al. [5], considering a vanished elastic region. Furthermore, Miriano [6] supplied the original version of the model in Abaqus.
The model is based on the bounding surface theory and is characterized by four different surfaces: yield, dilatancy, critical and bounding. The yield surface is represented by a cone, with the apex in the origin of axes in the p-q plane (Fig. 1a). On the other hand, the intersection of these surfaces with the π-plane (normalized with respect to the mean effective pressure) is reported in Figure 1b.

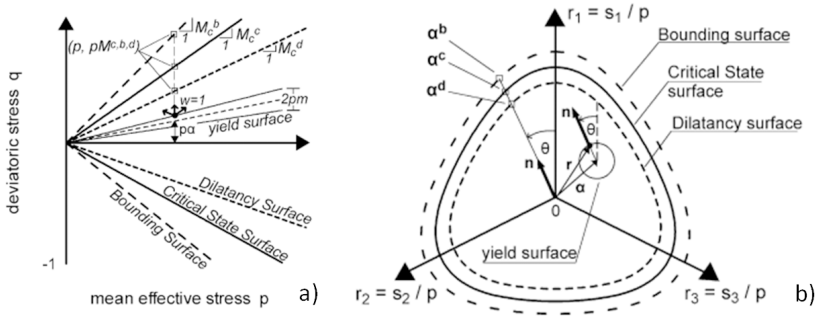

Fig. 1. Constitutive framework in both triaxial (a) and deviatoric (b) planes (from [2]).

Here, the yield surface is represented by a circle with a "diameter" directly related to the constitutive parameter m, while its location in the plane is defined through the back-stress ratio α. The parameter m assumes a constant value, while α can evolve so that only kinematic hardening can be activated and accounted for. Fig. 1b clearly shows that the former three surfaces (dilatancy, critical and bounding surfaces) are wedge-shaped in the π-plane. In particular, the openings of the dilatancy and the bounding surfaces depend on the stress ratio at the critical state, which is kept constant, and on the state parameter ψ, indicating "how far" the current void ratio is from the void ratio at the critical state. The loading direction is adopted to map the conjugate critical, bounding and dilatancy back-stress ratios α^i (cfr. Fig. 1b).
The main improvements provided by NTUASand02 with reference to the Manzari and Dafalias [1] model consist of a scalar value h_f that directly scales the plastic modulus in order to consider fabric tensor evolution and an elastic shear modulus that recalls the Ramberg-Osgood formulation to reproduce the response at low-shear strain

levels in a more reliable manner.
The implementation of the model has been carried out by taking advantage of the OpenSees platform [3]. OpenSees is an object-oriented, open-source software framework, mainly developed for research activities, which allows users to develop parallel and serial applications for systems subjected to earthquake loadings, among others. It is written in C++ and the source code is freely available. The implementation of a new multiaxial material requires the addition of a new subclass of the nDMaterial class.
The subclass not only contains all the mandatory methods required by the nDMaterial class, such as those useful for the definition of the current state, but also those aiming at describing the nonlinear response provided by the constitutive framework. For this reason, additional methods for the calculation of the state parameter, Lode angle and elastic moduli are implemented, among others.
Different integration scheme performances have been assessed, and Modified Euler with stress correction developed by Sloan et al. [7] was adopted.
The implementation of the constitutive model proposed by Papadimitriou and Bouckovalas [2] into the OpenSees framework is verified through the simulation of drained and undrained, monotonic and cyclic triaxial tests and direct simple shear tests (DSS) available in the literature. All the analyzed tests have been carried out within the VELACS [8] project on Nevada sand. The comparative analysis of the simulations performed by various Authors using different versions of the model will be discussed. Constitutive parameters calibrated by [2] for Nevada sand are adopted. In the following, for the sake of brevity, the undrained tests only are detailed.
As in the VELACS report [2], the undrained load-control cyclic direct shear test (DSS) adopted to calibrate and validate the model was performed at the element level on a soil sample of Nevada sand at 60% relative density which corresponds to a 0.66 initial void ratio. Firstly, the consolidation stage with effective consolidation stress equal to 160 kPa was performed and then cyclic shear stress with an amplitude of 13.7 kPa was applied. An initial load offset equal to 5.9 kPa was added before the cyclic load application. Loads are applied as nodal forces.
The τ-σ_y and τ-γ curves resulting from the simulations of the same DSS cyclic undrained test performed by Papadimitriou and Bouckovalas [2] and Miriano [6] are compared to those from the current implementation. In Fig. 2, the tests using the quadUP (Fig. 2a and Fig. 2b) element available in OpenSees are reported.

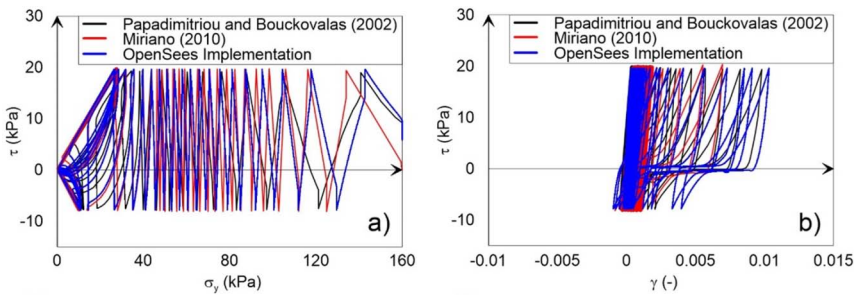

Fig. 2. τ-σ_y and τ-γ curves obtained using plane-strain formulation from [6] (red curves), [2] (black curves) and current implementation (blue curves) using quadUP (a)-(b) element.

The plots in Fig. 2 show valuable results provided by the current simulations in OpenSees (blue curves), if compared to those carried out by Papadimitirou and Bouckovalas (black curves). However, better performance is achieved by comparing simulations by Miriano [6] and the current implementation. The results obtained from the DSS test highlight a stiffer response at low strain levels exhibited by the current implementation and by the Abaqus User MATerial (UMAT) developed by Miriano [6] if compared to that resulting from the simulations provided by Papadimitriou and Bouckovalas [2]. On the contrary, good accordance is reached when the strain level increases. In addition, the classical butterfly-shaped τ-σ_y curve appears properly reproduced. It is worth noting that different numerical platforms are adopted for the simulations.
Then, a cyclic triaxial test is conducted on a sample with 40% relative density during the VELACS project [2]. In the current simulations, the 3D hexahedral SSPbrickUP element has been selected. The loading process consists of an anisotropic consolidation of 80 kPa mean effective pressure with 26 kPa offset followed by a cyclic deviatoric load of 43.1 kPa. The resulting stress paths and hysteretic loops are shown in Figure 3.
From the simulations of the cyclic triaxial test, general good accordance is observed. However, the comparison with the original implementation provided by Papadimitriou and Bouckovalas [2] highlights a higher strain reached by the OpenSees implementation. On the other hand, at a low strain level, the accordance is satisfactory.
In the end, three undrained monotonic triaxial tests performed with an initial void ratio equal to 0.66 at different confining pressures (p=40 kPa, 80 kPa, 160 kPa) are here simulated within the verification procedure. SSPbrickUP

hexahedral element is considered, and the resulting deviatoric stress-axial strain responses are shown in Figure 4.

The undrained monotonic tests show a complete agreement in the comparison between the results obtained in the current study and those resulting from [6].

In conclusion, element tests in different conditions have been performed in the verification procedure of the implementation of the constitutive model developed by Papadimitriou and Bouckovalas [2] for liquefaction analyses in OpenSees.

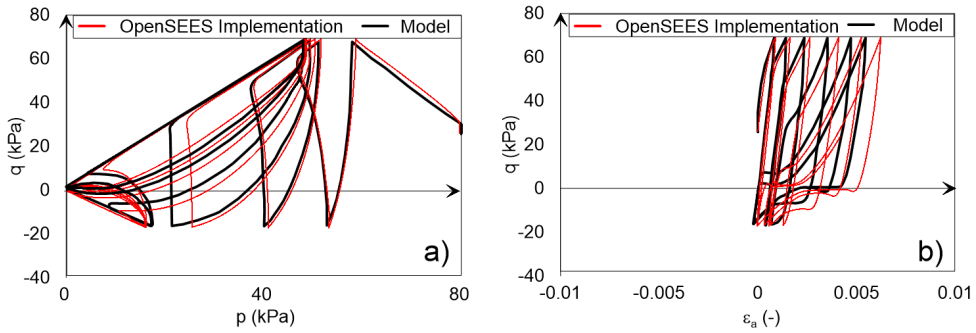

Fig. 3. Deviatoric stress-mean pressure (a) and deviatoric stress-axial strain (b) curves obtained from [2] (black curve) and current implementation adopting a 3D SSPbrickUP element (red curve).

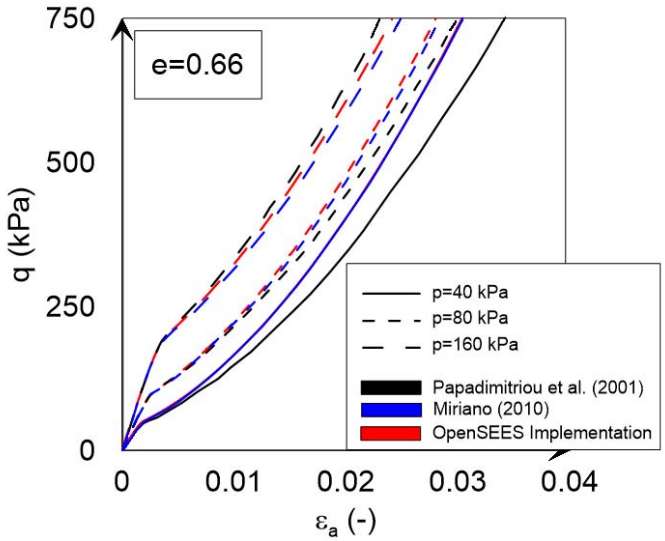

Fig. 4. Simulations of undrained monotonic triaxial tests using the implemented constitutive model (red curve).

Generally, good accordance between the OpenSees simulations and the results provided by various Authors is observed. The discrepancies can be related either to the different finite element frameworks (e.g., Abaqus and OpenSees) or to the different approaches (e.g., finite elements and finite differences).

The capabilities and the flexibility for the implementation of user-defined materials make OpenSees an extremely valuable tool for geotechnical earthquake engineering applications and for the evaluation of the performances of geotechnical systems.

Finally, it can be stated that the model is successfully verified and its application to boundary value problems is needed.

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Model updating of a historical structure

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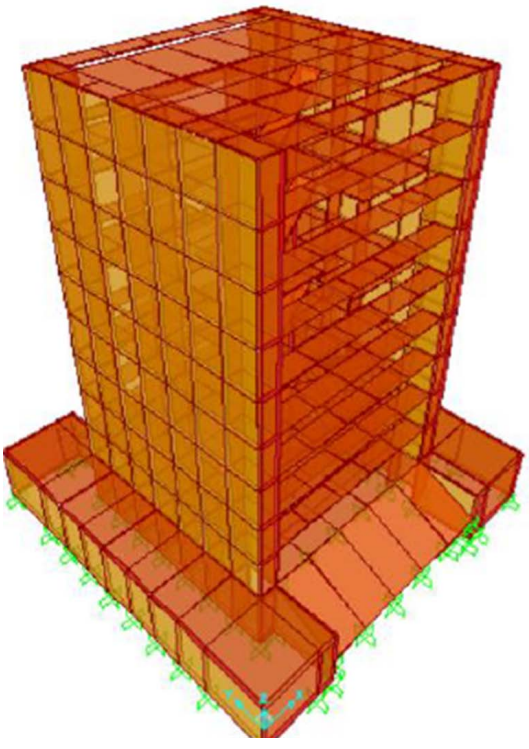
Knowledge of the state of health of historical structures is becoming increasingly important nowadays due to the great heritage value of the historic buildings. However, the use of destructive tests for material characterization is not allowed in most cases and different strategies must be adopted. In recent years the choice increasingly falls on the combination of finite element models (FEM) with vibration-based dynamic tests to reduce the gap between numerical and experimental modal parameters and indirectly determine the unknown structural mechanical parameters. However, although it is a frequently used method, solving an inverse problem requires considerable attention. They range from the correct construction of a reliable finite element model to the choice of the updating variables. An important problem is the high computational burdens that often characterize these procedures. For this purpose, surrogate models, capable of replacing the complete finite element model and reducing computational time, can be adopted as strategies. Models such as Douglas-Reid (DR) or Response Surfaces Method (RSM) are the most used in the literature, but no studies have been found in the literature in which the reliability of surrogate models is tested in an exhaustive manner [1, 2]. Therefore, the need to understand whether models such as Douglas Reid can be used in a continuous monitoring system to indirectly determine the mechanical parameters of the structure over time becomes increasingly binding [3].
The common thread of the present work is a simulated data analysis to test the reliability of Douglas Reid method to Structural Health Monitoring purpose. A real case study was selected to test the procedure, the Tower of the Nations located in the Mostra D'Oltremare urban park in Naples together with its Finite Element Model (see Fig. 1) and a natural frequency time series was generated considering temperature influence and noise measurements (see Fig. 2) [4].

(a)



(b)

Fig. 1. Tower of the Nations (a) and the FE model (b).

Temperature influence has been considered defining expressions for the values of the elastic moduli of concrete and tuff masonry as a function of temperature (Equations 1 and 2) and several analyses were repeated to calculate the corresponding natural frequencies considering a measured temperature time series [5-7]. The effect of experimental uncertainties has been taken into account by adding random noise to the simulated natural frequency time histories. DR method has been used for continuous model updating with the support of the simulated monitoring data in order to check its robustness to noise polluting the data and accuracy of structural parameter estimates under varying environmental conditions.

$$Ec(T) = -125T + 29130 \quad (1)$$

$$Et(T) = 9T + 920 \quad (2)$$

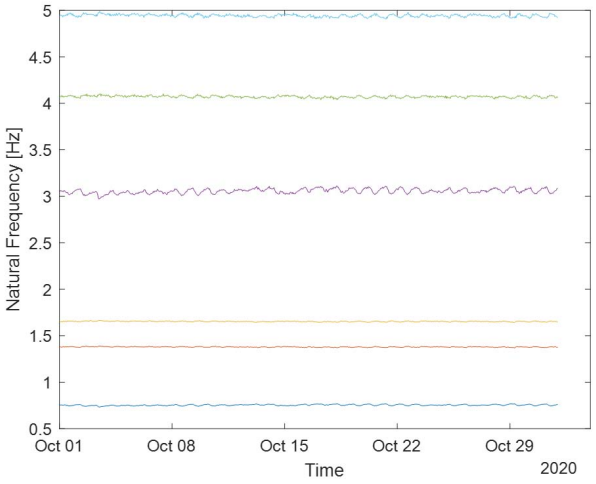
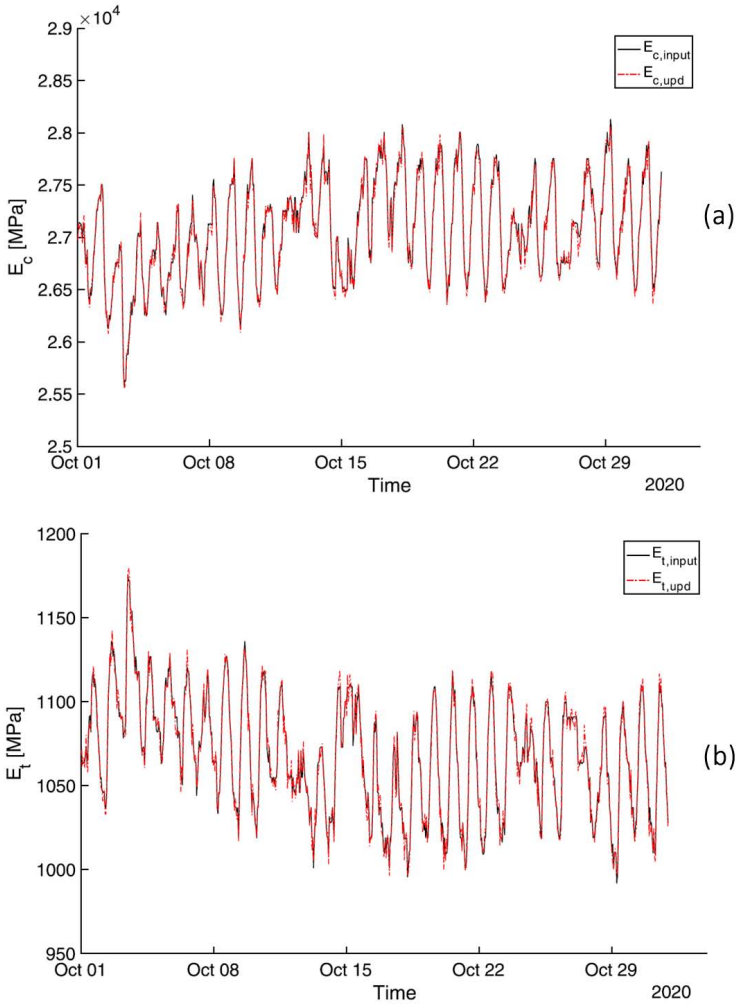


Fig. 2. Simulated natural frequency time series with added random noise.



As a results, accuracy is evaluated by comparing the results of continuous model updating in terms of optimized values of elastic moduli of the materials with the corresponding input values of the FE model which have been used for natural frequency time series simulation (see Figure 3). The results show a good agreement between the optimal values of the variables and the corresponding simulated variables with observed maximum deviations of about 0.8 % for the Young's modulus of concrete, and about 1.5 % for the Young's modulus of tuff masonry. The evolution of the updating variables over time can be effectively monitored provided that the corresponding values are within the predefined ranges for the setting of the DR model. In the future, it could be interesting to understand what happens if, in the hypothesis of structural damage, the update parameters do not fall within the ranges set for the update variables.

Fig. 3. Comparisons of time series of optimized updating variables and corresponding input values for data simulation for concrete (a) and tuff masonry (b).

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COMPUTATIONAL DESIGN METHODS FOR THE DIGITAL SURVEY OF THE HERITAGE

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The earlier Computer Aided Design (CAD) software, looking upon the computer as a digital drawing board, were the extension of traditional methods of the technical drawing. Afterwards, the 3D digital modelling tools widened the possibilities of the aided design. The first approach is called computerization, meaning a process whereas already-known entities are inputted, manipulated and memorized into an informatics system. On the other hand, the algorithmic modelling software allow the control of the process through which the computer is building the shape, leading to the computation as a new use stage of the computer founded on the shape's determination following logical-geometrical methods [1].
Whereas usually the average user of a 3D modelling software isn't able to take full advantage of the informatics potential at his disposal, there are already several Visual Programming Language (VPL) environments, featuring a node-programming where the building of the algorithmic definitions take place through the composition of objects representing the essential elements of the computer graphics, mixed to macro-elements for the manipulation of geometric entities.
Beyond the mere technological aspect, it is worth noting that an issue of process and methods arises. The modelling process in this way configures itself as a strong paradigm change, where the focus shifts from the act of drawing itself to the building and generation methods of it. Here the field of the Digital Explicit Design is accessed [2], whereas new creative ways in the procedure's construction can be explored.
Talking about the digital survey of the heritage, these computational design methods [3] are able to give a new perspective on the survey process, mostly in the post-processing phase of the raw data, in this case the point cloud. No matter if the cloud is proceeding from a Terrestrial Laser Scanner (TLS) or photogrammetry methods, it's still a numeric model: a huge number of coordinates, RGB and intensity values, to be converted in an architectural model, resulting from a detailed process of interpretation and so in a continuous model. In order to obtain valid results in this regard [4], it's crucial the issue of the computational design methods, whereas the target is not a mere-automatic meshing process of the points, but a supervised modelling leading to geometrical entities represented by mathematical 3D surfaces (NURBS) [5].

Fig. 1. Colegio de San Pío V, Valencia (source: https://museobellasartesvalencia.gva.es/va/).

This paper intends to show an application of these methods to a specific case of a column, an object of quite easy shape but still capable of many analysis and interpretations, in its role of ancestral architectural item [6]. Herein some aspects are discussed referring to the *Colegio de San Pío* V, a XIII century building housing the *Museo de Bellas Artes de Valencia* (Fig. 1), a very important art gallery in Spain, subject of a digital survey in September 2022. The experimentation is developed in Grasshopper, the VPL environment included in Rhinoceros, a NURBS modelling software.
The first Grasshopper definition (Fig. 2) is aimed to perform a segmentation of the point cloud in its three semantic parts: the basement, the shaft the capital. Giving in input the point cloud of the column, the first operation is to move it upon an XY plane placed on the origin of the coordinate system, so to deal with positive

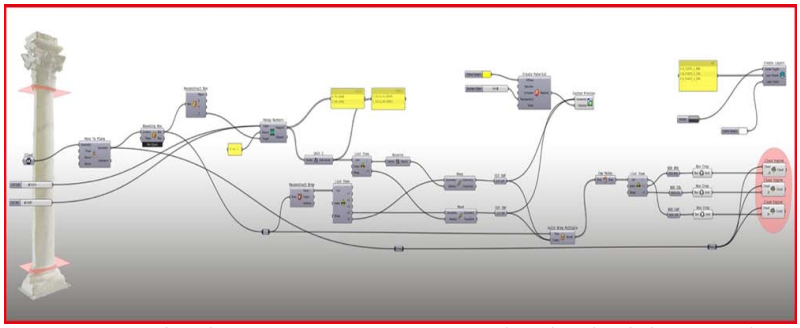

Fig. 2. Point cloud semantic segmentation (author's elaboration).

coordinates and make the elaboration process lighter. A bounding box of the cloud is created and- by deconstructing it- used to generate cut planes along the Z axis, remapping the domain of the box itself. By using two number sliders is so possible to explore the vertical domain of the column and place the cut planes where they're supposed to be. Afterwards, the main bounding box is splitted by these planes, and so are generated three boxes used to crop the main cloud, then automatically baked in Rhinoceros environment.

Right after, another definition has been implemented, aimed to perform different methods of modelling and so extract sensitive data to be useful in further analysis (Fig. 3). Giving in input the point cloud of the shaft – obtained earlier with the first script – the first phase is to divide its vertical domain in N parts (with N given in input through a number slider), identifying a series of horizontal frames to be used as section planes of the cloud. Once the N point cloud is achieved, the algorithm fits a circle through each one, so obtaining N circular sections; this operation is crucial, as represents the first shift from the numerical model to the NURBS field, as each section is a NURBS curve.

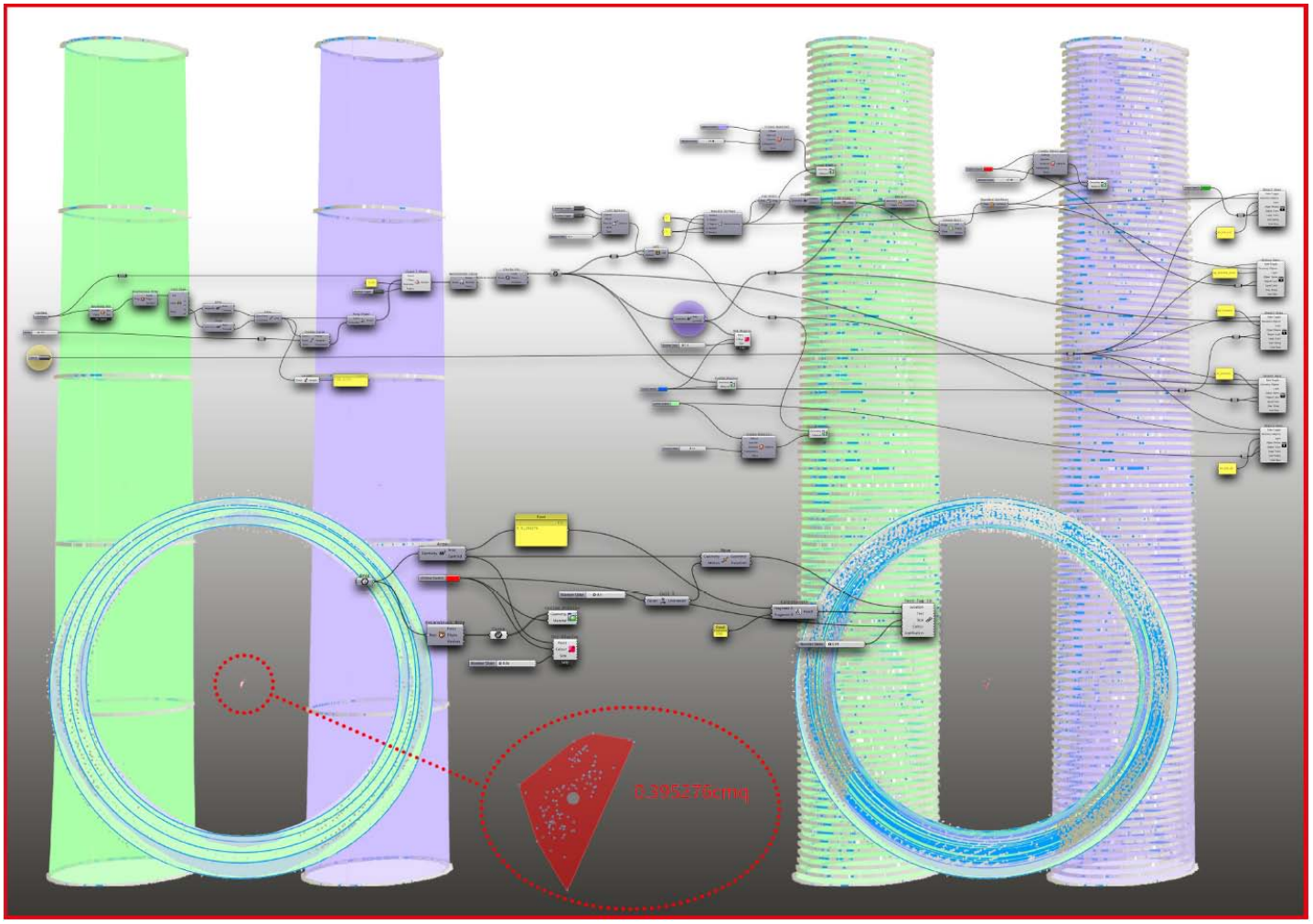

Fig. 3. Algorithmic modelling (author's elaboration).

Once obtained the circles, the definition starts with the modelling operations in two subsequent steps: firstly, performing a loft operation, so obtaining a NURBS surface that exactly fits the sections; right after, this surface is rebuilt as a NURBS surface with given grade and number of control points. These two surfaces are representing two possible ways of modelling and interpreting the raw data: the first one is an exact reconstruction, quite

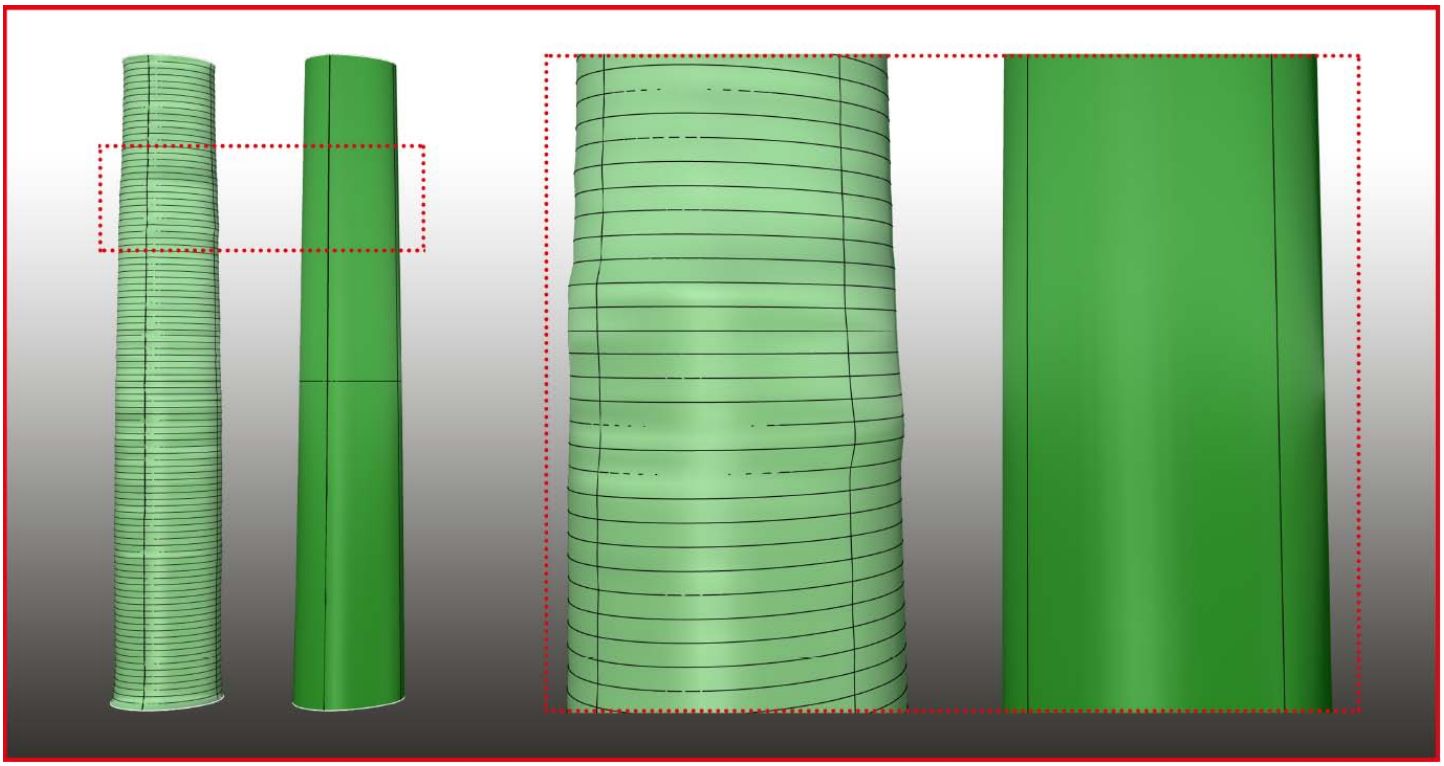

Fig. 4. Lofted and rebuilt NURBS surfaces (author's elaboration).

adherent to the numeric model (depending on the density of the sections), while the second it's an almost-critical reconstruction, less tight-fitting to the real column but with a geometrical connotation, to be describing an ideal shape [7] (Fig. 4). As the circles resulting from the sections are not concentric, the definition estimates a convex hull so to obtain a surface of dispersion of the centres. Finally, another bake operation delivers in the model environment the circles fitted through the point cloud sections, its centres as point objects, the lofted surface and the reconstructed regularized one.

Based on the processed NURBS elements, the last script (Fig. 5) is intended to analyse this post-processed data so to obtain focused interpretations: the aim is to calculate local deviations of the column's axis, so to identify irregularities in the shape which may proceed from local instabilities.

Giving in input the circles and the lofted surface, the first step is to build consecutive vectors, linking each centre with his consecutive, and at the same time placing vertical unit vectors on the points; having this vectors pairs is possible to compute the local angular deviation. These values are then remapped in colour scale, giving an RGB

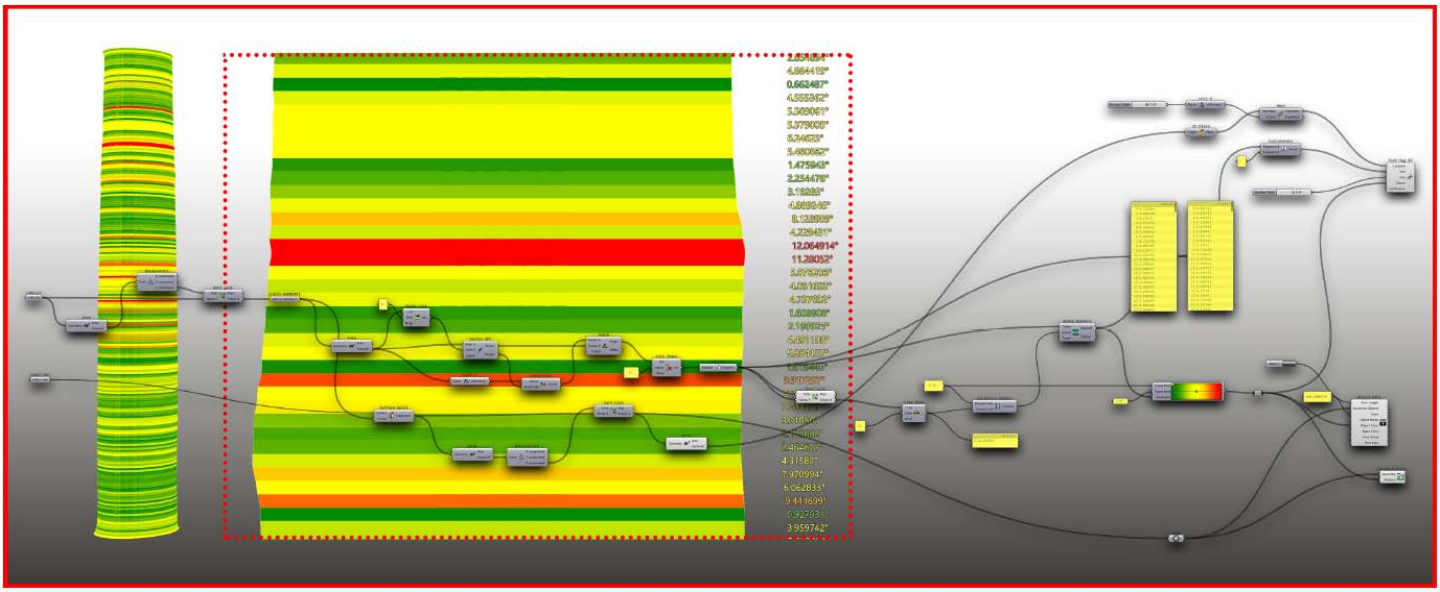

Fig. 5. Local angular deviations analysis (author's elaboration).

value to each section of the lofted surface, and visualizing in the model space the corresponding numeric values, in degrees.

These computational design methods are so intended to obtain critical and focused lectures [8] of a raw data – point clouds in the heritage case – suitable to be used within a framework of heritage conservation.

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DIGITAL TOOLS AND ARCHAEOLOGICAL ANALYSIS OF MASONRIES FOR HISTORIC BUILDINGS DIAGNOSTICS

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Starting from the assumption that a building, whether monumental or representative of the minor constructions that characterise the inner areas of our country, is the result of actions carried out over time, the knowledge process becomes necessary as well as preparatory to protection, conservation and enhancement interventions. Different disciplines (restoration, structural engineering, history of architecture and archaeology of building) have been confronting each other for years regarding the knowledge of historic buildings, so much so that we can consider the issue interdisciplinary. The complexity of the historical building soon made evident the need to integrate approaches, methods and tools and direct investigations towards shared languages, as demonstrated by previous conferences and works [1-3]. The application of the methods of the archaeology of building [4-7] supports the non-destructive diagnostic phase since, through the identification of the material traces still evident on the masonries, it allows us to recognise actions that have contributed to defining the history and evolutionary dynamics of these. This approach starts from the semantic decomposition of the object of study: from the Architectural Complex (CA), in which the building is inserted up to the single Masonries Stratigraphic Unit (USM), passing through the Building Unity (CF). Analytic analyses of the different USMs that compose the masonries, the identification of the relationships between them, illustrated in diagrams called matrix, and the comparison with documentary sources for relative and absolute dating allow identifying the construction phases by understanding the temporal evolution of the architectural heritage (Fig. 1).

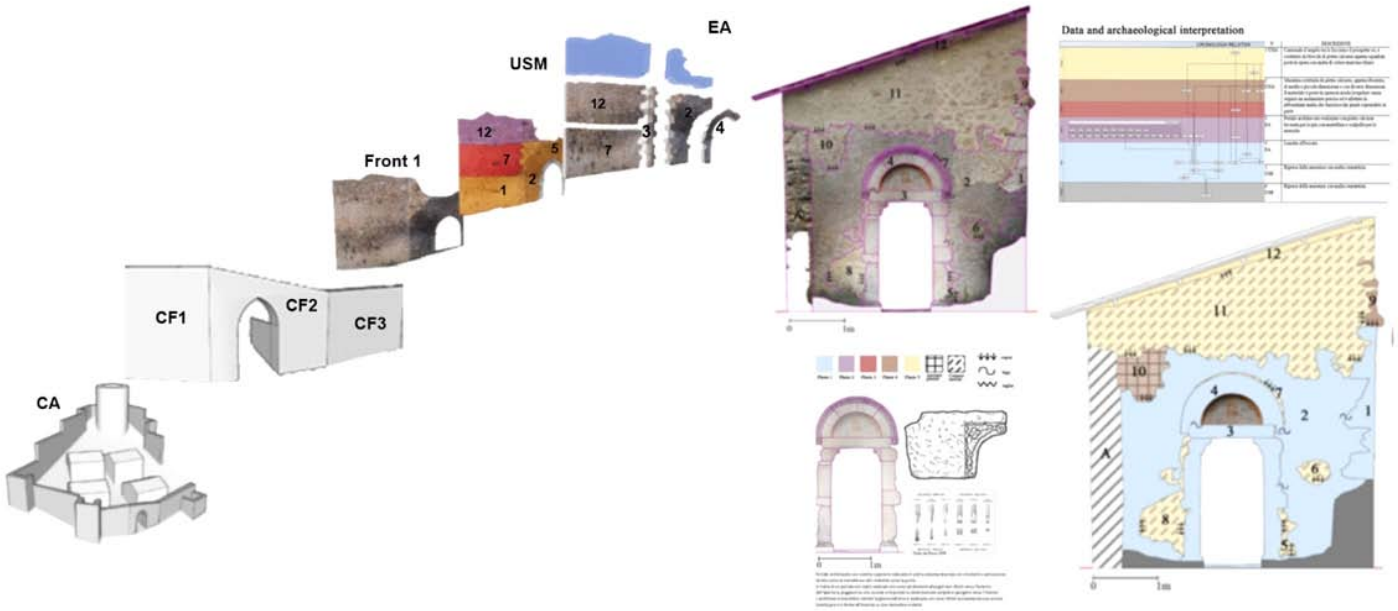

Fig. 1. Semantic decomposition of a historic building through the masonries archaeological analysis (left); an example of stratigraphic reading on masonries of St. Francis church (AQ) whit the connected matrix for relative and absolute dating (right).

This analytical approach produces many heterogeneous data that must be topologically linked to physical entities, three-dimensional representations of the real artefacts corrected in metric and colour data. This use of digital models is a topical issue in the field of research, and for about a decade now, questions have been raised about how to represent archaeological data as well [8-17]. The definition of digital tools for data management with a view to diagnostics, monitoring and conservation of the architectural heritage is the main focus of the research

carried out by our team.
In this regard, an operational workflow is being developed for the architectural heritage analysis, capable of integrating multidisciplinary studies to arrive at the definition of digital replicas that go beyond the mere three-dimensional representation (Fig. 2).

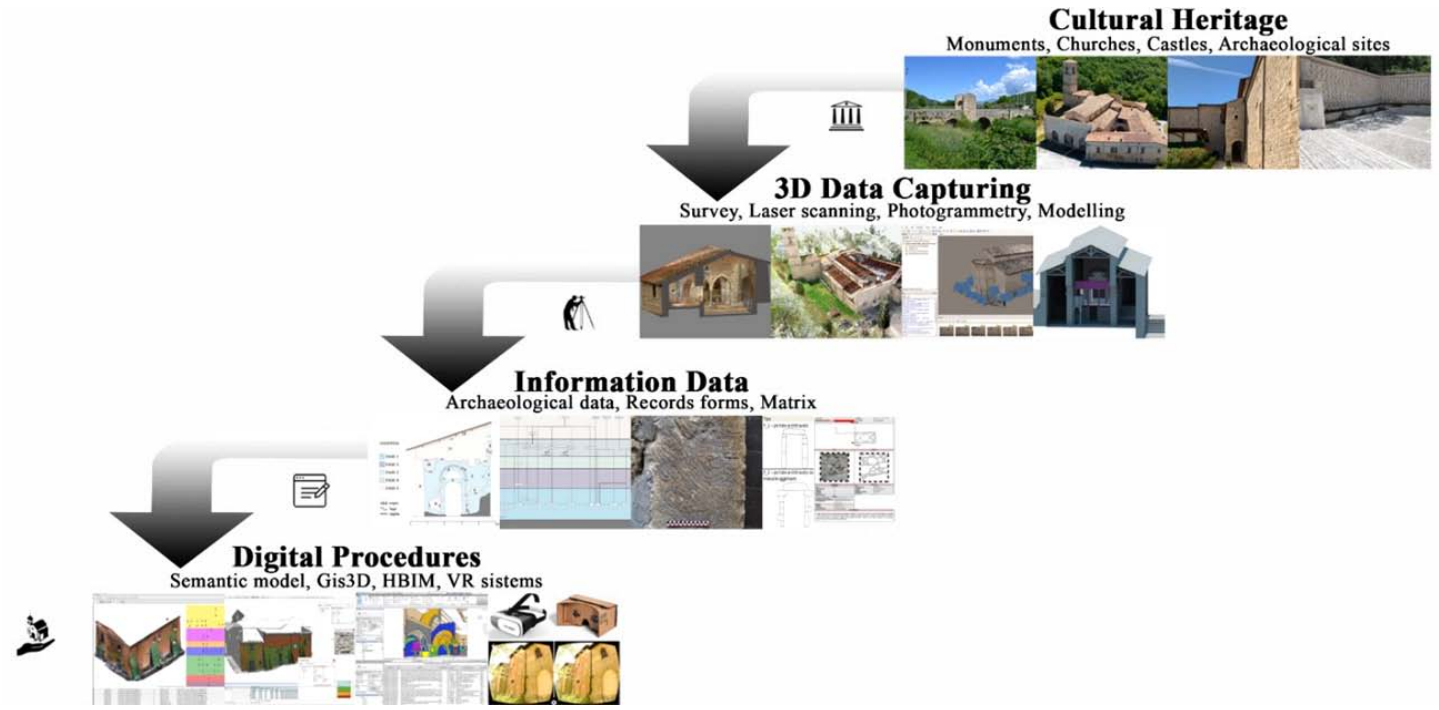

Fig. 2. Operational workflow for the analysis of architectural heritage and its digital representation.

These replicas are expected to meet the needs of artefact documentation and multi-data management for a correct knowledge of the historic built heritage. Therefore, several digital tools are being defined, such as the semantic models resulting from the integrated instrumental survey, the 3D GIS capable of managing geospatial data with punctual data concerning the built environment and the HBIM parametric models in which archaeological research results can be integrated (Fig. 3).

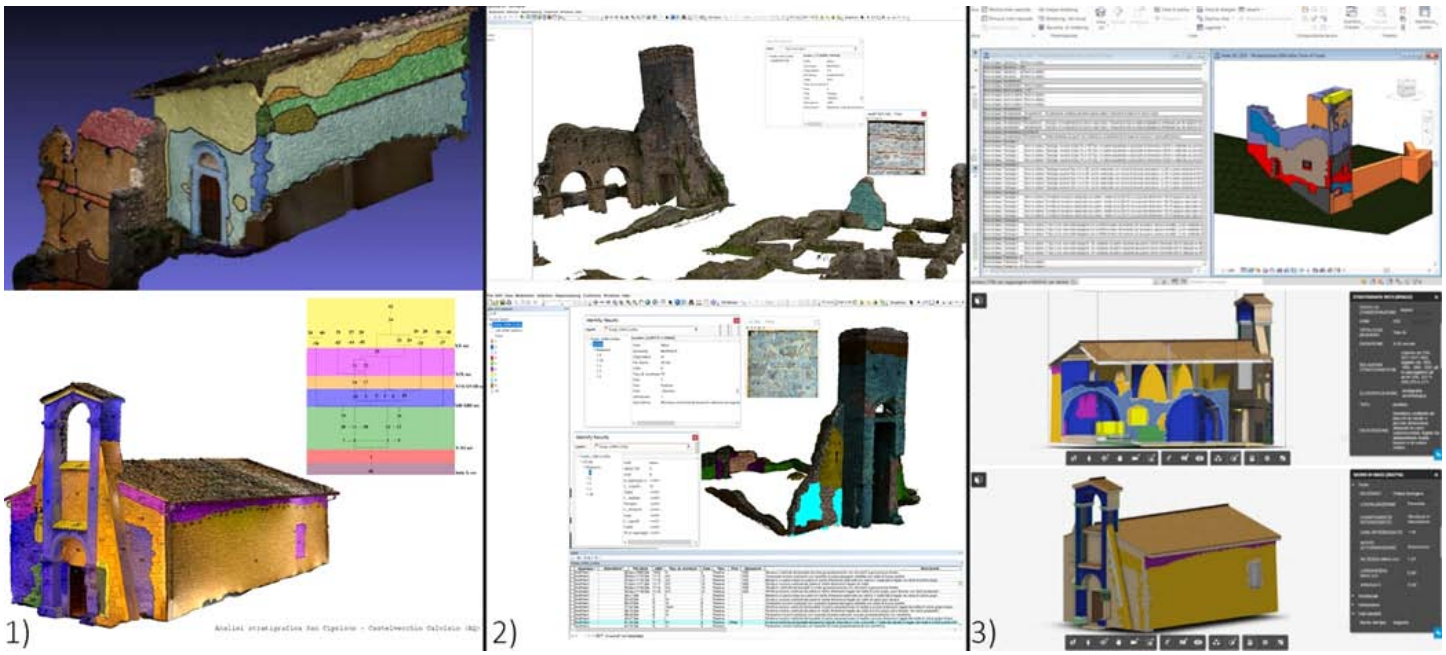

Fig. 3. Digital representation of the historical building and the archaeological data on the masonries: 1) semantic models; 2) 3D GIS; 3) HBIM.

Finally, to profitably use the multi-data connected to digital representation and make it key tool for the knowledge itself, the effectiveness of complex information systems is being tested using Virtual Tours, Virtual Reality and Augmented Reality (Fig. 4).

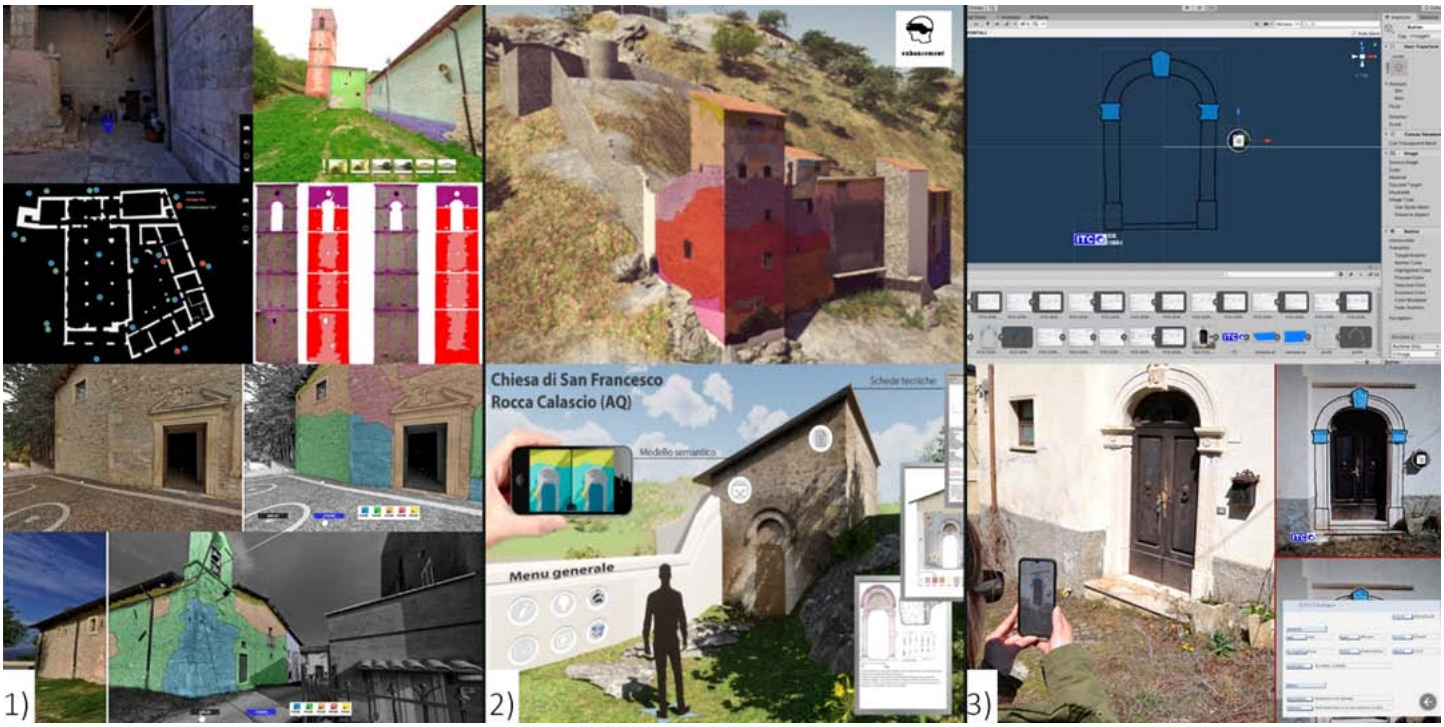

Fig. 4. Complex informative systems: 1) Virtual Tour; 2) Virtual Reality; 3) Augmented Reality.

The potential of the proposed approach, which integrates the results of archaeological research on masonries with the representation sciences to increase knowledge of the historic built environment, becomes exponential if it can dialogue with other disciplines, such as structural engineering; this has been demonstrated by the results of the research and investigations conducted by the team in the field of inspections and monitoring of existing bridges [18-21].

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Technological developments supporting qualitative research: The role of Nvivo for content analysis. An application study

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This contribution presents a study on the technologies supporting qualitative analysis in the field of scientific research. This investigation was inspired by a research work carried out as part of the author's doctoral thesis [1], in which an empirical investigation was conducted, supported by qualitative interviews [2][1] aimed at identifying and analyzing the relationships woven by foreign migrants in the places of migration. This reflection starts from an assumption premise, namely that the goal of every researcher has always been to seek a rigorous and convincing explanation of the phenomenon under study. As pointed out by Marradi [3, p. 83], for the so-called hard science, explanation finds its ideal model in the search for causes, of which the experimental method is the most complete operational translation, though certainly not free of limitations and problems. In the humanities and social sciences, instead, it is necessary to widen one's gaze to other "explanatory programs" [4, p. 89] and to provide different, more cognitive, types of explanation that can be able to integrate and shed light on the phenomena under investigation. As researchers, the aim that guides the collection of information is definitely analysis [5].
Analysis in social research is not immediate, nor so obvious, it has to deal with data coming from heterogeneous sources: biographies; case studies; personal transcriptions, photographed or video-recorded interactions (ethnographies); survey models, use of structured/semi-structured interviews (empirical investigation); official, institutional or administrative registers or texts (documents); interpretation of ideas, comments, forums, focus groups (arguments); literary and historical sources; etc. The information collected can be the result of different techniques and the list could be endless, because the ways through which men leave traces of their social relations are endless and ever-changing [5].
Therefore, in order to gather information, the researcher must necessarily establish relationships and comparisons between experiences and thus produce abstractions [6, p. 234]. The operational procedures that allow these conceptual abstractions to be translated into observable entities inevitably pass through a crucial distinction between quality and quantity, two opposing terms that have given rise, in the field of scientific research to two distinct methodological approaches, the qualitative and quantitative one. In particular, qualitative research has been attracting increasing interest, as it is capable of filling those gaps that traditional quantitative data (statistical and structured) sometimes fail to interpret on their own. From a technical point of view, it is possible to distinguish different methods of collecting qualitative data, in which the researcher is personally involved, and which can help the interpretation of the phenomenon under study[2].
In particular, the direct qualitative approach through the structured interview is a method of collection that is increasingly used in social research, especially when the problem is not suitable for being analyzed by means of precise analytical techniques, but can be better developed (or deepened) through a set of subjective assessments [8]. This type of approach is characterized by a strong attention to the experiential aspect and the characteristics of the subjects identified as privileged witnesses able to provide useful information for research purposes. To support qualitative investigation, software has been developed over the last twenty years that can support the researcher's categorization work during the content analysis of the available material. This is software dedicated to the lexical analysis of the text that, through procedures of coding the documents and their contents, are able

[1] According to Corbetta's definition [2, p. 405] the qualitative interview is a conversation provoked by the interviewer, addressed to subjects chosen on the basis of a survey plan and in substantial numbers, having cognitive purposes, guided by the interviewer, on the basis of a flexible and non-standard query scheme».
[2] Among these we include: the formal consensus technique; the Nominal Groups technique; the RAND technique; the Delphi method; the Focus Groups; and the interviews (structured/semi-structured). For further information, see: Gambacorti-Passerini et. al. [7, pp. 358-369].

to respond to the need of processing qualitative data collected during the research process.
Now, can the use of such software really help the researcher who makes use of qualitative investigation as his/her research methodology? In an attempt to answer this question, computer-based content analysis was applied to the processing of a part of the results from structured interviews, carried out during the empirical investigation planned within the doctoral thesis realized by the author. In this survey, the qualitative approach was particularly useful to understand the different nuances and factors that come into play in the complex dynamics between local communities and foreign organizations in their living places- and in particular the role of local institutions in these dynamics-, highlighting statistically undetectable data. Overall, the qualitative survey allowed: a) to analyze the migration dynamics in each context; b) to reconstruct the territorial dynamics of each association c) to identify projects and impacts generated among ethnic community members in the territories of immigration, but also in those of origin.
In order to support the process of analyzing the material collected, and in order to ensure the most rigorous possible return of the results, it was decided to use NVivo software: a program for analyzing texts, images and multimedia documentation, which is part of CAQDAS (Computer-Assisted Qualitative Data Analysis Software)[3]. The decision to use this tool was preceded by a critical evaluation of the possibility of using content analysis software in a consistent manner with the empirical method adopted. Before opting for the use of NVivo, a general knowledge of the program was considered in order to understand whether, on an epistemological level, it could effectively allow the study to remain faithful to the empirical analysis which considers the researcher's interpretive and creative thought as an indispensable element [8]. The Knowledge of the program and its functionalities allowed to deduce its potential and limits, so as not to create unfounded expectations of its possibilities. To this end, on the basis of the data actually available, a number of aspects were considered: i) the possibility of working out an analysis faithful to the objectives of empirical investigation; ii) the possibility of preserving the active, hermeneutic and reflective role of the researcher; iii) the possibility of archiving and analyzing the types of material collected (structured interviews). In particular, data storage and exploration practices allowed for an analytical approach centered on the construction of themes. Part of the information collected through the interviews was interrogated through the query tool[4], which, as the term suggests, methodologically corresponds to the way of "interrogating" data.
The analysis continued by working on the themes, following the conceptual scheme proposed by Van Manen [10], whereby the relations between the structured themes (nodes) were identified, highlighting those parts of text that could be part of a logical association between two or more themes. NVivo allows this step to be made by creating a further layer of themes, called "relations" (Sets), within which to insert the texts considered congruent according to specific characteristics. At the end of this second analytical level, it was possible to further interrogate the inserted material through the search functions offered by NVivo, which made it possible to trace certain words or expressions used by the respondents (Coding stripes). These searches allowed us not only to count the occurrences, but also to better understand the terms expressed through a focus on the chosen vocabulary (Attributes).
The comparative analysis work carried out within this socio-territorial survey has highlighted some positive aspects in the use of Nvivo, among these there is certainly that linked to its practicality and consistency with respect to qualitative research methods. In fact, in agreement with what Gambacorti-Passerini et al. [7] pointed out, the autonomy of storing the material collected within the software, allowed the researcher total freedom in both the exploration and interrogation phases of the same. In this regard, Zamawe [11] also emphasizes how precisely the possibility of being able to dispose of the material in an orderly and easily explorable manner, can create the conditions for more creative thinking on the part of the researcher, who is not heavily concentrated in the effort of governing the archiving and management of the complex and copious material collected.

[3] NVivo software was created in the mid-1980s by Tom and Lyn Richards and is now produced and distributed by QSR International (https://www.qsrinternational.com)
[4] NVivo software works with: a) Boolean (or logical) operators to search for elements to explore based on inclusion or exclusion criteria. These are linked to the OR commands for searching the items containing only one or both the selected items; AND for items where both of the selected terms are present; NOT for excluding the selected items from the search; b) semantic operators, to explore the relationships between codes defined by coding; c) proximity operators, to search for spatial or structural relations between text portions [9].

From this point of view, however, it is necessary to emphasize that the availability of software that facilitates the work of reconstructing the material collected by the researcher should not lead one to ignore the limitations of this type of analysis. After all, "computers don't analyze data, people do" [12, p. 34]. Even when planning to conduct part of the research through a computer-based content analysis, it is absolutely essential to read the raw data carefully and repeatedly, without blindly relying on automated software procedures. In this regard, Zamawe [11, p. 15] points out that CAQDAS software is an important part of qualitative data analysis. In particular, NVivo supports researchers in the 'time-consuming' transcription steps and increases the accuracy and speed of the analysis process. However, this is not enough to say that qualitative data analysis programs are perfect; just like other statistical packages, CAQDAS also has disadvantages.

In fact, although NVivo accepts most file formats, other similar software has more limitations that could be an obstacle for less computer-savvy users. Moreover, the software already presents structured nodes that in fact seem to limit the researcher's autonomy in sorting through the available material. Similarly, the queries present query characteristics that could partially mislead the researcher in analyzing the data. The use of multiple synonyms could also lead to only partial information retrieval. For example, in the thesis work, an attempt was made to understand the extent to which foreign immigrants' participation in social life in places of immigration can be considered an important variable for the realization of transnational projects, so it was created a node considering the word 'participation'. But examining the interviews in detail, it was possible to note that some statements concerning the sphere of 'participation' had not been identified in the query search. Obviously, this is only one of the cases that emerged during the content analysis, sometimes also depending on the researcher's lack of previous training in the correct use of the software.

However, what appears to be fundamental in this reflection on the use of technologies to support qualitative analysis is that, unlike statistical software, the main function of CAQDAS- and in the specific case of Nvivo- is not necessarily to analyze the data, but rather to aid the analysis process over which the researcher must always maintain control. In other words, researchers must be aware that no software can analyze qualitative data. NVivo and all other CAQDAS are basically data management packages, which are there to support the researcher during the process of analyzing the collected material.

Indeed, these tools allow the management of qualitative data while respecting its richness and heterogeneity. In this sense, the use of software in qualitative analysis may be even more appropriate than in other types of analysis.

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Augmented Reality. Forms and tools for a tourist use of invisible heritage

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Introduction

The paper deepens the meaning of invisibility linked to the cultural and environmental heritage of internal areas and the role of technological tools of augmented reality in the touristic enhancement of these areas. Invisibility and marginality are, in many cases, aspects shared by the local population, who perceive rurality – despite the strong sense of belonging to their roots – as an element of backwardness and not of development. In some cases, to fuel this cognitive invisibility, there is also a wrong and inadequate territorial management of these cultural heritages.

In recent years, despite all strategies aimed at enhancing inland areas such as the SNAI strategy, PNRR funds and other national and international actions, data on depopulation show negative signal, due to various natural and economic causes. This trend has led to a constant abandonment of internal areas, generating also the so-called ghost town villages.

Starting from these premises, it appears necessary support a sustainable tourist promotion of inland areas and their heritages in order to encourage the development of a new sense of place shared between inhabitants and visitors [1]. In a global context in which information moves quickly, technology represents a tool that can help to improve the usability, accessibility and promotion of the resources of inland areas, also contributing the reduction of the digital divide that characterize these territories. On this point of view is fundamental to remind the role of technology, that can generate also negative impacts, above all if it is not channeled in a shared planning. In effect, if there is an excessive technological development- disconnected from the socio-territorial characteristics of these areas – these could contribute to increase vulnerability; for instance, in terms of alteration of existing ecosystems; environmental pollution, lack of dialogue with local communities.

In this complex scenario, the work here presented intends to demonstrate the potentiality of augmented reality technologies in facilitating the discovery/rediscovery of local invisible heritages, technologies that can contribute to enhance these territories avoiding the risk of losing the cultural identity of local populations.

Cultural potentials of augmented reality

Augmented Reality (AR) technology is able to overlap different layers of information on reality combining information from different sources (such as cartographic, bibliographic and iconographic sources). These technologies can contribute to support touristic information, allowing the user to practice an experience enriched by virtual information and elements. The system can be implemented on various platforms such as mobile or stationary devices, viewing and listening and manipulation devices [2].

AR applications related to the enhancement of cultural heritage, produce new virtual cultural spaces defined Cultural Virtual Environment (CuVe); which enable real-time interactive navigation of environments, sites and monuments of historical, artistic or archaeological interest, even for those elements that have been strongly modified or partially destroyed [3]. In this field the technological aspect remains in the background, leaving to the historical-cultural dimension the main role of the simulation. The strengthens of CuVEs is in the multiple opportunities offered by virtual reconstruction through three-dimensionality, interactivity and spatial exploration. Such practices make it possible to reconstruct traditional cultural production and activities through the potential of the digital dimension. From technical point of view, this cultural environment is based on the acquisition of historical data, and of archaeological and geographical sources. The collected data are analyzed, cataloged and inserted into relational databases or GIS systems. The second stage is the identification of the most suitable tool to communicate the results obtained and to visualize them. The third stage refers to the treatment and optimization of data, this is possible with computer processing activities (Post processing) necessary to realize the virtual

environment. Fourth and final stage is the analysis of fruition, supported by audience surveys, that can validate the realized cultural virtual environment.

Case study and results

The potentiality of these applications for tourism enhancement has been applied on a case study, on a territory particularly significant for its rural characteristics linked, in particular, to transhumance culture.

GIS technologies and CuVE applications have been used in a geo-historical investigation focused on the study of transhumance routes located in central-southern Italy, especially that of Molise region. For this study has been produced a 3D virtual reconstruction model of the transhumance landscape, it consists of a prototype model with an educational aim: raise awareness of the heritage value of the transhumance landscape and its tourism potential.

The territorial context investigated is located in Central-Southern Eastern Italy [4], influenced by forms of territorial organization and social practices based on traditional cultural identities. The focus has been conducted in the territory of Molise, on an area called "Alto Molise" recognized by UNESCO as MaB (Man and Biosphere) reserve named "Collemeluccio-Montedimezzo" (Fig. 1).During the investigation have been investigated different aspects

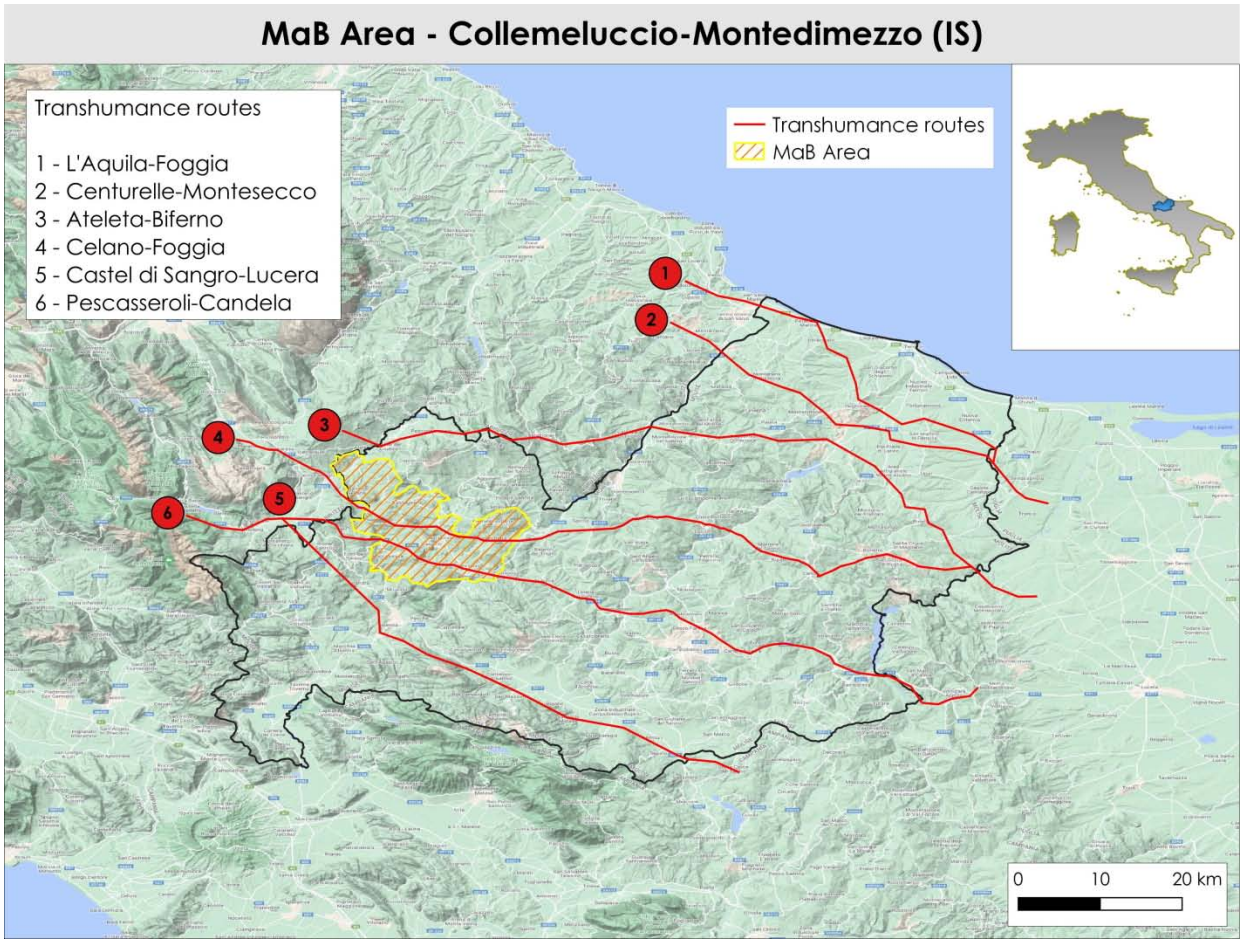

Fig. 1. Study area.

within a series of analyses, starting with an archivistic research on the documentation of "Dogana della Mena delle pecore di Foggia", then the construction of a geodatabase and the use of historical landscape rendering applications.

The temporal evolution of cartographic representations has been particularly useful deepen the landscape resources and changes happened during the period of the Doganas' management. In has been conducted also an extensive work using the Atlas linked to the different "Reintegre" made over the centuries. These geo-iconographic sources have been crucial for the reconstruction of transhumance practice.

The research started with an initial historical-geographical overview, followed by an analysis phase focused on the study of transhumance system and a final phase aimed at enhancing the transhumance landscape through CuVE applications for virtual rendering.

Augmented reality tools were used for 3d reconstruction of a portion of the Celano-Foggia transhumance routes located within the MaB area on the border between the villages of Vastogirardi and Carovilli, a relevant area interesting for its cultural heritage and the landscape elements that characterize it, particularly expendability for tourism purposes.
The compatible software used in this study are: ArcGis 10.1 for georeferencing and managing the maps of the 19th-century Atlas; Google Earth for acquiring the orthophotos of the area, SketchUp 2018 and Lumion 6.0 for 3D modelling, and for the rendering operations to produce a video in HD format. In detail, the operational phases involved: 1. the digitalization and georeferencing of historical cartographies identified in GIS environment; 2. the selection of a portion of the transhumance route in the MaB territory, a portion of 34 kmq; 3. the 3D modeling of the area.
The selected area was modelled using Sketchup 2018, a 3D modelling software, with which they were produced various models of both terrain and living elements, natural and artificial, typical of the transhumance landscapes. The last step was managed in Lumion environment in which was produced the 3D rendering. The final product is an HD[1] video that reproduces the transhumance routes and its landscape (Fig. 2), as well as a series of significant scenes of this practice. In accordance with the Virtual Cultural Heritage approach, this final product - although is a prototype model - aims to involve the public in a playful way on the heritage value of transhumance and its potential attraction for tourism.

Fig. 2. An example of an augmented reality scene.

Future Scenarios

This product of augmented reality, which is the result of research based non the landscapes of transhumance, certainly allows to perceive the potential that these tools can have in supporting the rediscovery of cultural heritage from a tourist perspective, but also encourages a series of constructive reflections for future scenarios in these contexts.
It must be said that beyond these technological tools, it will be fundamental as well as in any local enhancement initiative, the interaction with the territory throughout virtuous processes of local governance, focused to produce a complete and sustainable touristic experience.
Integration and digitalization for the enhancement of cultural heritage are two fundamental points of the 2017-

[1] Source: https://www.youtube.com/watch?v=fDGwaMCTDhk&ab_channel=congressogeografico

2022 Strategic Tourism Plan drawn up by the Standing Committee for the Promotion of Tourism with the coordination of the Directorate General for Tourism of the Mibact; new technologies such as augmented reality can support these territorial enhancement processes in terms of services, participation, preservation of cultural heritage [5].

Augmented reality applications can be part of this scenario, but only if they were included in a shared and inclusive path. They could become the basis of applications able to involve visitors in the interaction with the cultural and tourist heritage, also through gamification experiences, increasing their motivation through the stimulus provided by prizes and competitions, transforming tourists into positive ambassadors of the tourist location and its heritage. New products could be offered, differentiated according to the target with experiences aimed, for example, at schoolchildren through dedicated applications such as Minecraft, or at a target of cultural tourists with virtual reconstructions of artefacts, such as ancient monuments like the Colosseum or the Circus Maximus through the projection on 8K screens installed on tourist buses[2], or the reconstruction of the Arium MAXXI Aquila[3] digitally rebuilt after the destruction caused by the earthquake, or the interactive virtual navigation of the 77th Venice Biennale[4]. All these augmented or virtual realities may receive new stimulation in the era of blockchain technologies and, more specifically, of metaverses, particularly what we may call cultural metaverses, which may be configured as the new prospects for the rediscovery and valorisation of invisible cultural heritages.

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[2] Source: https://sovraintendenzaroma.it/content/roma-imperiale-virtual-reality-bus
[3] Source: https://arium.xyz/spaces/maxxi
[4] Source: https://www.youtube.com/watch?v=Kp4g0lE7ov8&ab_channel=BiennaleChannel

CPMdroid: Analysis of contextualized permission usage for malicious app detection in Android

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Today, Android is confirmed as the most popular operating system, with a market share exceeding 70% and nearly three million applications on the Play Store™, which collectively count tens of billions of downloads each year[1]. However, on the same store, many malicious apps are also available that attempt to steal users' data from their smartphones without their awareness [1]. These apps, classified as malware with malicious goals targeting sensitive data, are installed unintentionally by users directly from the Official Google Play Store™. Users are unconscious of the malicious behaviors that these apps attempt since they entrust the google security mechanism aimed at unpublish them. According to data, every day in 2019, there were almost 350,000 malware detections, and a new infection was created every seven seconds[2]. These findings unequivocally show the damage and danger smartphone users and businesses face. Therefore, it is crucial to have strong defenses against malicious behaviors. On the Google Play Store™, 36 new Android apps were infected with malware and adware in 2022. They have around 10 million downloads, and some are still accessible on the store. The apps' secondary objectives in every instance involve hijacking social network accounts, displaying intrusive adverts, enrolling the user in premium services, or, more in general, acquiring user data to profile them. To aim these goals, malicious developers misuse the Android permission model, requesting uncontextualized resource usage [2]. This is because one of the main weaknesses of the permission manager currently in use in Android is that approving a single permission request usually automatically enables to the entire application to inherit that permission globally. That means that once an app is granted access to the camera, it is allowed to access the resource without needing additional further approval in the near future and, potentially, even in background. For example, an application may present itself as a simple SMS manager. However, it exploits the SMS permission group to send messages to third-party sites in the background to secretly subscribe users to premium mobile services using the Android Joker malware.

In this paper, we introduce CPMdroid, a tool that helps identify the uses of resources with restricted access. We created a dynamic logging system that tracks user actions and links them to the requested functionality. The objective is to determine whether published applications use the scenario mentioned above. Using our tool, we can collect valuable data for detecting unexpected behaviors. Once the monitored application has launched, CPMdroid can record both system's calls to the access control and validation mechanism and all user activities taken at the interface level (independent of whether they exploit one or more permissions). The tool also takes a screenshot of each of these events, making it easier to identify and reconstruct the various usage scenarios retrospectively. Last but not least, the tool creates two exhaustive logs at the end of the app execution, one listing all user-generated events and the other listing all permissions accessed by the app, allowing us to determine, using timestamps, which activities led to access to crucial resources.

Two strategies [3, 4] are applied at a high level to perform analysis as part of the malware detection process. The first is a static analysis that examines the app's source code without launching it. This analysis focuses on comprehending the code's structure and functionality [5] it provides. Although this analysis method is effective, it can only identify known malware and cannot offer protection against zero-day risks. Additionally, malware that employs obfuscation cannot be detected using this method, and these tools need preliminary steps to work correctly. They first require APK decompilation and subsequent repackaging (which, of course, is done using a different signature than the original, implying a whole host of significant drawbacks, like the inability to update the application from the official Store directly). An example was given by Yang, that proposed a static analysis tool establishing the malicious behavior by extracting the context related to security-sensitive events [6]. In order to define the behavior of the application and identify malicious features using a classifier based on machine learning

[1] https://www.statista.com/statistics/272698/
[2] https://www.statista.com/statistics/680705/

methods, W. Wang retrieved static characteristics from the application, such as permissions and API calls [7]. A. Pektaş collected the API call sequences using static analysis tools, and using deep learning methods, they built the detection model [8]. Static analysis can detect the malicious behavior of an application at a low cost. However, in static analysis is challenging to detect applications that use anti-detection techniques to hide malicious behavior, such as malicious dynamic code loading, code obfuscation, and reflection. In addition to restrictions relating to code obfuscation already mentioned, these approaches may be unable to identify any stealth API-sensitive uses (i.e., occurring without first confirming that the program has the appropriate permissions but directly handling any SecurityException with a simple try-catch). Even if permission is denied, the program will still function correctly without crashing or alerting the user to attempt to access sensitive information.

In contrast, the dynamic analysis tracks the app's actions as it is being used. Dynamic analysis can also identify unknown malware by collecting data from monitoring. Wang built detection models using characteristics of HTTP packets and TCP flows to analyze the behavior of malicious applications by gathering network traffic while the applications were being executed [9]. However, they only considered permissions and network traffic data as criteria for detecting malware. Malware could violate users' privacy by also acquiring location data, audio records and even performing actions on the device's external storage. Bhatia et al. proposed a dynamic system aimed at detecting the behavior of android applications by analyzing the frequency of system calls made during their runtime [10]. They focus on classifying the behavior of android applications as malicious or non-malicious using the aggregated system calls as a feature set comprising all the applications that may or may not belong to the same family. However, they focused on system call stack trace without tracking user events on the UI, not contextualizing the actions that led to the malicious behavior. In order to identify malware, Li proposed a hybrid analysis technique in which the characteristics are mainly composed of both static data and dynamic runtime traffic queries [11]. This method requires much time and cannot detect malware that has employed anti-detection measures. The drawback of this type of investigation is that it uses many resources, which are typically unsuitable for mobile devices [12].

Unlike the abovementioned approaches, our tool can observe application behaviors without burdening smartphone resources. In addition, an application may not necessarily contain malware, but it may still have malicious behaviors or behaviors outside regular permission usage. This could be due to programming errors or design choices. For example, an instant transcription app might record audio before activating the recording feature to configure the language, or a fitness app might require location before starting a workout. While these are non-malicious behaviors within legitimate apps, they could be malware. Therefore, it would be appropriate to notify the user of the out-of-context use of the resource. In this paper, we want to assess the effectiveness of adopting the usage context to discriminate between different kinds of access to the same sensitive resource to detect malicious behaviors in malware apps. To this aim, we developed CPMdroid, a tool to monitor the execution of mobile applications using specific device resources that need dangerous permissions to be accessed and support linking resource usage and the access context. We define the usage context as a combination of the feature the app is executing and the actions performed on UI (made by the user or by the app behavior at runtime). CPMdroid Analyzer is thus a dynamic analysis tool that, when installed as an Xposed module on the Android device/emulator, allows obtaining helpful information in identifying the contexts in which permissions have been used, isolating permission usage to the identified contexts. This allows us to identify whether the running application is using permissions that have nothing to do with what is shown on the screen. Once the monitored application is started, CPMdroid Analyzer starts tracking any access to resources that require dangerous permission at the system level (e.g., the camera, microphone, storage, or location) through a dynamic injection mechanism based on hooking and callback techniques. These include all the actions performed by the user at the interface level and any system call to the access control and validation mechanism. Our approach allows the semi-automatic identification and *a posteriori* analysis of the usage contexts. To execute a completely automatic analysis, without the user interaction, the tool can be configured to execute random UI interactions on apps and observe the app's Android permission requests. In both cases, CPMdroid automatically associates the observed permission requests and UI actions to categorize each resource used within its own context.

We conducted a preliminary study to determine the feasibility of our method for identifying malicious behaviors based on usage contexts concerning the use of sensitive device features, i.e., those features that require explicit

user authorization before being accessed. We analyzed 7 malware apps identified in 2022 and downloadable from the Google Play Store™. The malicious behaviors of these apps are well-known, this helped us identify whether CPMdroid could contextualize malicious functionality within these apps. We ran each app on an Android emulator executing the actions on the GUI that would lead to the execution of the malicious functionality. In all apps, the tool allowed us to identify the context in which the malicious behavior was occurring by highlighting the usage number of dangerous permissions. The tool's outcomes have been evaluated by examining the log, screenshots, and screen records of the 7 apps; the details of some identified behaviors are shown below:

- *Codice Fiscale 2022* was downloaded in Italy thousands of times without users realizing its danger. It calculates taxes by asking us to access SMS to manage our account, but it hides the Sharkboat banking trojan capable of stealing personal data and SMS. Once downloaded and opened, the app prompts the download of an update, whereby the installation of the malware inside our smartphone takes place. CPMdroid identified uses of permissions to read user data and handle SMS (the latter mainly to track 2FA codes) in contexts where these permissions did not make sense. Finally, the app accesses the Internet to send user data to a remote server.
- With over 100,000 downloads, the apparently harmless and helpful *Symoo* app generates fake accounts once it is started. This app presents itself as an advanced SMS client for Android. In reality, it hides Android Joker malware that generates fake accounts [13]. The app requires access to send and read SMS messages when installed on any device. The first screen asks the user to provide his or her phone number. This request, at first, does not seem strange because it is a self-proclaimed "easy-to-use" SMS app. In reality, however, this permission starts its fraudulent processes. Then a new screen overlays the previous one, identified by CPMdroid, which generates numerous requests for SMS access. As time passes, despite the procedure's progress, the process is too long. Meanwhile, this allows the malware to send multiple 2FA SMS remotely to create accounts on various services.
- Another app analyzed was *Process Manager*. The app tricks the user into granting itself as many as 18 permissions. It can track precise location, record audio from the device, access files, read messages, access the camera, and modify some device settings. CPMdroid allowed us to identify these permissions while the app showed a list of processes on the GUI but simultaneously accessed write permission, recording audio, and accessing the location.
- *CLEANit* claims to be a junk file cleaner with millions of downloads on the Play Store™. The app not only needs a ton of permissions but also advertises services that are frivolous in modern Android devices with powerful hardware. The app in question is supposed to clean and optimize the device. However, via CPMdroid, we detected the use of permissions such as location access, multiple writes and reads to the device's external storage, and internet access (probably to send and download data).

The results confirm that malicious behavior is related to the context of resource use. This allows us to identify malicious apps by observing the relationship between user events on the GUI and uses of sensitive resources. In future work, we plan to conduct a large-scale analysis of the malicious apps published in the Play Store™. In addition, while in this work we aimed to analyze what was happening during the execution of an app, in the future, we want to implement a system that can notify the user that the app is accessing a resource in a different context than the one in which the permission was already authorized. In this way, we create a fine-grained system of permission management that would avoid checks by antimalware and limit the use of the app to only lawful functionality.

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Automatically Classifying Issues Reported in Gameplay Video Segments

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Introduction

The growth of the video game market, streaming services, and the increasing complexity of video games trigger the availability of video game-specific tools to assess performance and maintainability problems. Similarly any regular software, the presence of bugs in video games can cause considerable problems for game developers (e.g., lost sales, damaged public image). Despite the large efforts that the game developers invest in the development process of a game, it is not always possible to discover all bugs in a game before releasing it to the market. A possible strategy to involve the user to help the developer to obtain feedback on the user-perceived quality of a game is to allow gamers to submit bug reports that describe any encountered bugs during gameplay. Many studios employ discussion forums or specific features in their games for gamers to report bugs (e.g., Steam Community). Previous work shows that 80% of the Steam games release urgent updates to fix issues such as feature malfunctions or game crashes [1]. The large amount of gameplay videos continuously produced and publicly released by many gamers on platforms such as Twitch and YouTube could be helpful to developers: Sometimes, gamers indirectly report issues while they play.

Lin *et al*. [2] conducted an in-depth study of gameplay videos posted by players on the Steam platform aiming at automatically identifying the ones that report bugs. They observe that naïve approaches based on keywords matching are inaccurate. Therefore, they propose an approach that uses a Random Forest classifier [3] to categorize gameplay videos based on their probability of reporting a bug. Still, their approach (i) works at video-level, and a whole video can last even several hours, and (ii) they only distinguish bug-reporting videos from non-bug-reporting ones, without a more specific classification regarding the type of issue reported (e.g., glitch or logic bug).

We aim at filling this gap and further aid developers by classifying the video segment according to the type of problem encountered, and by trying to classify video segments (i.e., parts of videos) instead of whole videos. To achieve this goal, we rely on textual information (subtitles) from the videos. To determine meaningful categories in which it is worth categorizing video segments, we rely on a recent taxonomy of issues in video games introduced by Truelove *et al.* [4] (which extends the one by Lewis *et al*. [5]). In their taxonomy, the authors report 20 different kinds of issues. Using all such labels might be counterproductive since it is likely to observe a long-tail distribution (i.e., a few types of issues appear in most of the video fragments, while several other issues are quite rare or do not even appear). Therefore, starting from such a taxonomy, we define macro-categories by clustering similar fine-grained categories. We identified four labels, as reported in Table 1: Logic, Presentation, Balance, and Performance. In summary, we provide the following contributions: (i) we define a large labeled dataset of gameplay video segments labeled with their respective issues, and (ii) we introduce a machine-learning model for automatically categorizing such segments.

Dataset

To the best of our knowledge, there are no large-scale, publicly available databases of gameplay videos that provide meaningful information on the classification of problems in video games through subtitle analysis. Thus, we define a new dataset that can be used to both train and test the machine-learning models we aim at defining. To achieve this goal, we rely on gameplay videos from YouTube since they contain this piece of information. While other platforms, even more video game-oriented, could be used (e.g., Twitch), YouTube provides APIs for searching videos of interest and it also allows to download videos including subtitles. Our premise is that several gameplay videos report issues. However, we also expect that the issues-reporting videos represent a minority of the entire gameplay videos population (thus the relevance of our research). Therefore, to support the construction of the dataset, we use the approach defined by Lin *et al.* [2] and filter out videos identified as non-issue-reporting. We

randomly selected YouTube videos that match a generic query containing the six keywords defined by Lin *et al.* [2] (e.g., "gameplay bug") to ensure that the final sample contains all the types of issue we aim at identifying. The first author manually split each video into meaningful segments and labeled each segment as "logic", "presentation", "balance", "performance", or "non-informative" (when the segment does not report any issue). At this stage, we do not handle segments reporting more than an issue at a time. In other words, we assume that each segment has exactly one label.

As a result, in this first phase, we analyzed a total of 170 gameplay videos, totaling about 17 hours of gameplay. As a result, we identified 1250 video segments. Each video segment was categorized according to the information it contained. Specifically, we obtained 696 non-informative video segments, 302 video segments representing persistence problems, 168 video segments reporting logic problems, 47 video segments with balance problems, and 37 video segments highlighting performance problems.

Given the nature of the problem at hand, as we expected, the dataset is imbalanced, with a great majority of segments being non-informative (~55.7%) and a very small percentage of them reporting balance (~3.8%) and performance (~3.0%) issues.

Tab. 1. Mapping between types of issues identified and categories from the taxonomy by Truelove et al. [4].

Issue type	Description	Categories [4]
Logic	Issues related to the game logic, regardless of how information is presented to the player.	Object Persistence Collision of Objects Inter. Btw Obj. Prop Position of Object Context State Crash Event Occurrence Interrupted Event Triggered Event Action Value
Presentation	Issues related to the game interface (graphical- or audio-related).	Game graphics Information Bounds Camera Audio User Interface
Balance	Detrimental aspects in term of "fun"	Artificial Intelligence Exploit
Performance	Performance-related issues (e.g., *FPS drops*)	Implem. Response

Categorizing Gameplay Video Fragments

A key feature of gameplay videos are live comments from streamer describing their experience of the game. To this end, through subtitle analysis, it is possible to extract keywords from the text that refer to issues encountered within the game. Words such as "bugs", "glitches", "stuttering", *etc* allow the developer to identify the type of issue encountered by the streamers. To divide the video into meaningful segments, we analyze the subtitles with the streamers' comments. To this end, we define as significant the part of the video containing subtitles that provide information on the problems encountered by the player during streaming.

We manually extracted and categorized segments through NLP techniques based only on textual features extracted from subtitles (i.e., what the gamer says). Specifically, we explore two types of NLP features extracted through doc2vec [6] and word2vec [7].

Using the doc2vec model [6], we extract a vector of features for the subtitles of each segment in the dataset. Such a model allows to automatically represent a document (sequence of words) as a vector. We chose to represent each subtitle string as a vector composed of 40 features. Then, we train and test two machine-learning models using 10-fold cross validation: We randomly split the dataset in ten folds and we use each of them, in turn, as test set, while the others are merged and used as training set. We use as machine-learning algorithms Random Forest [3] and SMO [8]. With the former we obtain a 56.8% accuracy with an average AUC of 0.53. On the other hand, with the latter, we obtain an accuracy of 56.6% and an average AUC of 0.53. What we could observe is that the features mined through doc2vec do not allow us to obtain satisfactory results, considering that a similar level of accuracy can be obtained by simply classifying all the istances as "non-informative."

In a second attempt, we used word2vec [7] to extract features from text. Differently from doc2vec, which works at document-level, such a model maps single words to vectors of features. Given all the words in a given instance, we extract the vectors through word2vec and we compute the average of each feature (300 features). We used the same experimental setting used for the doc2vec (10-fold cross validation with two ML algorithms). In this case, we obtained a 57.6% accuracy, and an average AUC of 0.71 with Random Forest. On the other hand, with SMO,

we can observe that the results improve, albeit slightly, as we can obtain 60.9% accuracy, with an average AUC of 0.66. In summary, we obtained non-satisfactory results with both the techniques. We analyzed more in depth the data to try to understand why the classifiers do not achieve good results. We observed that, often, the mined sentence segments are incomplete and ambiguous (*e.g., "logics, bro. Well, I talk all"* as logic, "*they are lower than that"* as presentation, *"less well-known logic that's arguably one"* as performance). This happens because the comment corresponding to the portion of the video in which the issue appears might not be in sync with the issue itself: Gamers might talk about the issues even several minutes after it appears.
Also, streamers comment on their gaming experience in an irregular manner, often even through simple exclamations *(e.g. "the glitch myself?"* as performance, *"BAM!"* as logic, *"and there!"* as presentation). This clearly makes the automated categorization based on textual information a challenging task, for which more work is required.

Conclusion and Future Work

In recent years, there has been a growing interest in video games. During game development, many bugs go undetected prior to release because of the difficulty of fully testing all aspects of a video
game. We propose a novel approach for detecting anomalies in video games from gameplay videos to support developers through the analysis of subtitles by (i) providing a labeled dataset that contains the issues found in gameplay videos and (ii) proposing an approach to automatically identify issues in the gameplay videos. However, the analysis of the subtitles is complex as, following the segmentation of the video, the sentences are incomplete and ambiguous also due to the use of slang. Based on the qualitative analysis we conducted, future work will be aimed at evaluating further techniques for textual analysis of subtitles (e.g., Bag of Words). Besides, we will try to get broader context for the subtitles to try to capture the use of interesting keywords that might be pronounced after the issue appears. We will also evaluate additional features that can be extracted through the analysis of gameplay videos, including audio and game frame analysis.

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Best Poster Award

At the end of the day, prizes were awarded for the three best posters. The prizes were offered by the company *Datasound* srl, a spin-off of the University of Molise.
Based on the votes obtained from the research day participants, the competition for the best posters had this result:

First place

The sea and the coastline: development of the territory

Checchia L

Second place

CPMdroid: Analysis of contextualized permission usage for malicious app detection in Android

Guerra M, Fasano F, Scalabrino S, Oliveto R

Third place

A comparative study on chemical composition and antioxidant activities of essential oil and methanol extracts from seeds of *Daucus carota* L. subsp. *carota* growing in the Central Apennines (Italy)

Fantasma F, Fortini P, Chini MG, De Felice V, Iorizzi M, Saviano G

Image by everesd_design from Pixabay, modified

Working sessions

The Working session was conceived as a moment of the Research Day in which each member of the department had the opportunity to present a project idea or project in its initial stages, to be nurtured and developed through the contribution of other skills and knowledge present within the department. It was thus configured as a constructive stage of contamination of knowledge and skills for the identification of new interdisciplinary research paths.

Landscape and environmental crisis. Genesis and perspectives

Rossano Pazzagli

The landscape is both a mirror and an aspect of the environmental crisis, a planetary crisis linked to the imbalance between population and resources. In Italy, this crisis has been accompanied by a widespread territorial problem, which finds in geographical disparities and social inequalities the most eloquent expression of the imbalance between man and nature. Climate change, the energy issue and the loss of biodiversity impose new and urgent responsibilities on politics and spatial planning. This calls for an integrated vision of environment and landscape, avoiding the risks of conflict between the environmental and landscape dimensions, also in the context of the recent amendment of Article 9 of the Constitution.
The research presented here, also using multidisciplinary methodologies, aims to trace the main stages of environmental history - from the development of agriculture to the industrialisation process - using landscape as a framework for analysing the impact, meaning and economic and social relevance of the transformations, as well as their impact on the relations between the different territorial components: city and countryside, mountains and plains, coast and hinterland.

Pandemics and territory. Interdisciplinary research paths

Marco Petrella

The proposal is the result of a research project, started in the first months of the spread of Covid-19 in Italy and worldwide, on the contribution of geographical sciences, and more generally of spatial and territorial sciences, to the study of the dynamics of contagion in relation to possible spatial and territorial indicators. At the same time, there was a reflection on the processes of cartographic representation of the evolution of the pandemic and on the value of cartography as a tool for analysis in the scientific field, as well as for the knowledge and awareness of broad sections of the population.
From this experience emerged the need for an interdisciplinary reflection on the multiple implications of geographical analysis and cartographic practice, within the framework of a critical rethinking of the phases of elaboration of interpretative models, analysis and representation of infectious diseases.
The proposed session is therefore aimed at all those interested in working constructively towards the following objectives:
- to compare models of analysis that focus on the territorial dimension of contagion, particularly that linked to social interactions,
- to develop skills in identifying significant territorial variables and indicators for the implementation of existing models,
- Identifying new elements for discussion and reflection on cartographic representations.

Conclusions

The annual meeting of researchers from the Department of Biosciences and Land was a key moment to discuss research, dialogue and exchange views on the analyses, lines of action and activities carried out.

The day, which was attended by more than 100 participants, was marked by a packed scientific program:

- in the morning there were presentations, by the coordinators, of the activities of each of the Department's five research sections (*Biology - Nature, Environment, and Forests - Landscape, Economy, and Planning - Engineering - Computer science and Mathematics*);
- in the afternoon there were two different activities:
  1) a long and extensive poster session in which more than 70 young researchers described the new lines of research that will characterize the Department. Numerous research projects were presented, many of them intercepting the development lines of the PNRR, demonstrating once again the fundamental role of the University of Molise in national and regional development;
  2) two working sessions: *Landscape and environmental crisis - Genesis and perspectives* and *Pandemics and territory - Interdisciplinary research paths*.

It was also an opportunity - looking at the many themes, areas, sectors and research projects described - to highlight the Department's multidisciplinary educational-scientific format; a variety of scientific areas covered ranging from biology to sustainable territorial development, with particular reference to the environment, landscape, economics and planning, to applied sciences such as engineering, computer science and computational mathematics.

But that's not all. The day was also the ideal setting to relaunch and reaffirm one of the several important new features that will characterize UniMol's educational offerings for the coming academic year, particularly for the Termoli branch of the Department of Biosciences and Territory. In fact, it will host, in addition to the Courses of Study on Tourism, the new curriculum of the Bachelor of Science in Computer Science course, oriented to the training of "software technology specialist," a profession in high demand by the market and by the pressing need to create and train figures with strong skills, knowledge and competencies able to manage and govern the process of digital transformation.

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