Conferenza CNR IRET 2025

RISULTATI E PROSPETTIVE PER LA GESTIONE INTEGRATA DEGLI ECOSISTEMI DEL FUTURO

ABSTRACT BOOK

18 - 19 FEBBRAIO
2025

SALA CONVEGNI, SEDE CENTRALE CNR

PIAZZALE ALDO MORO 7, ROMA

CNR IRET
NATIONAL BIODIVERSITY FUTURE CENTER
ITINERIS

Conferenza CNR-IRET 2025: risultati e prospettive per la gestione integrata degli ecosistemi del futuro

18 - 19 febbraio 2025

Sala Convegni, Sede Centrale CNR

Piazzale Aldo Moro, Roma

Book of Abstracts

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Redazione a cura di **Ilaria Rosati, Alexandra Nicoleta Muresan e Davide Raho**.
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Foto di Copertina a cura di Paolo Colangelo, Cristina Di Muri, Laura Loru, Elena Marra, Gregorio Sgrigna.
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National Research Council
Research Institute on Terrestrial Ecosystems

Piazzale Aldo Moro, 7 - 00185 Roma
ISBN (ed. digitale) 978-88-8080-742-1
DOI https://doi.org/10.61008/CONFIRET2025

Required citation
Rosati I., Muresan A. N., De Leo F., Gavrichkova O., Macci C., Pioli S., Traversari S., Zenone T., Raho D. eds. 2025. CNR-IRET 2025: risultati e prospettive per la gestione integrata degli ecosistemi del futuro. Book of Abstract. 18 – 19 Febbraio 2025. Rome, CNR.
https://doi.org/10.61008/CONFIRET2025

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Preface

The environmental issues threatening our society and the optimization of ecosystem services are among the most pressing challenges that require immediate attention and actions. Climate change, biodiversity loss, deforestation, environmental pollution, and soil degradation are just some of the crucial issues that our society is facing now and for the future decades. While some progress towards environmental target have been achieved, more efforts are needed to reach climate neutrality, reduce pollution and preserve biodiversity.

Environmental challenges are strictly related to the sustainability concept, representing the intersection point of social, environmental, and economic needs: an interdisciplinary strategy that integrates conservation, sustainable development, ecological resilience, and optimization of ecosystem functionality is needed to face these issues. In this context, national and international research networks and infrastructures play a crucial role to advance the scientific knowledge and provide practical solutions to the policy makers. The multidisciplinary approach of environmental research has been the main theme of the CNR-IRET 2025 Conference dedicated to highlight the Institute's most recent projects, including those within the National Recovery and Resilience Plan (PNRR) and other national and international initiatives.

The event has provided a valuable opportunity for dialogue, fostering interaction among different research groups and the entire scientific community. The exchange of ideas, experiences, and best practices emerged during the conference aimed at shaping the future of environmental sustainability. Key topics included habitat restoration, the integration of technologies in environmental management, global and local biogeochemical cycles, and the One Health approach.

This Abstract Book serves as an invitation to reflection and action, with the awareness that only through a collective commitment we can ensure the protection and well-being of ecosystems.

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Sessione I: Progetti PNRR nell'ambito delle tematiche del GdL "Biodiversità"

Small mammals and bats as bioindicators of habitat quality in urban environments: experiences from Italian cities

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***Keywords**: Urban biodiversity; Mammals; Nature based solutions; Ecosystem; Monitoring*

Cities are often considered as degraded and ecologically poor areas due to extensive loss of natural habitats and pollution. However, a growing body of research highlights that cities may still play a crucial role in biodiversity conservation by supporting a surprisingly rich array of taxa, including species that can cope with the harsh ecological conditions found in urban contexts. How urban areas are structured in terms of e.g., urbanistic patterns and development history, as well as the abundance and quality of their green spaces, strongly shape biodiversity levels and patterns of occurrence within the boundaries of cities. Nonetheless, many cities worldwide feature key elements that allow species – sometimes conservation-relevant ones – to persist or even thrive in urban areas, thus representing poorly considered wildlife havens.

This shift in perspective challenges the conventional view of urban areas as biodiversity sinks, and underscores the need to understand the factors fostering richer urban assemblages, in accordance with the objectives of the EU Nature Restoration Law in terms of habitat restoration and valorisation in urban areas. Within Spoke 5 ("Urban Biodiversity") of the National Plan for Resilience and Recovery (PNRR), we focused on selected taxa to test the relationships between wildlife diversity and urban landscape structure at different spatial and temporal scales, using three Italian cities as study area. Namely, we sampled small mammals (rodents and shrews) and bats in Milan, Florence, and Rome, adopting a consistent and stratified sampling scheme, by using indirect non-invasive techniques (hair-tubes for small mammals, bioacoustics for bats). We then paired land-use mapping at a 1-km square grid cell scale and microhabitat assessment in the immediate surroundings of sampling sites with a generalized linear mixed model approach to quantify the main drivers of small mammal and bat diversity in the investigated cities. The biological and ecological differences between small mammals and bats allow testing a set of different hypotheses and spatiotemporal horizons in urban ecology. Rodents and shrews are in fact characterized by low mobility and high demographic turnover, thus being highly sensitive to local and immediate environmental changes; conversely, bats are highly mobile and long-lived mammals that perceive the environment at large spatial scales and over longer periods.

Overall, we here disclose relationships between small wildlife diversity and both landscape and microhabitat features in the three investigated Italian cities, highlighting the importance of unmanaged remnants in urban greening, as well as a key role of deadwood and of specific undergrowth plant species in fostering higher diversity and better-structured assemblages of small mammals. For bats, past land use was a major driver of species diversity and probability of persistence over long periods within cities, again supporting our finding on the importance of natural remnants within urban green spaces; nonetheless, long-lived mammals such as bats also revealed that many species may be subjected to extinction debt dynamics, highlighting how recent and current reforestations may take longer before having positive effects on these mammals.

By combining expertise on different taxonomic groups with a collaborative network across Italian cities and institutions we provide a first comprehensive analysis of the relationships between wildlife diversity and urban landscape, including different spatial and temporal scales. Such knowledge may be profitable for both conservationists and urban managers and authorities for optimizing urban development and green space managing not only for recreational purposes, but also for securing biodiversity and the key services provided by wildlife and functioning ecosystems.

***Acknowledgments**: Funded by the National Recovery and Resilience Plan(NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union– NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC"*

Genomics of Mediterranean forest trees

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***Keywords**: Genomics; Forest trees; Mediterranean endemic species; Genetic diversity; Biodiversity conservation*

The Mediterranean Basin represents one of the world's major biodiversity hotspots, both in terms of species and genetic diversity. The objective of Task 4.3.1, "Genetic-Molecular Toolkits for the Conservation of Intra-Specific Genetic Diversity," within Spoke 4 of the National Biodiversity Future Center (NBFC PNRR), is to assess patterns of neutral and adaptive genetic diversity in Mediterranean species, with the ultimate goal of developing genetically-informed conservation strategies. Within this context, at the forest genetic lab of CNR-IRET (Porano) we applied genomics and phylogenomics approaches focusing on the following Mediterranean forest trees: (1) the Italian alder (*Alnus cordata*); (2) the Macedonian oak (*Quercus trojana*) and other Mediterranean cork oaks (*Quercus* section *Cerris*), (3) Mediterranean sclerophyllous oaks (*Quercus* sect. *Ilex*); and (4) the sweet chestnut (*Castanea sativa*).

For the Italian alder (*Alnus cordata*), endemic to the Tyrrhenian side of the Apennines and Corsica, we adopted a whole-genome re-sequencing approach (20x), aimed at assessing patterns of genome-wide variation within and among populations across the whole Italian native range, and shedding light on potential introgressive hybridizationevents with the partially syntopic common alder (*A. glutinosa*). We generated whole-genome sequence reads for a total number of 89 individuals for *A. cordata* and 26 for *A. glutinosa*. In addition, we are involved in the de novo assembly of the reference genome for *A. cordata*, in collaboration with University of Udine.

For the Macedonian oak (*Quercus trojana*), a Mediterranean endemic tree with fragmented distribution, we carried out a phylogenomic study with the objectives of (1) assessing the degree of genetic differentiation between geographically disjunct subregions, and (2) shedding light on its evolutionary history in the context of other cork oaks. Based of genome-skimming data, we assembled the whole plastome sequence (Fig. 1) for 20 *Q. trojana* individuals sampled across the whole distribution, and for other 18 individuals belonging to 11 Mediterranean cork oak species. A similar phylogenomic study was implemented for Mediterranean sclerophyllous oaks (12 sequenced individuals, corresponding to 4 species), in collaboration with University of Tuscia (Viterbo). The de novo assembly of the reference genome for *Q. trojana* is also planned for the next future.

For the sweet chestnut (*Castanea sativa*), a multipurpose Mediterranean tree of particular importance, we applied whole-genome resequencing (15x) to investigate patterns of nuclear and chloroplast diversity in monumental trees, natural stands and cultivated varieties, sampled mainly in central-southern Italy, one of the hypothesized glacial refugia for the species. The first reference genome for the species was also assembled, as a result of a collaboration involving different Italian universities and research institutions.

Here, we will present an overview of the progress status for the reported genomic activities, highlighting preliminary results.

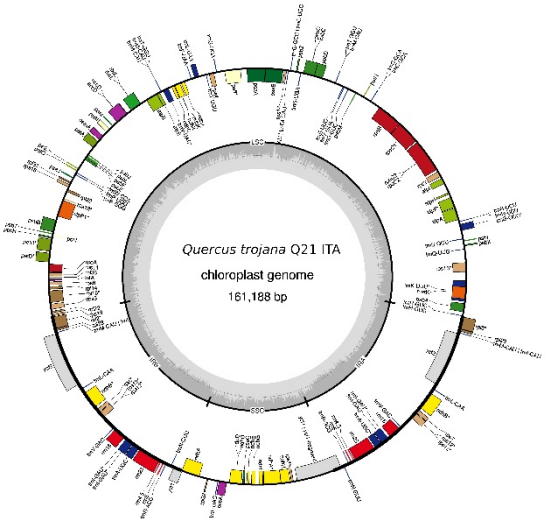

Figure 1. Whole chloroplast genome of *Quercus trojana*.

***Acknowledgments**: Funded by the National Recovery and Resilience Plan(NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union– NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC*

Soil health indicators to assess the effectiveness of nature-based solutions and restoration actions in degraded areas

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***Keywords**: Post-fire; Soil indicators; Urban forestry; Ecosystem services; Post-storm sites*

The National Biodiversity Future Centre (NBFC), founded by Italian National Recovery and Resilience Plan (PNRR), focuses on valorization and conservation of Italian and Mediterranean biodiversity. One of the main goals of this centre is to improve the application of nature-based solutions (NbS) and restoration actions in natural and anthropized areas. The research activities, carried out within the framework of the NBFC, include the selection of suitable soil indicators for the evaluation of NbS and restoration actions in natural and anthropized areas. The aim of this work was to assess the suitability of stable isotope composition and soil enzyme activities as indicators of soil health and validate their sensitivity to disturbance as fire, urbanization, flooding, and storms. Afterwards, the selected soil indicators were used to assess the NbS and restoration action suitability in the soil health recovery. The impact of fire events and intensity on soil isotope composition and soil enzyme activities were studied in pine and chestnut forests in Monte Pisano (Pisa), to assess the suitability of these soil indicators in monitoring soil health during natural post-fire revegetation. After validation, the soil enzymes were applied in the monitoring of soil health recovery after the plantations of *Quercus suber* L. as NbS in a post-fire site on La Verruca, Monte Pisano (Pisa). The sensitivity of soil stable isotope composition and soil enzyme activities to urbanization were also investigated in green areas dominated by *Quercus ilex* L. from natural to urban sites (Pisa and Livorno). Afterwards, they were applied to evaluate the suitability of green infrastructure as NbS in providing ecosystem services in urban areas, linking soil health to air quality. Concerning the restoration actions, the studied soil indicators were investigated in riparian woodlands (Parco Naturale del Po Piemontese) with different managements to define their role as drivers of plant richness as well as to select the best management practices. In addition, the soil enzyme and stable isotopes will be used for assessing the suitability of poplar-vicia consortium as NbS for soil health recovery in riparian ecosystem. The application and validation of soil indicators in the post-storm sites are still ongoing. In particular, post-Vaia sites (Asiago, VI), have been selected to investigate the soil indicators in four different environments: inner woodland stands not affected by the storm, transition areas, storm damaged areas with new plantations and damaged areas with spontaneous evolution of vegetation. Thanks to NBCF research activities, strategies and guidelines as well as databases and tools will be provided for the designing and application of NbS aimed at promoting biodiversity, preserving and recovering soil health and resilience in urban and natural areas.

***Acknowledgments**: This work was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC".*

Monitoring, functional characterization and traceability of resilient basic propagation material for ecosystem restoration and nature-based solutions: from seed sources to seedlings

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***Keywords**: Adaptation; Mitigation; Plant establishment, Genetic, Biodiversity*

The success of restoration or nature-based solutions that can mitigate the effects of disturbance is linked to the use of resilient plant material. The quality of seedlings or saplings depends on the seed source, the procedures of the nursery chain as well as the prediction of the suitability of sites in relation to climate change. We present some of the activities of Task 4.5.2 in the NBFC related to the selection of species and provenances resilient to climate change and suitable for restoring biodiversity in sites affected by extreme events of chronic stress conditions. The characterization of the forest nursery chain has allowed us to define some bottlenecks that reduce the potential production of plants resilient to climate change and suitable to grow in harsh environments such as disturbed sites (storms, floods, post-fire, pollution, pest attacks). We report the main results obtained through the main activities related to: 1) setting innovative approaches to characterize seed sources (defined by DL 386/2003) including genetic studies (introgression and interspecific hybridization) and germination through seed priming treatments (orthodox seeds); 2) quantifying the genetic variability decrease due to nursery practices selection; 3) mature tree, nursery and laboratory experimental trials to characterize the response to environmental and biotic stresses; 4) selection of herbaceous species suitable to use in actions of nature-based solutions and ecosystem restoration.

Acknowledgments: *This work was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC".*

Absorption and translocation of polyethylene terephthalate micro(nano)plastics derived from synthetic textile in *Arabidopsis thaliana*

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***Keywords**: Gene expression, Microplastics, Nanoplastics, Phytohormones, Root development*

Microplastics (MPs) and nanoplastics (NPs), collectively referred to as MNPs, are an emerging threat raising concerns about their impact on the environment and human health. Synthetic textiles account for more than half of global plastic pollution, leading to a massive spread of MNPs in different ecosystems. Depending on the polymer type, size and shape, MNPs may trigger various physiological responses in different plant species. Studies have shown that root growth is induced in *Allium fistulosum* plants treated using polystyrene (PS) and polyethylene terephthalate (PET) MPs, while treatments using PS nanobeads were shown to inhibit root development in *Arabidopsis thaliana* seedlings. Limited data is available on the uptake of MNPs in plants, as one of the main challenges for these studies is the detection of MNPs inside plant tissues. Recent research has demonstrated that different plant species, such as *A. thaliana*, can uptake PS nanobeads through the roots, accumulating them in the vascular tissues. However, most of these studies have been conducted using round-shaped fluorescently labelled PS micro- and nanobeads, while less data is available on the uptake of true-to-life MNPs. Furthermore, the molecular responses induced by MNPs in plants have been poorly investigated. To date, a few authors have shown the impairment of gene expression in *A. thaliana* seedlings treated using PS nanobeads, suggesting the involvement of phytohormones signalling pathways.

To delve into the impact of true-to-life MNPs contamination on plant development, this study focused on the uptake and effects of a powder derived from a reflective safety vest.

The powder was characterized by Raman spectroscopy, scanning Electron Microscopy (SEM) and Transmission Electron Microscope (TEM), revealing it was made of PET and contained both MPs and NPs. Due to the fluorescent dye of the safety vest, the MNPs contained in the PET powder were detected by fluorescence optical microscopy. Thus, *A. thaliana* seedlings were grown on a medium supplied with increasing concentrations of PET powder. By means of confocal microscopy, fluorescence optical microscopy and SEM, the fluorescent MNPs were detected inside the vascular tissues of roots and shoots, suggesting the uptake and translocation of MNPs along the plant. Seeds germination percentage was unaffected by the PET powder, while after 5 days the seedlings grown on PET powder-containing medium developed roots significantly longer compared to controls. Root elongation relies on the development of the root apical meristem (RAM), which provides new cells for root growth. The analysis of the RAM showed an increased length and an increased number of cortex cells in the PET powder-treated seedlings, confirming the increase in root length. The dimension of the RAM is mainly regulated by the interplay between the phytohormones auxin, which promotes cell division and root elongation, and the cytokinins, that repress RAM elongation and auxin signalling. In seedlings treated with PET powder, the expression of cytokinins responsive genes, such as *SHY2* and *GH3.17*, was significantly downregulated compared to controls, suggesting that the absence of the repression of the auxin signalling caused the enhanced RAM and root elongation. Additional insights into the effects of MNPs on plant development were obtained using Laser ablation ICP-MS, which provided first evidence for the correlation between MNPs uptake and the re-distribution of macro- and micro-nutrients in plants. Altogether, these results unveiled new details for the uptake, physiological and molecular effects of PET MNPs in plants, shedding light on the consequences of textile-derived pollution.

***Acknowledgments**: National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC".*

Making Digital Objects FAIR and Interoperable Across Environmental Research Infrastructures: Insights from the ITINERIS Project

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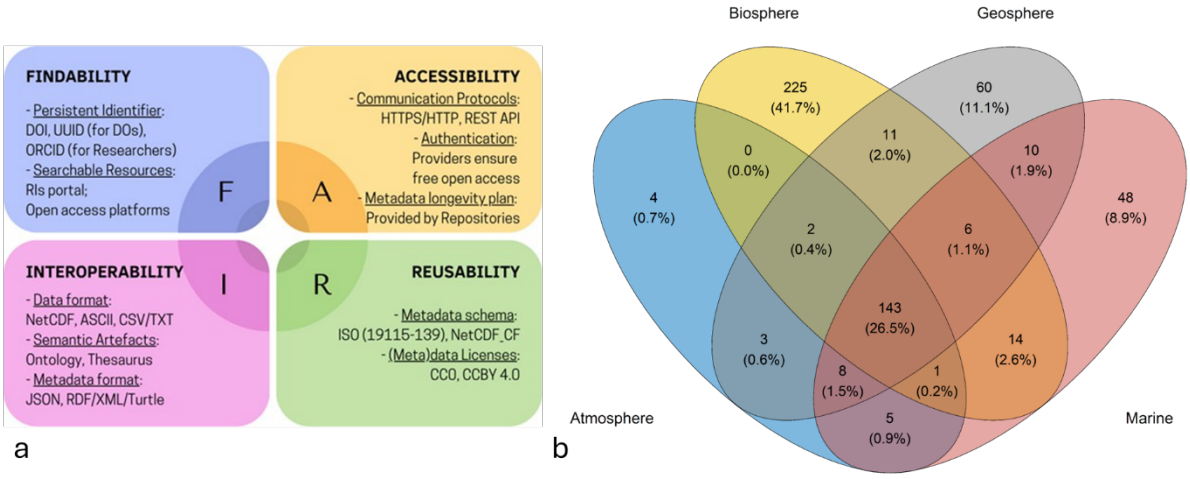
***Keywords**: FAIR principles, Semantic interoperability, Digital objects*

The ITINERIS project is establishing a unified gateway to the scientific data and services (e.g. facilities, instruments, platforms, etc.) offered by an Italian network of 22 Research Infrastructures (RIs) encompassing all four environmental domains, i.e. atmosphere, marine, terrestrial biosphere, and geosphere landsurface. The heterogeneity of implementation practices adopted, and technologies used by the ITINERIS RIs poses a significant challenge to the sharing, interoperability, and integration of interdisciplinary scientific information, ultimately limiting the capacity to face current and future environmental concerns. In this regard, the adoption and implementation of FAIR principles (Findable, Accessible, Interoperable, Reusable) and Open Science practices is crucial to address these issues and to increase the impact of environmental research. Within ITINERIS, the Operative Unit of CNR-IRET in Lecce facilitates the effective integration of the FAIR principles by, for example, developing a FAIR-compliant data management plan, evaluating the implementation of FAIR practices, and promoting semantic interoperability.

While running these activities, we found a significant heterogeneity in the implementation of FAIR practices related to the different stages of development of the ITINERIS RIs and to the specific needs of the involved scientific communities (Fig. 1a). Particularly for interoperability, we found more than 500 semantic artefacts, resulting in a complex network of resources (Fig. 1b) holding redundancies and, at times, incomplete adherence to the FAIR principles. However, evidence of convergence in FAIR strategies was also identified as evidenced by the marine RIs. The result of our analysis offers valuable examples of FAIR implementation practices to be adopted by evolving RIs and the ITINERIS HUB. In addition, the integration of FAIR semantic artefacts and services within the hub will enhance the search, discovery, and reuse of interdisciplinary scientific information. By improving the interoperability, it will enable researchers to integrate diverse datasets and methodologies across domains, fostering global



collaboration and accelerating insights.

Figure 1. Summary of the most common FAIR implementation practices used by the Italian Environmental Research Infrastructures participating in the ITINERIS project (a) and Venn diagram showing the 540 semantic artefacts and their distribution on the four environmental domains (b).

***Acknowledgments**: The publication has been funded by EU - Next Generation EU Mission 4 "Education and Research" - Component 2: "From research to business" - Investment 3.1: "Fund for the realisation of an integrated system of research and innovation infrastructures" - Project IR0000032 – ITINERIS - Italian Integrated Environmental Research Infrastructures System - CUP B53C22002150006. The authors acknowledge the Research Infrastructures participating in the ITINERIS project with their Italian nodes: ACTRIS, ANAEE, ATLaS, CeTRA, DANUBIUS, DISSCO, e-LTER, ECORD, EMPHASIS, EMSO, EUFAR, Euro-Argo, EuroFleets, Geoscience, IBISBA, ICOS, JERICO, LIFEWATCH, LNS, N/R Laura Bassi, SIOS, SMINO.*

Progetti PNRR nell'ambito delle tematiche dei GdL "Ambiente e Salute"; "Ciclo del Carbonio"; "Infrastrutture di Ricerca" e "Altro"

Advancing Sustainability and Health: The role of microbial-derived copolymers in circular economy and biomedical applications

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***Keywords**: Biodegradable copolymers; Endocrine-disrupting chemicals (EDCs); LAHB nanoparticles; Oxidative stress response; Pharmaceutical and biomedical applications*

Recently, "environmentally sustainable" plastic materials made with biodegradable polymers, such as polylactic acid (PLA), polycaprolactone (PCL), and poly(lactic-co-glycolic acid) (PLGA), have been designed for pharmaceutical and biomedical applications to function as transient (bioresorbable) polymers while maintaining mechanical properties similar to those of their non-biodegradable counterparts. These bioplastics exhibit accelerated environmental degradation, facilitating a controlled and sustained release of the encapsulated drug, thereby extending its therapeutic efficacy. However, the proliferation of bioplastic materials requires careful examination, as the accelerated biodegradation of polymers raises concerns about the rapid accumulation of particles in living tissues, which could act as endocrine disruptors. Indeed, endocrine-disrupting chemicals (EDCs), imitating natural hormonal activities may potentially modify the endocrine system's physiological functions. Disruption of the endocrine system results in hormonal imbalance, potentially affecting the development and pathophysiology of metabolic illnesses, including non-communicable diseases (i.e., type 2 diabetic mellitus (T2DM), obesity). Numerous in vitro, in vivo, and epidemiological investigations indicate that engineered nanomaterials (ENMs) typically have harmful effects on the molecular and hormonal pathways, as well as the organ systems implicated in the pathogenesis of type 2 diabetes mellitus (T2DM).

To overcome these issues, the present study describes the application of a novel biodegradable copolymer, defined LAHB, obtained from PLA and polyhydroxybutyrate (PHB) a bio-based aliphatic polyester which is degraded by human microbiota without the formation of toxic products. The synthesis of LAHB nanoparticles was achieved using the nanoprecipitation method, which ensures uniform particle size and stability. The nanoparticles were characterized through dynamic light scattering (DLS) to determine their hydrodynamic size and polydispersity index, and the values were confirmed by scanning electron microscopy (SEM). In addition, infrared (IR) spectroscopy was employed to confirm the chemical structure and functional group integrity.

Cytotoxicity, oxidative stress response, and endocrine activity were performed according to an ISO guideline on LAHB-NPs. PLA-NPs were used as control. The obtained data demonstrated that LAHB-NPs did not have any effect in the Microtox assay, while PLA-NPs induced baseline toxicity. Moreover, PLA-NPs activate Nrf2-ARE pathway involved in oxidative stress response and inhibit the androgen receptor at the highest non-cytotoxic concentration, while no estrogenicity was detected. In contrast, LAHB-NPs showed no antiandrogenic or antiestrogenic activity at all tested concentrations.

These findings underscore the potential of LAHB as promising alternative to conventional bioplastics for pharmaceutical and biomedical applications, with a specific advantage in mitigating concerns related to the endocrine-disrupting effects of existing bioplastics.

Enhancing Urban Air Quality: Integrating Vegetation Strategies and Plant Stress Research

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***Keywords**: Urban Green Infrastructure; Air Pollution Mitigation; Tree Selection*

Urban areas face multifaceted environmental challenges, necessitating the identification of city-specific priorities to develop effective mitigation strategies. The deployment of urban green infrastructure represents a promising avenue for enhancing public health by mitigating air pollution. Vegetation plays a pivotal role in regulating pollutant dynamics, with certain tree species demonstrating superior efficacy in capturing airborne contaminants. Critical traits influencing this capacity include high canopy density, extended foliage lifespan, water-use efficiency, and elevated stomatal conductance, which facilitates the uptake of gaseous pollutants. However, vegetation can also contribute to secondary pollution by emitting volatile organic compounds (VOCs), which react in the atmosphere to form ground-level ozone (O_3). Consequently, selecting tree species with low VOC emission potential is particularly vital in residential and high-traffic areas. Advanced modelling tools in remote sensing used for recognizing tree species in urban environments (Figure 1) and ecological assessment frameworks offer valuable support for estimating ecosystem services and guiding the selection of optimal vegetation species. These tools empower urban planners, public institutions, and citizens to make informed decisions that maximize air pollution reduction benefits. The effectiveness of urban green spaces in mitigating air pollution is influenced by factors such as habitat fragmentation, overall green cover, and the specific characteristics of plant species. While selecting vegetation with traits conducive to pollutant capture is essential, it must be balanced against ecological adaptability and stress tolerance considerations. Particularly in roadside and other high-stress urban environments, the resilience of plant species to local conditions is a critical determinant of their long-term efficacy and suitability.

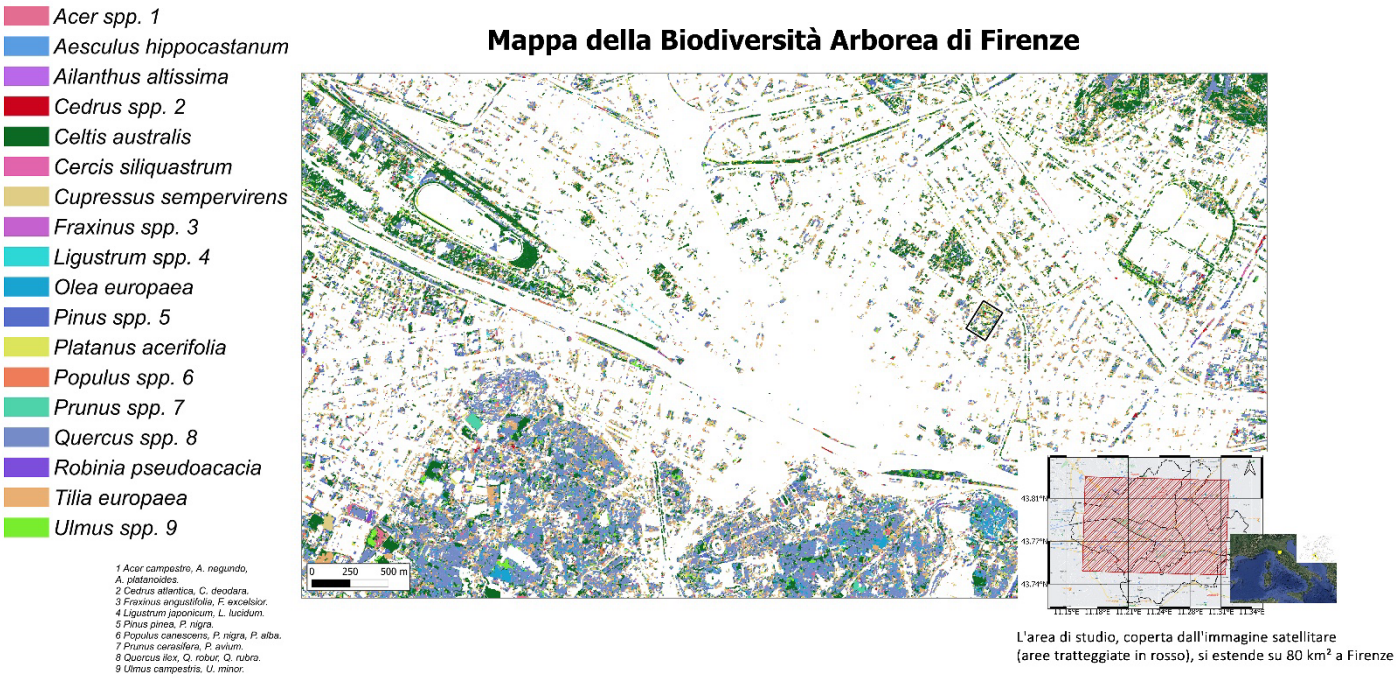

Figure 1. Florence Tree Urban Biodiversity

Extracellular Vesicles as Multi-Bioactive Complex and mediators of Inter-kingdom communication: Their Versatile ecosystem Effects

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***Keywords**: Extracellular vesicles; Inter-kingdom communication; Ecosystem; human health*

Extracellular vesicles (EVs) are lipid bilayer-enclosed vesicles of endosomal origin, which initially considered as garbage bins to dispose unwanted cellular components, but they have now emerged as an intercellular communication system involved in several physiological and pathological conditions.

Exciting recent studies demonstrate that EVs are emerging as essential mediators of intercellular communication within complex biological ecosystems. These nanoscale vesicles, secreted by various cells across different species, facilitate the transfer of bioactive molecules such as proteins, lipids, nucleic acids (DNA, mRNA, small and long non-coding RNA), and metabolites, enabling dynamic, decentralized communication networks. EVs function as a pivotal dialogue mechanism, coordinating cellular responses to environmental stimuli, maintaining homeostasis, and supporting tissue repair and regeneration. Within multicellular organisms, EVs enable communication between different cell types and organs, supporting integrated physiological functions. Beyond the individual organism, EVs also play a significant role in interspecies communication, influencing interactions between microbiota, plants, and animals, and helping ecosystems adapt to changing conditions. This work explores the emerging understanding of EVs as a fundamental component of ecological networks, mediating both intra- and inter-organismal signalling to preserve ecosystem stability and health. Through their capacity to exchange molecular information across species barriers, EVs represent a novel paradigm in ecological communication, with broad implications for human health, environmental sustainability, and disease prevention. In addition, this work explores the concept of interspecies communication between plants and humans through EVs, highlighting the potential therapeutic implications, including novel plant-based interventions for disease prevention and treatment. Importantly, EVs from different eukaryotic and prokaryotic kingdoms are involved in many processes including host-pathogen interactions, spreading of resistances, and plant diseases. More extensive knowledge of inter-species and interkingdom regulation could provide advantages for preventing and treating pests and pathogens. By understanding the molecular mechanisms underlying this communication, we can explore new avenues for harnessing the power of plants to improve human health and well-being. The role of EVs in these complex dialogues will open new avenues for therapeutic strategies aimed at restoring ecological balance and enhancing health resilience, since it is becoming increasingly evident that these nano-vesicles play important roles in the global ecosystem.

Trait syndromes and intraspecific responses to fire regimes of Mediterranean reseeder and resprouter woody plants

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***Keywords**: Fire disturbance; Mediterrenean Basin; Plant functional traits; Reseeders and resprouters; Wody plants*

Fire has occurred on the Earth's system for more than 420Myr so representing an eco-evolutionary force influencing the dynamics of ecosystems and biological assemblages from the local up to the global scale. Fire disturbance affects especially open biomes' vegetation, e.g. globally distributed tropical savannas and mediterranean shrublands. In these ecosystems, plants have evolved a variety of strategies that allow them to cope (more or less effectively) with certain fire regimes. Among these strategies, reseeder and resprouter plants is the most used dichotomy. Yet, populations and species are not homogeneous entities, rather are composed of individuals characterized by having variable traits (intraspecific trait variability), hence the strict dichotomy between reseeder vs resprouter strategies may be more nuanced (captured by distinct trait syndromes). Additionally, ontogeny can shape trait expression and (co)variation. All these elements can largely affect plant responses to the environment, including to fire regimes. However, these sources of within-species variability remain largely underexplored in fire ecology and functional ecology of plants, particularly in mediterranen-type ecosystems.

In this research, we set out to address four questions: (Q1) Do resprouter and reseeder species differ in their trait coordination, while resprouters tend to be characterized by similar trait coordination? (Q2) Do intraspecific trait patterns indicate fire-modulated plant responses to changes in fire regimes? Do these patterns differ between reseeder and resprouter species, as well as between resprouter species? (Q3) Does fire regime affect key intraspecific bivariate trait coordination relationships? (Q4) Intraspecifically, does ontogeny affect key relationships between functional traits and fire regime?

To tackle these questions, during Spring and Summer of 2024, we conducted fieldwork focusing on three species typical of, and widely distributed in the Mediterranean Basin, namely *Cistus salviifolius* (reseeder), *Erica arborea* and *Quercus ilex* (both resprouters) at three sites (Montiferru, Sardinia; Mt Morrone, Abruzzo; Mt Pisano, Tuscany). We selected 12 morphological, structural and architectural traits informing on 1) plant ability to cope with fire disturbance and resource (mainly water) availability, 2) size, 3) mortality, 4) allocation to sexual reproduction effort, and 5) developmental stage. Within each site, we identified a fire regime gradient, based primarily on time since last fire event and number of fires in the last 50+ years. Overall, we sampled 120 individuals, and conducted a set of multivariate and bivariate linear regressions to answer our questions.

Results indicate: (A1) High species- and trait-specific patterns (i.e. pairwise bivariate and multivariate correlation), such as *E. arborea* is characterized by a much tighter syndrome (stronger trait correlation) than *Q. ilex* and *C. salviifolius*. (A2) Intraspecific analysis suggests that fire regime modulates plant functional responses, yet these are not always aligning with the reseeder-resprouter dichotomy, e.g. *C. salviifolius* at times responds similarly to one of the two resprouter species (while these may differ between them). (A3) Fire can affect some key intraspecific bivariate trait coordination (e.g. a negative interaction effect for growth-related traits), and this is observed for *C. salviifolius* and *E. arborea* while *Q. ilex* remains unaffected. (A4) Ontogeny can affect some key trait-fire regime links (e.g. allocation to canopy of the main stem, related to light caption exerted by single or multi-stemmed individuals), and these interactions emerge for *C. salviifolius* and *E. arborea* while *Q. ilex* remains unaffected. The more relaxed trait syndrome and less responsive trait patterns in *Q. ilex* can be also related to the considerably longer lifespan of this species (centuries; compared to that of *E. arborea* and *C. salviifolius*; decades), implying that we could not sample mature individuals (most *Q. ilex* plants have indeed 0 to 2 forks).

Overall, our findings suggest that the strict dichotomy between reseeder and resprouter plant strategies can be seen as a gradient, and less fixed/more nuanced than previously thought – when intraspecific trait variability and the effect of ontogeny are taken into account. While *a priori* classifications of life histories (e.g. reseeders vs resprouters, life forms, growth forms) can be powerful tools to examine functional patterns at coarse (e.g. regional, global) scales, at fine (local) scales considering within-species variability in life history classification can refine our understanding of how plants can cope with their changing environments (e.g. fire disturbance).

***Acknowledgments**: This research is supported by NBFC (National Biodiversity Future Center) funded by the Italian Ministry of University and Research, P.N.R.R., Missione 4 Componente 2, "Dalla ricerca all'impresa", Investimento 1.4, Project CN00000033 (financed by the European Union – NextGenerationEU).*

Comparing C and N stable isotopes analysis between urban and peri-urban Mediterranean parks along a latitudinal transect

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Keywords: *Isotope analyses, Carbon, Nitrogen, Human impact, Urban parks*

As one of the most vulnerable areas on our planet, Mediterranean region represents a hotspot for climate change and biodiversity. Prolonged droughts and heatwaves are increasingly affecting water availability, which is crucial for the resilience and adaptation of the local vegetation. For over 10,000 years, human activities such as agriculture, urbanization, and deforestation have altered the Mediterranean landscape. Understanding the response of the vegetation to the dynamics between natural and human induced changes is essential for conservation and management efforts. Mediterranean vegetation responds differently to environmental changes and anthropogenic pressure such as urbanization and the related stressors. Even within the same species, different compartments (e.g. leaves, roots, and branches) may exhibit diverse responses to these stressors and can be used as valuable bioindicators. Stable isotopes, particularly carbon (C) and nitrogen (N), have been widely used as powerful tools to investigate plant responses to environmental gradients such as plant water-use efficiency, nitrogen-use strategies and ecosystem functioning.

In this study, conducted within ITINERIS (Italian integrated environmental research infrastructures system) project, we present preliminary results of a comparison between $\delta^{13}C$ and $\delta^{15}N$ values in soil and vegetation samples of coastal Mediterranean urban and peri-urban parks along a latitudinal gradient in Italy. To assess the relationship between isotopic signatures and environmental changes, soil and vegetation from the most abundant evergreen and broadleaf species were collected in three different ICOS (Integrated Carbon Observation System) stations located along the coastal latitudinal transect (Pisa, Rome and Naples). Holm oak (*Quercus ilex*) was chosen as potential bioindicator of environmental changes and anthropogenic disturbances, since abundantly present in all the three sites. Soil and plant compartments (e.g., leaves of different ages, branches, pollen, fine roots) were collected and analysed at the beginning and at the end of the growing season to investigate and provide insights on the response of Mediterranean species up to organ level to thermo-pluviometric gradients.

The main findings of this study so far, have underlined differences in C and N concentrations and isotope compositions along the latitudinal gradient and seasons. Capodimonte, the most southern site, reveals an enrichment in $\delta^{13}C$ and $\delta^{15}N$ in leaves compatible with a response to hot and dry climate, and a much higher degree of anthropization. Castel Porziano shows a similar trend but with lower $\delta^{15}N$ and N concentration. San Rossore, the most northern site, appears to be less exposed to summer water stress and consequently shows more diluted $\delta^{13}C$ and $\delta^{15}N$ values. Leaves during the fall showed higher $\delta^{13}C$ and N concentration compared to spring. Branches were about 1 ‰ enriched in ^{13}C compared to leaves due to post-photosynthetic isotope fractionations. Further analyses are in progress on other plant compounds, including pollen extracts and soil samples, to identify the most suitable bioindicators of Mediterranean species response to climate change and human impact and consequently, potential applications in management and conservation strategies in the region.

Acknowledgments*: The work was funded by IR0000032 – ITINERIS, Italian Integrated Environmental Research Infrastructures System (D.D. n. 130/2022 – CUP B53C22002150006) Funded by EU – Next Generation EU PNRR-Mission 4 "Education and Research" – Component 2: "From research to business"-Investment 3.1: "Fund for the realization of an integrated system of research and innovation infrastructures".*

An experimental infrastructure for realistic experiments in Mediterranean Europe - FO_3X (Free air O_3 eXposure)

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Keywords: *Tropospheric ozone; ozone FACE; forest health; technological development*

Tropospheric ozone (O_3) is a secondary pollutant gas that causes a significant adverse effect on forest tree species. Damage to vegetation occurs when O_3 enters the stomata following gas exchanges, which results in a reduced photosynthetic rate and impairment of biomass development. Plant sensitivity to O_3 is species-specific, although an exposure-based assessment approach using AOT40 (Accumulated exposure Over Threshold of 40 ppb), which is applied for the European regulation, does not consider the difference of species-specific response to the O_3 polluted condition. Considering a particular climate and relatively high biodiversity in Mediterranean Europe, a proper risk assessment should be implemented based on a metric that effectively considers stomatal O_3 uptake for the Mediterranean plant species. Free-air controlled exposure (FACE) facilities are considered an ideal tool that can provide realistic estimates of tree response to O_3 under real-world conditions. Since 2015, the last generation of the O_3 FACE facility has been available at the CNR experimental garden in central Italy (FO_3X - Free air O_3 eXposure), a unique facility in Europe within an AnaEE (Analysis and Experimentation on Ecosystems) European research platform. FO_3X also participates in the activity of the PNRR project ITINERIS (Italian Integrated Environmental Research Infrastructures System) which aims to involve establishing common protocols, methodologies, and standards to enable seamless data integrations. FO_3X hosted 44 visiting researchers from 13 countries with 22 joint scientific papers published in peer-reviewed ISI journals since 2015. This facility permits the exposure of plants to three levels of O_3 concentrations (ambient, 1.5- and 2.0-times ambient concentration, denoted as AA, 1.5×AA and 2.0×AA, respectively), with main environmental variables continuously monitored. Plants were fumigated at FO_3X to assess the effects of only O_3 or combined with other stressors on plant attributes (drought, nitrogen) evaluated O_3 damages. During the presentation, it will be introduced how studies on varying ozone (O_3) treatments have provided insights into the relationship between O_3 levels and plant biomass/yield responses. Through the parameterization of species-specific stomatal conductance (gs), Critical Levels (CLs) have been established, indicating a 4–5% reduction in biomass or yield via the Phytotoxic Ozone Dose (POD). A three-year free-air experiment (2020–2022) on *Vitis vinifera* demonstrated significant yield reductions under elevated O_3 exposure. Additionally, deciduous trees were identified as highly sensitive, along with evergreen species like Aleppo pine. These findings emphasize the importance of studying ozone effects in FACE experiments, as they allow for realistic, controlled assessments of O_3 impacts on plant systems under field-like conditions.

Iniziative e percorsi per un futuro sostenibile

Sessione II: Progetti nazionali e internazionali nell'ambito delle tematiche dei GdL "Biodiversità" e "Ciclo del carbonio"

Intraspecific epigenomics divergence in brown bears (*Ursus arctos*): insights from genome-wide DNA methylation patterns

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***Keywords**: RRBS, Methylome, Non-model, Gene ontology, Conservation, Population*

Brown bears (*Ursus arctos*) are spread across Europe, with populations spanning northern, central-eastern, and southern regions. This flagship species exhibits striking variations in their physical traits (phenotypes). A particularly intriguing population resides in central Italy: these bears stand out due to their distinctive skull shape and behavior, possibly shaped by isolation and adaptation to their local environment. What causes these differences? While genetics plays a role, it is important to consider the impact of epigenetics—mechanisms like DNA methylation that regulate how genes are turned on or off in response to environmental pressures. Could epigenetics be the key to understanding such unique traits? Until now, epigenetic studies were mainly limited to laboratory experiments, and were rarely performed on wildlife, leaving many questions unanswered about how these mechanisms influence population differences.

To address this knowledge gap, we used a method called Reduced Representation Bisulfite Sequencing (RRBS) to examine DNA methylation—the addition of methyl groups to DNA—in brown bear populations from Central Italy, Northern Italy, and Scandinavia. The study analyzed blood and muscle samples to determine which tissue provides better insights and explored how epigenetic patterns might link to local environmental factors. Methylation patterns are highly dynamic and can vary across tissues, age, seasons, and sexes. These patterns regulate gene expression and may shift in response to external environmental conditions. To determine the role of population as a driving factor in shaping the methylation profiles, samples were collected from adult and sub-adult bears to minimize age-related biases. Blood and muscle tissues were analyzed separately, and autosomes and X chromosomes were also examined independently to account for potential sex-related influences. We found that: *i)* Brown bears from different populations showed distinct methylation patterns in both blood and muscle tissues. These patterns were also evident on the X chromosome, highlighting clear epigenetic differences tied to their populations. *ii)* Blood samples exhibited a higher number of differentially methylated regions compared to muscle samples, making blood the preferred tissue for such studies. This finding is particularly significant because it enables sampling from live animals, whereas muscle sampling would require deceased specimens, making it more challenging to collect sufficient samples. *iii)* Many of the identified methylation differences were associated with genes involved in cellular and anatomical development. This finding aligns with the unique skull shape and other traits observed in the Central Italian bears.

Epigenetics provides a new lens through which to study how animals adapt to their environment. This research highlights its potential to explain the remarkable differences among brown bear populations and underscores the importance of such studies in wildlife conservation. For instance, understanding population-specific adaptations can help identify Evolutionarily Significant Units (ESUs)—key groups that are crucial to preserving biodiversity.

In short, studying epigenetics in brown bears not only helps unravel the mysteries of their adaptation but also offers powerful tools for ensuring their survival in a rapidly changing world.

A non-invasive genetics insight into population structure and recolonization dynamics of the Eurasian otter (*Lutra lutra*) at the boundary of its Italian core range

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***Keywords**: Microsatellite, Inbreeding, Admixture, Non-invasive genetics, Recolonization route.*

Once widespread across the Italian peninsula, the Eurasian otter (*Lutra lutra*) underwent a severe decline in the 20th century, surviving only in two disjunct cores in Southern Italy. Since the 2000s, the species has been recovering in its former range, leading to the re- joining of the two isolated populations and, specifically in the last 15 years, along rivers of Abruzzo region. The expansion along the Sangro river enabled its recolonization upstream the lake Barrea's dam in the Abruzzo, Lazio and Molise National Park (PNALM) after 40 years from the extinction. This area is fundamental for the persistence and northward expansion of the species.To investigate the dispersal dynamics from Southern Italy to Abruzzo and identify potential factors limiting gene flow at the boundary of the Italian core range – particularly between the PNALM and surrounding areas – we collected samples from Central and Southern Italy. DNA was extracted from 52 specimens, including faeces (spraints), anal jellies and tissue samples, collected across the regions of Abruzzo, Molise, Campania, Apulia and Basilicata (Fig. 1). The extracted DNA was then used to amplify a panel of 11 microsatellite loci.

Identity analysis identified 18 unique genotypes within the PNALM (15 males and three females). The estimated census population size (N_c, CI95% 31-75) upstream the dam suggested a minimum population of at least 31 individuals. The observed heterozygosity in the PNALM was moderate (H_o=0.50), but consistent with literature values, and the level of inbreeding in this area was relatively low.

spatial Principal Component Analysis (sPCA) and admixture analysis revealed a geographically structured genetic diversity in the entire sample. Specifically, there is a clear genetic differentiation between Southern and Central Italy. Notably, specimens from the Adriatic coast of Abruzzo (nearby the mouths of Foro, Sinello and Pescara rivers to the north of the PNALM) showed high genetic similarity with those from the Adriatic coast of Apulia. Gene flow among Abruzzo's populations (PNALM, Sangro and Volturno rivers downstream lake Barrea's dam, and Foro-Aterno-Pescara rivers) was relatively high, except from Sangro-Volturno toward the PNALM.

Population structure in Abruzzo is weak, and genetic diversity in the PNALM remains low, consistent with a founder effect. The 18 unique genotypes detected upstream the dam, and the N_c estimate suggest the population in this area could host more individuals than previously assumed. The level of inbreeding suggests that the number of founders was not low, or that immigration from neighboring areas over time may have helped to limit inbreeding. Moreover, results revealed a significant genetic differentiation between Southern and Central Italy, suggesting a stepping-stone recolonization process of Abruzzo river basins from Southern Italy. Furthermore, the high genetic similarity between specimens from the coastal areas of Abruzzo and Apulia suggests otters followed different recolonization routes and possibly dispersed along coastal areas, using the sea as corridors. Finally, gene flow estimates indicate a possible reduction of connectivity within the PNALM, likely due to the barrier effect of the dam.Although the otter population upstream the dam in the PNALM shows a favorable conservation status, its low genetic diversity makes it vulnerable to demographic and environmental stochastic factors. Accordingly, interventions to enhance connectivity and facilitate otter movements are highly recommended to stabilize and expand Central Italy's population.

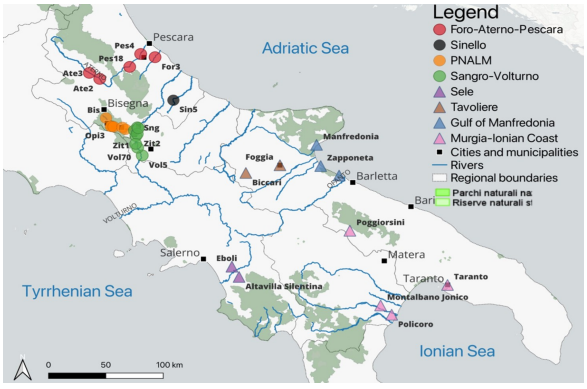

Figure 1. Distribution of the sampling sites, where genotyped specimens come from, in the whole study area. Coloured circles or triangles discriminate between sampling sites of spraints/anal jellies and tissue samples, respectively.

National programs of classical biological control of the invasive alien pests *Halyomorpha halys* and *Drosophila suzukii* in Sardinia (Italy)

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***Keywords**: Natural enemies; Parasitoids; Pest management; Establishment; Host-specific*

Biological invasions are a global consequence of an increasingly interconnected world and the rise in human population. The number of invasive alien species, non-native species introduced to areas outside their native range through human activities, is increasing. Global warming exacerbates current invasions and facilitate new ones, amplifying their ecological, economic, and human-health impacts. Insects are one of the taxa with the highest frequency of introduction due to their high diversity, biological properties, and close association with human activities.

Integrated pest management tools often fail to effectively reduce invasive pest infestations. Moreover, current pest management techniques rely on non-selective insecticides, which negatively affect the biocoenosis while increasing the production costs. Classical biological control, the introduction, release, and establishment of host-specific co-evolved natural enemies, offers a sustainable solution by permanently suppressing invasive insect pest populations across wide areas, often reducing them to less harmful levels.

Two invasive pests, *Drosophila suzukii* (Diptera Drosophilidae) and *Halyomorpha halys* (Hemiptera Pentatomidae), native to east Asia, were first recorded in Italy in 2009 and 2012, respectively. *D. suzukii* is a pest of soft- fruits and cherries, while *H. halys* attacks a wide range of fruits, horticultural and ornamentals crops. Since its first detection in 2012, *D. suzukii* has become the primary pest of sweet cherry in Sardinia, whereas *H. halys,* detected in Sardinia in 2016, has spread throughout the island without causing significant damage so far.

To set up national classical biological control programs targeting *H. halys* and *D. suzukii*, two national Technical Committees were established within the frame of the action of the Ministry of Agriculture, Food Sovereignty and Forests. These Committees, coordinated by Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria (CREA), comprise scientific experts and representatives of the regional plant protection services. The *H. halys* Technical Committee was formed in 2020 and operate in the introduction of *Trissolcus japonicus* (Hymenoptera Scelionidae), the exotic egg parasitoid of the stink bug. The *D. suzukii* Technical Committee was formed in 2021 and operate in the release of *Ganaspis brasiliensis* G1 (Hymenoptera Figitidae), the exotic larval parasitoid of *D. suzukii*. The objectives of the Technical Committees include: i) securing annual permissions for the release of the exotic parasitoids from the Ministry of Ecological Transition, ii) developing rearing and distribution systems for parasitoids to support field releases, and iii) coordinating release and monitoring activities across participating regions.

Since 2020 and 2024 respectively, the Research Institute on Terrestrial Ecosystems has been participating as scientific responsible for the implementation of the *H. halys* and *D. suzukii* classical biological control programs in Sardinia within the respective Technical Committees. Between 2021 and 2024, *T. japonicus* was released at five sites with each release involving 100 mated females reared in the laboratory, and conducted twice per year. Pre- and post-release samplings of egg masses were performed according to national protocols. Egg masses of *H. halys* and native pentatomids were isolated in plastic tubes and reared in laboratory until bug or parasitoid emergence. Laboratory analyses confirmed the emergence of *T. japonicus* from three *H. halys* egg masses collected during post-release samplings in 2022 at two sites and from one sentinel egg mass exposed in 2023 at another site. No emergence was observed from non-target pentatomid egg masses. In 2024, *G. brasiliensis* G1 was released at one site, with three releases per year, each involving 100 mated females. Pre- and post-release fruit samplings followed the national protocol. In laboratory, fruits were incubated for at least 35 days at 22°C and drosophilid pupae were regularly collected and isolated in plastic tubes for emerging fly or parasitoids. *G. brasiliensis* G1 did not emerge from either *D. suzukii* or non-target drosophilid pupae.

Although the establishment of efficient and specific biological control agents takes several seasons, once it occurs it could promote long-term control of invasive species that is less dependent on the use of chemical pesticides, mitigating the negative effects associated with them.

***Acknowledgments**: Work supported by Regione Autonoma Sardegna, Settore Fitosanitario: Programma Nazionale di lotta biologica per il controllo in Italia di Halyomorpha halys mediante l'impiego del suo antagonista naturale Trissolcus japonicus and Programma Nazionale di lotta biologica per il controllo in Italia di Drosophila suzukii mediante l'impiego del suo antagonista naturale Ganaspis brasiliensis and by CNR: Research project USEit - Utilizzo di sinergie operative per lo studio e la gestione integrata di specie aliene invasive in Italia.*

The Arctic tundra: key drivers of the Carbon cycle under changing climate

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Keywords: *Arctic; Carbon cycle; Carbon fluxes; Stable isotopes*

Arctic terrestrial ecosystems play a pivotal role in the global Carbon cycle, holding approximately 50% of the world's below-ground organic carbon in permafrost soils. Accelerated warming of Arctic leads to significant increase in emissions of CO_2 and CH_4 after the permafrost degradation. Simultaneously, Arctic is undergoing "greening", characterized by an increase in plant productivity and shifts in species composition. These changes are further altering carbon (C) and nitrogen (N) cycles across the atmosphere-biosphere continuum, leading to increased ecosystem complexity driven by fine-scale ecological interactions and climate change

Since 2013, researchers from our institute have been investigating different aspects of High Arctic tundra ecology, focusing on the interplay between climate and biological drivers. The research is conducted in different locations of Brøgger peninsula (Ny-Ålesund), in Svalbard Islands Archipelago (Norway). Principal objectives of these studies are:

- Assessing the sensitivity of dominant tundra species to variations in climatic drivers (e.g. temperature, soil water content, light, CO_2 concentration);
- Examining the impact of biological drivers (e.g., presence of herbivores) on ecosystem functioning, and the interactions between soil microorganisms and vegetation communities;
- Exploring shifts in C and N allocation strategies between plants and soil in response to vegetation composition changes, including shrubs, sedges, mosses, and lichens;
- Investigating the long-term effects of simulated warming via Open Top Chambers (OTC) on tundra ecosystem processes.

Using diverse measurement methodologies, we cover a range of spatial and temporal scales, from the leaf level to community and ecosystem dynamics, and from discrete point measurements to time-integrated observations. At the leaf scale, we conduct precise measurements of photosynthetic efficiency and spectral reflectance, analyzing their responses to variations in key climatic parameters simulated locally. At higher spatial levels, we measure CO_2 fluxes across different plant communities. Carbon and nutrient transfer within plant-soil-atmosphere continuum is assessed by means of stable isotope pulse labeling technique. Community-scale data could be integrated and compared with ecosystem-scale flux measurements.

Preliminary findings from our multi-year study reveal that cloud cover and soil temperature are key factors driving the carbon balance in dominant tundra plant communities, specifically *Dryas octopetala* and *Salix polaris*. Increased cloudiness limits photosynthetically active radiation, reducing the capacity for carbon assimilation by plants, while rising soil temperatures enhance microbial respiration, leading to higher carbon losses. Together, these factors suggest a potential shift in tundra ecosystems from carbon sinks to carbon sources under future climate scenarios. Furthermore, the presence of vascular plants significantly influenced soil microbial functionality, enabling the decomposition of recalcitrant compounds within the rhizosphere. Hence, arctic greening will interact with the capacity of microbial community to degrade complex substrates affecting soil carbon stability. Ulterior complexity is added by the impact of climate change on the population of big grazers (arctic reindeer). We demonstrated that reduced herbivory modifies soil edaphic conditions, declines nutrients availability and fosters the dominance of mosses and lichens, with cascading effects on C cycle. By integrating these findings across spatial and temporal scales, our research provides critical insights into the interconnected effects of climate change, herbivory, and vegetation dynamics on Arctic ecosystem functioning.

Acknowledgments*: This research was supported by the Research Council of Norway, project "GrazeAct", Svalbard Integrated Arctic Earth Observing System - Knowledge Centre (SIOS-KC).*

The project CAROLINA (ClimAte Resilience Over Landuse change In semi-Natural grAsslands, PRIN 2022 PNRR)

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Keywords: *plant biodiversity; soil health; climate extremes; vegetation succession; grasslands abandonment*

Humans have always shaped the landscape to meet their needs, with the results of favouring some habitats as the case of semi-natural grasslands employed to feed the livestock. In mountain areas, the gradual abandonment of traditional agricultural activities, such as grazing and mowing, has led to the expansion of forests to the detriment of meadows and other open habitats. These habitats are particularly biodiverse and, after the abandonment, the colonisation by woody species can result in the net reduction of vascular plant richness. These changes also reflect on ecosystem services, defined by the Millennium Ecosystem Assessment as the benefits that people receive from ecosystems. These include the provision of goods and resources, the conservation of biodiversity and soil fertility, the capacity to sequester carbon and the mitigation of climate change.

The CAROLINA PRIN PNRR project, launched in 2023, investigates the consequences of pasture abandonment on biodiversity and climate resilience. The research focuses on both, the short-term effects of grazing cessation on soil, vascular plants and spectral properties as well as the long-term effects of the transition from grasslands to forest. To achieve these goals, two types of study areas have been chosen: 1) experimental fields and 2) chronosequences, across three different climatic zones: Mediterranean plains, Central Apennine hills and Alpine mountains. The experimental fields are designed to study the short-term effects of grazing suppression through fencing and the impact of reduced precipitations simulated with rainshelters. Chronosequences are represented by five stages of the vegetation succession from grasslands to forests. Planned activities include the study of: a) taxonomic, morphological and physiological plant functional diversity, b) soil functional diversity and c) spectral diversity and climate resilience. Taxonomic and morphological plant functional diversities are studied through the evaluation of selected plant functional traits, as well as with classical metrics to define the functional space occupied by the investigated communities and the related adaptive strategies. To carry out the study of the vegetation physiological functional diversity we are assessing indicators of photosynthetic efficiency and plant health status through the non-destructive analysis of chlorophyll a fluorescence and leaf pigments. Moreover, the compound specific analyses of green tissues coupled to carbon (C) and nitrogen (N) isotope compositions are performed with the intention to acquire data on the time-integrated intrinsic water use-efficiency and the variation in N economic spectrum of species, respectively. Soil properties investigated include soil organic matter and its main fractions, the particulate organic matter and the mineral-associated organic matter. Activity of soil microorganisms is addressed through the enzymatic approach which is a measure of microbial functional diversity and allows estimating the relative resource limitations. Soil CO_2 efflux is monitored in the three experimental fields throughout the year to obtain an overview of changes in soil biological activity. In addition, other main soil physical and chemical parameters such as pH, electrical conductivity, soil texture and aggregate size and stability are evaluated. The study of spectral diversity, carried out through the analysis of time series of specific indexes, will allow to explore the links between vascular plant diversity, land use change and ecosystem resilience in relation to climatic extremes. Care is given to communicating the project aims, scopes and results to local stakeholders and decision-makers, as well as to other kind of audience, also through social media. Among the other deliverables provided, the guidelines based on the multifunctional sustainability index will be realized for the stakeholders relevant for the aims and the activities of the project.

Project outcomes will represent an important step towards the comprehension of the dynamics that link the three types of functional diversity, and to the identification of the best management and conservation practices for these valuable grassland habitats and their characterizing plant communities.

Acknowledgments: *The project is funded by Italian Ministry of University and Research (European Union, Next Generation EU), project PRIN 2022 PNRR n. P20228JYWN*

Progetti nazionali e internazionali nell'ambito delle tematiche dei GdL "Ambiente e Salute"; "Infrastrutture di Ricerca"; "Siccità, Scarsità e Crisi Idriche" e "Altro"

Terahertz Imaging for Agriculture and Non-Invasive Food Inspection

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***Keywords**: Eurobioimaging Node*

The integration of advanced technologies in agriculture is essential for addressing critical global challenges, including biodiversity preservation, environmental sustainability, food security, and efficient resource management. In this context, hazelnut cultivation has seen growing significance in recent years, marked by an expansion in cultivated areas and rising yield projections. Despite this growth, in agriculture currently lacks a reliable automated and non-invasive method to differentiate between healthy and unhealthy hazelnuts. Terahertz (THz) radiation (0.1-10 THz), however, could offer a promising solution. As a non-ionizing radiation, THz waves carry low-energy photons, making them entirely safe for non-invasive biological analysis. This work presents a pilot study designed to test a continuous-wave terahertz transmission imaging system for detecting unhealthy hazelnuts, along with a subsequent project focused on exploring the potential of this technology for other fruits and scenarios. The proposed non-invasive method has the potential to be adapted for real-time application. The terahertz imaging system was composed of an impact avalanche transit time diode source (IMPATT-100-H/F, TeraSense, San Jose, USA) with a nominal output power of 30 mW and an output frequency of 140 GHz, and a THz camera (T15/32/32, TeraSense, San Jose, USA) composed by a square matrix of sensors (32 x 32 pixels). The trial was conducted on a sample of 200 hazelnuts. For each THz image data, we calculated the average attenuation and performed a statistical analysis on these results. Results show that injured nuts tend to have lower attenuation levels. We developed a classification model and evaluated its performance. To achieve this, we utilized MATLAB's probability plot function. The distributions of healthy and damaged samples were plotted, and each was fitted with a third-degree polynomial curve. Once the calibration curves were established, data from an unknown hazelnut sample were introduced as a new data point in both distributions. The minimum distance between this point and each fitted curve was calculated, and the sample was assigned to the distribution with the smaller distance. The model showed an excellent prediction ability, with a True Negative Rate (TNR) of 100% and a False Positive Rate (FPR) of 0%. This means that all the unhealthy hazelnuts in the sample were correctly identified. On the other hand, the True Positive Rate (TPR) and False Negative Rate (FNR) were 75% and 25%, respectively, thus showing a somewhat lower sensitivity. These results indicate that continuous-wave terahertz transmission imaging is a promising method for identifying defective dry fruits prior to commercialization or processing. Furthermore, this approach is simple, requires inexpensive equipment, and has the potential for real-time application, making it particularly appealing to the food inspection. These findings inspired the development of a related project titled *"Fruit recognition using terahertz imaging for swift contamination assessment and non-invasive inspection (FRUITSCAN)".* This initiative, linked to the international call by "Eurobioimaging Node - Terahertz Plant Imaging, THzI @ Italy - Molecular Imaging Italian Node," explores the synergy between terahertz (THz) imaging and artificial intelligence to classify hazelnuts as healthy or unhealthy in a non-invasive manner. Furthermore, by employing terahertz imaging, the project promotes this method for early pest detection, aiming to minimize chemical interventions and foster a positive environmental impact.

One method to rule them all: a FAIR and Open approach for the study and integrated management of Invasive Alien Species

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***Keywords**: Invasive alien species; Open science; FAIR principles; Data management; Best practices*

The implementation of FAIR (Findable, Accessible, Interoperable, Reusable) principles and Open Science practices can greatly enhance the capacity to integrate and reuse multi-disciplinary research products obtained by various monitoring approaches and data collection methods. Such ability allows a better transfer of knowledge-based scientific information to different stakeholders, especially those involved in policymaking, and to the society.

The opportunity to test the adoption of FAIR and Open Science practices at the national Italian level in a multi-disciplinary, multi-ecosystem, and multi-taxonomic context was provided by USEit, a project focusing on Invasive Alien Species (IAS), funded by the National Research Council, and involving scientists from three different CNR Institutes.

The scope of USEit was to combine expertise from scientists studying IAS in different ecosystems (aquatic and terrestrial) and find synergies across different data collection and data management methods, ultimately, outlining operational and technical guidelines suitable for adoption within and beyond the project (Fig. 1).

The operational guidelines for IAS data collection were drafted based on an analysis of the responses to a national survey. They were subsequently reviewed by national experts of biological invasions and, finally, validated through SWOT analysis. In addition, technical guidelines on IAS data management were defined collaboratively and shared within a Data Management Plan describing standards and schemas used for a diverse array of (meta)data (*i.e.*, occurrences, stable isotopes, elemental composition, remote sensing, DNA metabarcoding, citizen science, acoustic telemetry; Fig. 1). These guidelines are shared within a unique landing page representing the main access point of all research products generated and/or published within USEit as well as to any communication material and event associated to the project activities.

Overall, data, services and related metadata are published into canonical repositories and/or available within the LifeWatch Italy Data Portal and Metadata Catalogue (Fig. 1). All research products are assigned with persistent identifiers, described by clear metadata and enriched with controlled vocabularies to enable a facilitated interpretation, reuse and interoperation of products and their outcomes.

These results could more readily be used to develop practical solutions for managing IAS, demonstrating how the implementation of community-shared FAIR practices in multi-disciplinary contexts can lead to an improved capacity to manage natural resources efficiently and create novel opportunities for growth.

Figure 1. USEit project workflow.

***Acknowledgments**: We are grateful to all members and collaborators of the USEit team and to A.N. Muresan for helping with the graphical abstract.*

Evaluating the Impact of Drought Events on Grassland and Forest Ecosystems in Northern Italian Mountains

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Keywords: *Climate variability, Mann-Kendell test; Meteorological drought, NDVI, SPEI, Vegetation responses*

Investigating how vegetation ecosystems respond to climate variability has become a growing research priority. In European mountains, a prominent land-use change trend involves abandoning traditional pastoral activities, leading to the rewilding of grasslands and their gradual conversion to forests. This study examines the responses of managed grasslands and forests established since grassland's abandonment to climate variability, in the eastern Italian Alps. To realize this study time series climate and satellite observation data were employed. Time series precipitation, minimum temperature (Tmin), and maximum temperature (Tmax) covering 43 years (1981 to 2023) were used to assess drought events. These climate variables were extracted from the European Center for Medium-Range Weather Forecasts (ECMWF-ERA.v5) grid climate data. The Standardized Precipitation Evapotranspiration Index (SPEI) was employed for drought assessment, which was calculated at 1-, 3-,4-,6-, and 12-month time scales to get into vegetation responses at various time lags. Similarly, the Mann-Kendall (MK) trend test and Sen's slope estimator were applied to examine trends in monthly precipitation and Tmin and Tmax. The Moderate Resolution Imaging Spectroradiometer (MODIS) 16-day NDVI time series (2001-2023) was used to characterize how the forest and grassland ecosystems respond to climate variability. The drought assessment and associated vegetation responses focused on May to August (MJJA), which encompasses the principal phases of vegetation phenological development. The result revealed a significant increasing trend ($\alpha<0.05$) in Tmin and Tmax for JJA over 43 years, with Sen's slope coefficients of 0.0438 to 0.0497 °c/year. The area has also experienced several drought events during MJJA from 2001 to 2023 although dominantly of moderate intensities and short-durations. The number of times the vegetation was characterized as stressed was much lower, and only a few drought events could be attributed as direct causes of vegetation stress in the forest and grassland sites. We may conclude that alpine Picea forest and managed grasslands are largely resistant to moderate drought events. However, combined with the increasing trends of Tmin and Tmax, such drought events could have a growing future impact on vegetation productivity.

Acknowledgments*: The project is funded by Italian Ministry of University and Research (European Union, Next Generation EU), project PRIN 2022 PNRR n. P20228JYWN*

A metabolic approach to investigate the effect of increasing salinity and temperature on groundwater fauna

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***Keywords**: Stygofauna; Metabolism; Salinity; Temperature*

The PRIN 2022 STIGE – CLIMAQUIFERI project, conducted in collaboration between CNR – IRET and the University of Milan Statale, investigates behavioral and energetic processes in groundwater ecosystems and springs. Groundwater, along with the deep ocean, constitutes the largest unexplored ecological domain. The slow pace of life in underground environments makes hypogean organisms excellent models for studying the metabolic-behavioral syndrome and for biomonitoring groundwater quality. Springs represent a crucial connection point between surface aquatic and subterranean domains, serving as an interface between different life paradigms. Despite their enormous scientific interest and the fascination that underground environments have always exerted on humans, only a small portion of society is aware of the existence and importance of subterranean animals.

Recently, it has been highlighted that climate change may severely impact even the relatively isolated subterranean aquatic ecosystems and its stygofauna, especially in the Mediterranean area. Predictions of drought in the Mediterranean region suggest the potential for saltwater intrusion from the sea, leading to increased salinity of continental waters. Many aquatic species in the caves of the Mediterranean area already inhabit brackish waters and have thus developed physiological adaptations to cope with temporary variations in salinity and temperature. The Pace of Life Syndrome (POLS) posits that closely related species or populations experiencing different ecological conditions should exhibit differences in a suite of energetic (i.e. metabolic), physiological, and behavioural traits. Investigating the implications of POLS on stygofauna facing varying levels of water temperature and salinity regime (e.g., variation in behaviour, resource and space use) can enhance our understanding of subterranean ecosystem responses to ongoing climate change. Within the Apulian endemic stygofauna, the mysidacea *Spelaeomysis bottazzi* can serve as a valuable model for studying how animals regulate their metabolic rate and behaviour under different salinity levels and temperature conditions due to its widespread distribution within the Apulian ecoregion. Here, we present a research plan to measure *S. bottazzi* physiological responses using a closed respirometry system. Additionally, we will examine whether individuals from different populations, already accustomed to higher salinities, exhibit osmotic stress that affects their metabolic rate, behaviour, and survival. Specifically, we observed that increasing temperature shifts physiology and behaviour via their effect on organism physiology, energy and behaviour. Our goal is to develop a predictive model that illustrates how salinity and temperature individually impact the metabolism and survival of animals differently than when these factors interact, which is actually what is happening in nature.

***Acknowledgments**: We sincerely thank the Gruppo Speleologico Leccese "Ndronico" and Gruppo Speleologico Tricase (GST) for their valuable support. Special thanks to the Company of Biologists for providing the travel grant (Grant No. JEBTF24081567). This research was funded by the PRIN 2022 STIGE-CLIMAQUIFERI project (DTA.PN010.014 2022MM8P88_LS8_PRIN2022 - CUP: B53D23012170006).*

Tree physiology response under different light environments in a Mediterranean *Quercus frainetto* forest exposed to dieback phenomena

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Keywords: *Broadleaf forest decline; Drought resilience; Irradiance variation; Leaf physiological response; Light adaptation*

Drought alters forest architecture, increasing canopy gaps and changing tree exposure to sunlight. While several studies address plant light adaptation and drought-related dieback responses, there is limited understanding of how trees respond to varying light exposure under decline conditions. A pure *Quercus frainetto* stand affected by dieback phenomena was selected in southern Italy as an experimental site. We assessed the physiological responses of declining (D) and non-declining (ND) trees to different light environments using the following methods: leaf structural traits, nitrogen concentration, photosynthetic pigments determination, carbon isotope composition, gas exchange, chlorophyll fluorescence and optical measurements. Results indicate that D trees exhibit characteristics similar to those of high-light adapted plants, as demonstrated by morphophysiological and ecophysiological parameters. Compared to ND trees, D trees present lower leaf nitrogen concentration per unit dry mass and higher leaf mass area, chlorophyll a/b ratio, carotenoids to total chlorophyll ratio and maximum electron transport rate. However, for other parameters, the D trees do not fully behave as high-light-adapted plants. In fact, no significant differences were found between the two groups for maximum photosynthetic rate, stomatal conductance, intercellular CO2 concentration, transpiration rate, and carbon isotope composition of leaf soluble sugars. Optical measurements identified the visible (525-600 nm) and red edge (700-725 nm) regions as the most informative zones of the leaf spectral signature for distinguishing D and ND trees. Similar photosynthetic rates between D and ND trees contrast with the significantly higher electron transport rate values for D plants, suggesting that part of the electron transport is directed to alternative electron sinks rather than photosynthesis. This mechanism likely prevents photodamage caused by the over-reduction of PSII reaction centres, which leads to the formation of reactive oxygen species. This can be ascribed to the fact that D plants lack the necessary resources to enhance their photosynthetic system, making them less efficient than expected if they were purely high-light-adapted plants.

Acknowledgments*: This research was carried out in the framework of the project "Advanced EO Technologies for studying Climate Change impacts on the environment - OT4CLIMA" which was funded by the Italian Ministry of Education, University and Research (D.D. 2261 del 6.9.2018, PON R&I 2014-2020 e FSC; project cod. ARS01_00405).*

Greening of Svalbard in the twentieth century driven by sea ice loss and glaciers retreat

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Keywords: *Arctic greening; Climate change; Tundra vegetation; Biomarkers*

The greening of previously barren landscapes in the Arctic is one of the most relevant responses of terrestrial ecosystem to climate change. Analyses of satellite data (available since ~1980) have revealed a widespread tundra advance consistent with recent global warming, but the length of the time-series is insufficient to resolve the long-term variability and the precise timing of the greening onset. Here, we measured plant-derived biomarkers from an Arctic fjord sediment core as proxies for reconstructing past changes in tundra vegetation during the transition from the Little Ice Age to modern warming. Our findings revealed a rapid expansion of the tundra since the beginning of the twentieth century, largely coinciding with the decline of summer sea ice extent and glacier retreat. The greening trend inferred from biomarker analysis peaked significantly in the late 1990s, along with a shift in the tundra community towards a more mature successional stage. Most of these signals were consistent with the biomolecular fingerprints of vascular plant species that are more adapted to warmer conditions and have widely expanded in proglacial areas during recent decades. Our results suggest that the greening of Arctic fjords may have occurred earlier than previously thought, improving our mechanistic understanding of vegetation-climate-cryosphere interactions that will shape tundra vegetation under future warming projections.

Acknowledgments*: This work has been principally funded and conducted in the framework of theJoint Research Center ENI–CNR "Aldo Pontremoli" of Lecce, within the ENI-CNR Joint Research Agreement. We also thank "Premio Dirigibile Italia" and"Fondazione Carisbo" (2017/0334) for their financial contribution. The ItalianArtic Station "Dirigibile Italia" is kindly acknowledged for the logistic support. Finally, we thank the Italian-German partnership on "Chronologies for Polar Paleoclimate Archives (PAIGE)" and the funding from the Helmholtz European Partnering.*

Biological valorisation of a vermicompost as biostimulant for horticulture

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Keywords: *Agricolture; Microbial biostimulant*

The new Regulation (EU) 2019/1009 established rules related to the placement of fertilizers on the EU market by amending previous regulations. The new regulation introduces a new category of fertilizers, microbial biostimulants for plants, defined as products containing one or more bacterial strains belonging to the genera *Azotobacter spp*., *Rhizobium spp*., *Azospirillum spp*, or mycorrhizal fungi, which enhance one or more of the following characteristics: nutrient use efficiency, abiotic stress tolerance, qualitative characteristics; availability of nutrients confined in the soil or rhizosphere.

In this context, CNR IRET in Pisa carried out an experiment, funded by Bioagrotech srl, aimed at developing a microbial biostimulant product starting from a substrate made of vermicompost from animal manure (vermicompost-based substrate). Over the last two years, various plant species capable of enriching the vermicompost-based substrate with N-fixing bacteria were tested, specifically rice (*Oryza sativa L.*), alfalfa (*Medicago sativa L.*), calendula (*Calendula officinalis*), lentil (*Lens culinaris Medik.*), and the rice + lentil mix, to determine which species could contribute more to the enrichment in nitrogen fixers bacteria in the vermicompost-based substrate. The alfalfa resulted the best plant in increasing N-fixing bacteria growth. Afterward, the protocols necessary for the integration and maintenance of bacterial populations in the substrate with alfalfa were defined and the derived product (biostimulant) were tested on the productive yield and quality of *Lactuga sativa capitata* (iceberg lettuce). This species was included in the eligible group of vegetable crops according to the technical document FprCEN/TS/17700-1 of the Regulation (EU) 2019/1009. The experimentation was carried out under controlled greenhouse conditions and various vegetational, and chemical and biochemical substrate parameters, using both established and innovative analytical methods such as the TraitFinder F600 phenotyper were monitored.

The results demonstrated that the biostimulant product significantly improves the growth and biomass of the lettuce, highlighting an increase in health status and greater photosynthetic activity. Moreover, chemical analysis of substrate revealed a more effective nutrient management in the tests with the biostimulant, with particular attention to nitrogen and phosphorus. Significant increases were also observed in enzymatic activities linked to the nutrient cycle (C, N, P), showing that the biostimulant product is able to make larger quantities of available nutrients to the plants. In summary, the application of the biostimulant product not only promoted plant growth but also contributed to more sustainable agricultural practices.

Figure 1. image of the experimental phase on iceberg lettuce and TraitFinder phenotyper.

Poster e Flash talks nell'ambito delle tematiche dei GdL "Ambiente e Salute"; "Biodiversità"; "Ciclo del Carbonio" "Infrastrutture di Ricerca" e "Siccità, Scarsità e Crisi Idriche" e "Altro"

Effects of the Byproduct of tomato processing on Aging in *Drosophila melanogaster*

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***Keywords**: Drosophila; Healthy aging; Tomato waste; Insulin signaling; Autophagy dynamics*

Industrial processing of tomatoes generates a significant amount of waste, including peels, seeds, and pomace. These by-products are often discarded, leading to environmental concerns and resource wastage. However, these discards have potential health benefits, focusing on its bioactive compounds such as polyphenols, carotenoid, proteins, and dietary fibers.

For this reason, tomato seeds and peels waste are sources of bioactive compounds, including flavonoids, phenolic acids, and carotenoids, which possess antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. These compounds have garnered interest for their potential health-promoting effects, such as cardiovascular protection, anti-aging, and immune system enhancement

Our study presents a comprehensive examination of the effects of Tondo di Caserta Tomato Seed Extract (TSE) and Tomato Peel Extract (TPE) on various cellular pathways *in Drosophila melanogaster*, and on their collective impact on aging-related processes. By investigating proteostasis, insulin signaling, carnitine metabolism, and autophagy dynamics, we uncover the intricate interplay between dietary components and cellular homeostasis, with implications for healthy aging and disease management.

Our findings demonstrate that TSE and TPE supplementation significantly extend lifespan and enhance resistance to oxidative stress in Drosophila models. These effects are consistent with previous studies highlighting the longevity-enhancing properties of dietary interventions and the antioxidant properties of tomato-derived bioactive compounds. The observed improvements in neuromuscular activity further underscore the potential neuroprotective effects of TSE and TPE, suggesting a multifaceted mechanism underlying their beneficial effects on aging-related phenotypes.

Our investigation into proteasomal activity and autophagy dynamics reveals a coordinated cellular response to TSE and TPE consumption. Our findings have significant implications for developing targeted dietary interventions for promoting healthy aging and combating age-related diseases, particularly neurodegenerative disorders

In conclusion, our study underscores the importance of a complete approaches in understanding the complex interactions between dietary components, cellular pathways, and aging-related processes. By integrating insights from proteostasis, insulin signaling, carnitine metabolism, and autophagy dynamics, we provide a framework for harnessing the potential of dietary interventions in promoting healthy aging and enhancing resilience against age-related pathologies.

Peat forming mosses (*Sphagnopsida*) for peatland's preservation, phytoremediation and pollution biomonitoring

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***Keywords**: Biomonitoring; Plant stresses; Mosses; Carbon cycle; Air pollution*

Thanks to their peculiar morphology and physiology, *Sphagnum* mosses significantly contribute to the carbon cycle, as they can store up to 30% of the global soil carbon in form of partially decomposed organic matter, i.e. peat, making peatlands efficient C sinks in a greater extent than any other terrestrial ecosystem.

However, peat forming mosses are highly sensitive to climatic changes and human activities. Under stresses, peat decomposition is accelerated with a consequent releasing of both CO_2 and methane (CH_4), a potent greenhouse gas, into the atmosphere. Therefore, the preservation of sphagnum mosses and the study of their functionality are fundamental issues to be addressed.

Besides their ecological relevance, peat mosses possess are efficient to adsorb a wide variety of airborne or aquatic pollutants (e.g. metals, metalloids, PAHs, radionuclides, PMs), hence working as phytoremediation and biomonitoring organisms. In particular, the exposure of mosses inside nylon bags (i.e. moss bags) is an economic, easy-to-manage and eco-friendly method for the evaluation of the air and water quality.

Acknowledgments: National Recovery and Resilience Plan(NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union– NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC"

GReen ENgineering solutions: a new LIFE for SEdiments And Shells

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***Keywords**: Shells; dredged sediments, Landfarming, Innovative products*

GreenLife4Seas is an EU-funded project which addresses pressing environmental issues by developing sustainable solutions for two highly impacting marine wastes in the EU: *dredged sediments* and *shells*. Europe-wide, the volume of dredged sediments is estimated at 200 million cubic metres per year. The fate of such a massive amount of material dredged every year, with its hazardous characteristics, is a well-recognised primary concern for the whole society in the EU. The second problem that the project faces is the management of shells coming from the aquaculture and fishery industries. The global production of marine bivalves for human consumption is more than 15 million tonnes annually, about 14% of the total marine production. In Europe, the production of marine bivalves decreased from about 600 to 465 thousand tonnes in the last five years, mainly due to enormous difficulties and costs connected to the disposal of shells; many of the shells are still disposed of illegally and dumped into the sea.

The project responds to these needs through a triple-helix model of Consortium, where synergy among research capitalisation, industrial symbiosis and societal participation is the key to innovation. The green and engineering-based solutions developed by the project partners will transform bivalve shells and dredged sediments into resources, giving them a new life. The *shell powder,* as eco-design by-product from marine shells, will be used in a mixture with *decontaminated* or uncontaminated *dredged sediments* to create three *innovative building sector products*, i.e., paving blocks, breakwaters and mass stabilization. Realization and put in the operation of the new building products will be carried out via a prototype of a super mobile-plant directly in the four ports where sediments will be dredged: Bari, Barletta and La Spezia ports in Italy and Piraeus port in Greece (Fig. 1).

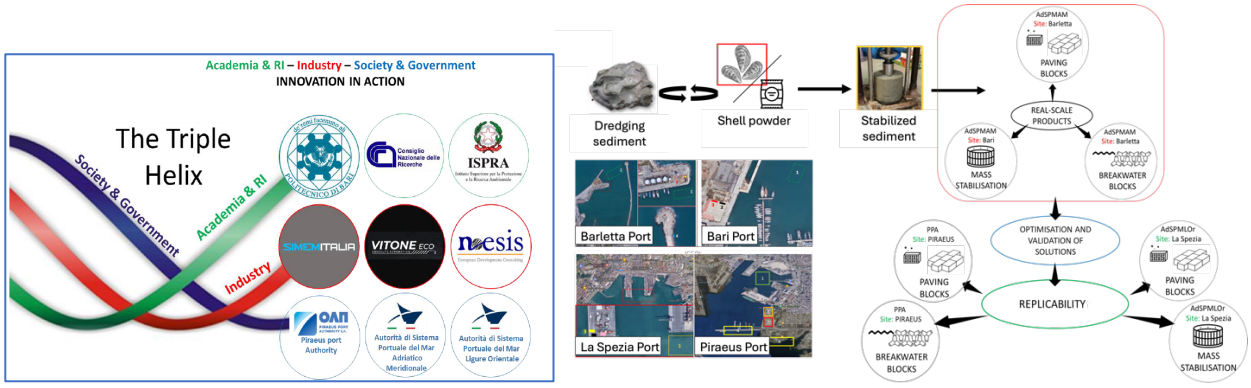

Figure 1. Project layout

Sediment decontamination technology, developed by the CNR-IRET unit of Pisa, will involve the assisted landfarming process. Contaminated sediments will be spread in piles and periodically turned over to facilitate the degradation of toxic organic compounds. The addition of specific commercial bio-activators for petroleum hydrocarbon degradation will allow to reach the target established in the Italian legislative framework to obtain the target of End of Waste status. The maintenance of the optimal conditions for biological activity (e.g., water content, redox potential, C/N/P ratio, salinity, pH) and the evolution of biological activity will also be monitored during the assisted landfarming process. Today, the shift to a circular economy for dredging sediments is necessary and supported by an increasing number of initiatives and transdisciplinary skills; however, political and societal barriers still exist that could hinder its practical implementation. The definition of a specific authorization protocol for the decontamination and reuse of dredged sediments as pilot by-products will allow to overcome the regulatory barriers.

***Acknowledgments**: Co-funded by the European Union. Project 101114177 — LIFE22-ENV-IT-LIFE GREENLIFE4SEAS. Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or CINEA. Neither the European Union nor the granting authority can be held responsible for them.*

Leveraging Urban Tree Cover to Combat Heatwaves: Modeling Intensity, Duration, and Mortality in Italian Cities

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***Keywords**: Urban Forests, Nature-Based Solutions, Urban Heatwave, i-Tree Cool Air*

Cities are increasingly adopting nature-based solutions (NbS) to reduce the impacts of heatwaves, and there is a need for tools to support the strategic design and management of these solutions. This study utilized the i-Tree Cool Air soil-vegetation-atmosphere transfer model to assess how expanding urban tree cover enhances evaporative cooling, thus reducing exposure to heatwaves and associated mortality. The research simulated heatwave events and heatwave degree days (a measure of intensity and duration) for 10 Italian cities during the entire summer of 2003, with hourly time steps. Two scenarios were modeled: a base case and an alternative scenario in which tree cover was increased to reach a minimum of 30% tree cover target to overlap with European goals. In the base case, heatwave events and excess mortality among individuals aged 65 and older were estimated for each city, with data distributed across neighborhoods based on heatwave degree days and the size of the elderly population. The alternative 30% tree cover scenario resulted in an average reduction of 40% in both heatwave degree days and excess mortality, with cooling benefits derived from evapotranspiration of water that was previously runoff in the base case. This modeling approach can assist in prioritizing where to implement NbS for maximum impact.

This study received funding support from the National Biodiversity Future Centre and was supported by a sabbatical leave for TE from SUNY ESF to CNR IRET.

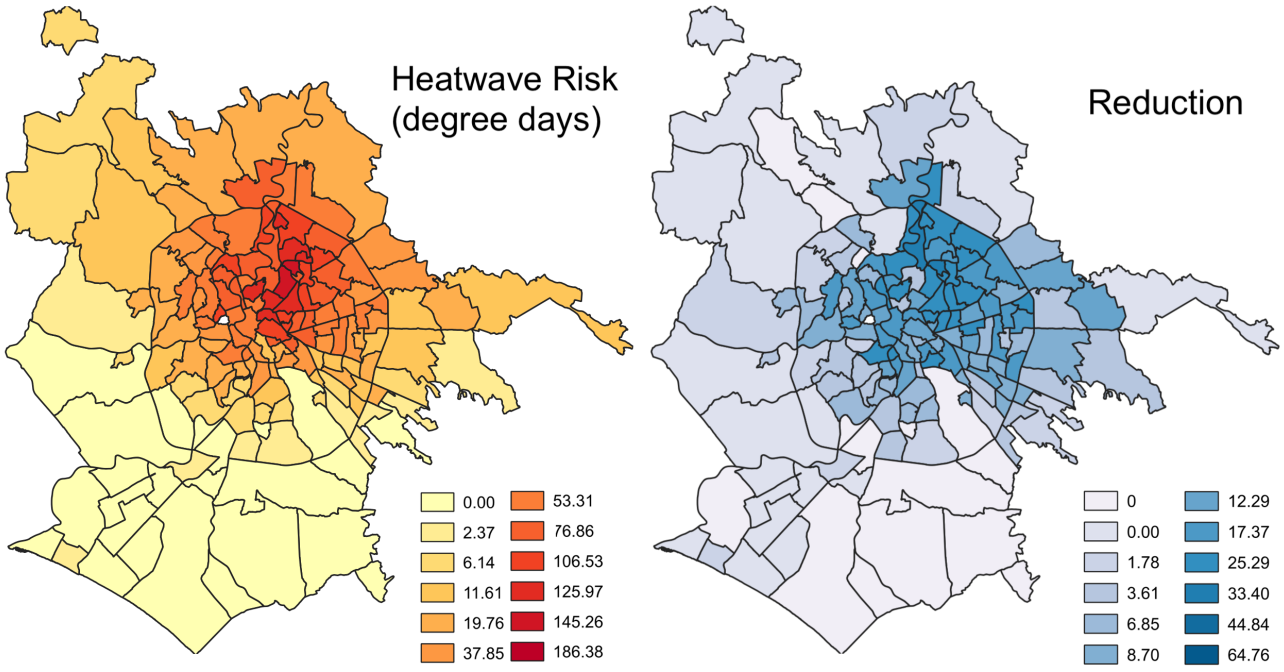

Figure 1. Heatwave risk and risk reduction in Rome.

Qualitative X-ray fluorescence spectroscopy characterization of ground electronic waste

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***Keywords**: XRF, electronic waste, fungi, circular economy, strategic elements*

This activity is part of the PRIN 2022-PNRR project, titled "*Fungal interaction with metals (FUN METALS): transformation and mechanisms for biorecovery*", aimed at investigating the mechanisms and strategies through which fungi interact with Indium, Gallium, Germanium and Yttrium, elements of industrial and technological interest.

The rising amounts of Waste from Electrical and Electronic Equipment (WEEE) pose significant environmental and health risks due to the hazardous materials contained within discarded electronics. Their effective management and recycling are essential for protecting human health and the environment, as well as recovering valuable resources and supporting the circular economy. Furthermore, the growing global demand for critical strategic elements is driving increased research into methods for their recover from electronic waste (e-waste). In this context, fungi could represent strategic tools for innovative, sustainable and nature-inspired solution to targeted elements recovery. In fact, as fungi can tolerate high concentrations of toxic compounds, they can also bioaccumulate and transform them through direct or indirect mechanisms, which can also be selective for some elements.

The potential of fungi to assimilate or mobilize metals and metalloids can be investigated using robust analytical methods, including X-ray fluorescence (XRF) spectroscopy. Knowing the chemical composition of WEEE is crucial for studying the mechanisms of interaction with fungi and, consequently, for selecting efficient fungal strains for biorecovery applications. In this study, multi-metal substrates obtained from ground electronic devices and provided by a private company, specialized in WEEE recycling, were subjected to granulometric separation through sieving ($x > 2.80$ mm; 2.80 mm $> x >$ 710 µm; 710 µm $> x >$ 125 µm; $x <$ 125 µm). Each fraction was then characterized using XRF spectroscopy, using Leit adhesive carbon tabs of 25 mm diameter, to assess composition and element enrichment across the fractions.

XRF technique operates by exciting a sample with high-energy X-rays, which results in the emission of characteristic secondary X-rays from the material. The intensity and energy of fluorescent X-rays were measured to produce an XRF spectrum, displaying peaks corresponding to different elements and their concentrations within the sample. The X-ray source operated at 60 kV and 0.4 mA with a beam diameter on the sample of just over 10 mm. An energy-dispersive X-ray fluorescence (EDXRF) detector was positioned to directly capture the energies of the X-rays emitted by the sample. The acquisition time was set to 300 s to improve the signal-to-noise ratio.

The results indicated an abundance of Cu, Sr, Zr, Sn, Ba, Br, Zn, Fe and Ni. In addition, the analysis of the finest-grained sample revealed trace amounts of Ga, Y, and In, elements of particular interest for this project. The XRF technique combined with the EDXRF detector has proven to be highly effective, enabling a rapid, non-destructive and simultaneous identification of the elements within the sample.

Acknowledgments: PRIN 2022 PNRR Project funded by European Union-NextGenerationEU-Project Prot. P2022ENEWL-Title "Fungal interaction with metals (FUN METALS): transformation and mechanisms for biorecovery" CUP B53D23032130001.

Advancing Agroforestry Innovation in Europe: the role of IRET in the AF4EU project

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***Keywords**: Regional agroforestry networks; Multiactor approach; Sustainable food systems; Biodiversity; Innovation in agroecology*

Agroforestry Business Model Innovation Network (AF4EU) is an EU-funded project that promotes agroforestry in Europe through the development of a multi-actor approach linked to interactive and innovation-driven expanded agroforestry networks: the Regional Agroforestry Innovation Networks (RAINs). The project builds on the results of the previous AFINET project and aims to expand the knowledge already gained in previous European RAINs, as well as to complement the innovations by developing business models and enhancing extension services.

The AF4EU consortium consists of 12 partners from 10 different European countries and five national associations.

In each country, the Regional Agroforestry Innovative Network (RAIN), through the key role of the Innovation Broker, should promote Agroforestry as a multifaceted approach that combines trees, crops, and livestock in integrated farming systems, while improving farms sustainability, promoting biodiversity and enhancing soil quality. E.g., the Italian RAIN bases on the stakeholder community (farmers, extenders, multipliers, researchers and policy makers) acting within the Bolsena Lake Biodistrict.

Building on the experience of three Lighthouse RAINs, namely the Finnish, the Italian and the Spanish ones, created during the AFINET project, AF4EU reflects the agroforestry conditions and perspectives in countries of the Continental – Boreal, Mediterranean and Atlantic regions, IRET is acting to establish a methodological framework following a twining scheme for construction of the new RAINs. (Fig. 1). Within this framework. IRET elaborates the guidelines to identify (1) the RAIN management procedures; (2) the RAINs governance structure, (3) the role and activities of the IB, (4) the involvement of actors/stakeholders in each RAIN, (5) the composition, objective and timing for each RAIN workshop and (6) the thematic issues to be addressed by validating and extending the most promising land management innovations collected in AFINET towards the development of business models.

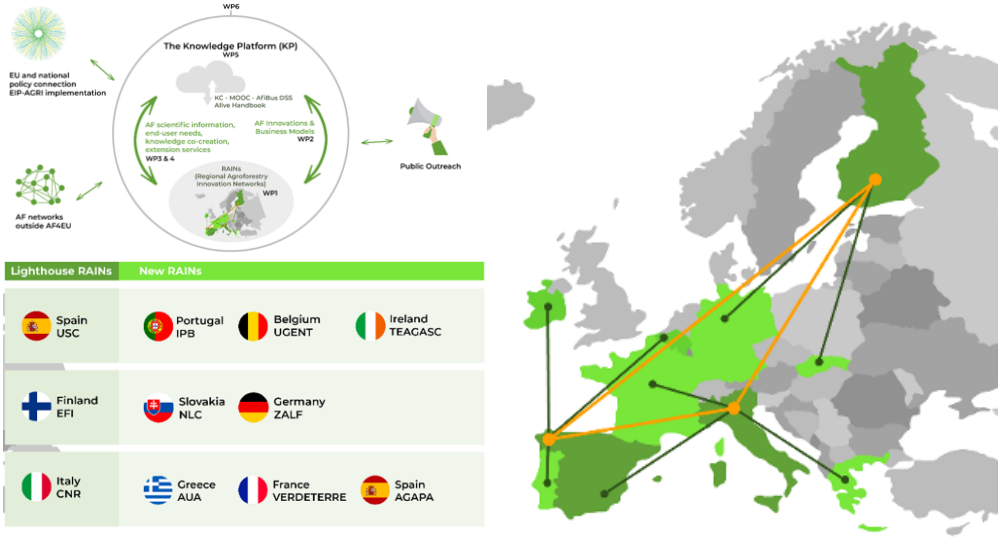

Figure 1. AF4EU Overall concept; RAINs distribution and Network Twining scheme

Food Waste Valorization from Olive Oil Production Chains: New Applications in Regenerative Medicine

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***Keywords**: Circular Economy; Hydroxytyrosol-Chitosan Nanoparticles; Thermo-responsive Hydrogel; Tissue Regeneration*

Olive oil by-products have long been considered as a challenging waste material impacting environmental protection, but this view has recently been reformed, leading to their recognition as a source of high-added value compounds that can be re-used as natural additives in the food, cosmetic and as adjuvants in medicine. Nowadays, these waste products represent a crucial strategy to promote the circular economy and environmental sustainability. In olive oil production chains, olive leaves, pomace and olive oil sediment are often discarded as worthless waste, conversely, they are materials rich in bioactive molecules with high therapeutic potential. These waste materials are rich in polyphenols, including hydroxytyrosol, oleuropein, tyrosol, ligstroside and flavonoids, known for their remarkable antioxidant, anti-inflammatory, and regenerative properties. Hydroxytyrosol is known for its extraordinary ability to neutralize free radicals, reduce inflammation and protect cells from oxidative damage, making it a promising candidate for regenerative medicine, especially in the treatment of osteoarthritis (OA), a degenerative disease characterized by progressive loss of cartilage, chronic inflammation and pain. Current therapies for OA are limited to palliative care, and surgery in the most severe cases is required. Therefore, one of the main challenges in developing new therapies is to create approaches that not only alleviate symptoms but also promote cartilage tissue regeneration. However, the therapeutic application of hydroxytyrosol is limited by its poor stability and bioavailability.

The present study proposes an innovative solution in this field: the development of a localized hydroxytyrosol release system based on chitosan nanoparticles loaded with hydroxytyrosol (Hyt-NPs) and integrated in a thermo-responsive hydrogel. The completed formulation is composed by Pluronic F-127, hyaluronic acid and Hyt-NPs, which was called Hyt@tgel. Hyt@tgel can be injected into a target region as a freely flowing solution at room temperature, transforming into a gel at body temperature. This advanced drug delivery system allows a gradual release of the bioactive ingredient directly into the damaged area, maximizing its therapeutic effectiveness.

In vitro results, tested on human chondrocyte cells, have demonstrated that the controlled release of hydroxytyrosol improved cell proliferation and vitality, exerting anti-inflammatory and antioxidant effects, and reducing the number of senescent cells treated with H_2O_2. In addition, Hyt@tgel stimulated the production of type II collagen and proteoglycans, essential components of the extracellular matrix. Furthermore, Hyt@tgel limited the expression of critical OA- related genes in human chondrocytes treated with stressors promoting OA-like characteristics, suggesting a potential regenerative effects. These data suggest that Hyt@tgel could represent a sustainable solution for Hyt delivery leading to cartilage regeneration for OA treatment.

In conclusion, food waste processing into valuable resources for the development of advanced medical technologies offers a path towards a more sustainable development model. This approach integrates agriculture, industry and healthcare, improving human well-being through effective regenerative treatments while addressing environmental challenges leading to an eco-friendlier future.

Certified sustainable forest and life cycle management to support the implementation of an ecosystem service-based crediting mechanism

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***Keywords**: Certification; Credit; Ecosystem service; Environmental impact; Life cycle management*

Sustainable forest management is critical at global, national, and regional levels in order to maintain its multifunctional role for the well-being of people and the conservation of biodiversity. Further to regional or (inter)national regulations on forest management, certification schemes exist to ensure that sustainable practices are implemented in forest restoration and conservation interventions. The most popular examples are the Programme for Endorsement of Forest Certification (PEFC) and the Forest Stewardship Council (FSC) schemes. Both standards focus on the assessment and monitoring of carbon uptake and stock as a measure of post-intervention enhancement of ecosystem services (ES), as well as on increasing the supply of other ES such as the protection of biodiversity or the implementation of recreational functions. However, ES quantification is performed in different ways within the PEFC and FSC procedures. Furthermore, while PEFC explicitly allows for the generation of carbon credits, FSC does not, and such different perspectives spread uncertainty and reduce transparency in the process of carbon trading when carbon sequestration and related credits originate from certified forests. Concerning the carbon crediting system in general −thus not only the sole PEFC-based carbon credits −, a lack occurs of a rigorous, durable and transparent solution to ensure the quantification, traceability and durability of credit itself, which does not prevent the risk of generating greenwashing mechanisms. On top of this, out of the carbon sequestration, other ES are scarcely considered for the calculation of impact compensation credits. All these issues are the focal point to support the implementation of a broad-spectrum environmental crediting system based on a reproducible, verifiable and traceable protocol of actions that would ensure long-term sustainable management of forest natural capital.

The aim of this contribution is to illustrate the development of a testable mechanism of forest credits development starting from a step-by-step application of contextual rules, which can be implemented and replicated at different spatial scales. The proposed mechanism has the ambition to become a reference system for natural capital credits generated from forests of the Catholic Church. These represent in Italy an impressively extensive natural capital asset (of around one million hectares). While historically those forested areas represented vital resources for local economies, nowadays they are in many cases unmanaged of even abandoned land. The set of principles underpinning the proposed credits' certification protocol was applied to some forest properties belonging to the Diocesan Institute for the Support of the Clergy (IDSC) of Asti, in the Region of Piedmont, Italy. The entire case study covers ~90 ha of very fragmented forests, which face severe management problems. The area is indeed constituted by more than 100 small and diversified forest sites with an average extent of less than 1 ha. A sustainable forest management project was recently implemented in the area according to the PEFC scheme, identifying best management practices to quantify, increase, conserve and monitor specific key forest ES. However, so far, only ~11% of the certified forest area has been the object of specific forest interventions. The proposed ES-based crediting mechanism was initially applied on this area through sustainable forest cuts aiming at wooden resource harvesting, ecotourism solutions, and plant biodiversity enrichment (ex-post analysis). It was then applied to the rest of the project area that will undergo management interventions in the next future (ex-ante analysis). The number of generated credits was obtained from the ratio between the *net costs* of sustainability measures implementation and management, and 50 € (value per credit established by the IDSC after a market analysis for concurrent credits pricing). Net costs were retrieved from the balance between una tantum (e.g., certification costs, costs for forest interventions, administrative costs, etc.) and yearly management (e.g., for auditing, for communication, for maintenance, etc.) *costs*, and *benefits* represented by profits calculated as 10% rate of the implementation costs (average earning margin observed in the regional forest management inventory) and wood resources sales.

Results show that, in the context of the ex-post analysis, ~16,400 € / ha is the average total value of the environmental credits generated over five years of forest management and use, which reflects the supply of ~330 credits / ha. For the totality of ~10 ha of forest sites, an amount of ~3,300 credits are ideally generated, which can be sold in the market. It can be noticed that those credits do not only incorporate carbon sequestration services (and thus the value of carbon credits), but also the value of many other ES. By extending the analysis with a prospective ex-ante approach to the rest of the project sites, net costs and number of generated credits substantially decrease, by ~36%. Among the principles established in the mechanism, a key one states that those credits shall be sold to citizens or private/public companies aiming to support or sponsoring nature restoration projects following for example corporate social responsibility goals. But they can also be sold to companies aiming to compensate for their residual and unavoidable environmental impact in order to help them reaching a condition of impact neutrality. In those cases, however, in order to prevent the upsurge of greenwashing effects, buyer companies shall demonstrate to have planned or already implemented specific life cycle management measures to reduce or avoid environmental impacts. Last but not least, it is expected to work on the calculation of a physical (not only monetary) quantification of the components of the environmental credit, so to incorporate more consistently, legitimately and unambiguously the intrinsic and sometimes instrumental value of ES into the cost-benefit balance that determines the price of the credit.

Functional Plant-Based Beverage Fortified with Hazelnut Cuticle Polyphenols: Antioxidant and Phenolic Content Characterization

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***Keywords**: Natural deep eutectic solvents (NADES); Hazelnut Cuticle; Bioaccessibility; Polyphenols*

The heightened awareness of contemporary consumers regarding the potential health benefits of their diet has resulted in a greater demand for foods containing biologically active substances. The new challenge in the food sector is to enhance items for human consumption with molecules possessing functional qualities, such as antioxidants. Polyphenols are garnering significant attention for their possible beneficial impacts on human health, including anticancer, antioxidant, and anti-inflammatory properties. Research in food science aims to identify novel and sustainable sources of antioxidants, enhance extraction and purification techniques, and create innovative functional foods that support health. The initial phase in the development of functional food enriched with physiologically active substances is the extraction of the desired molecules. This is frequently associated with shortcomings such as excessive organic solvent usage, elevated energy consumption, and substantial waste generation. Natural deep eutectic solvents (NADES) represent a valid alternative to canonical extraction solvents as they are ecological, sustainable, and with a high safety profile.

In this study, antioxidant properties of hazelnut cuticles extract rich in polyphenols were evaluated and subsequently used for fortification of plant-based milk. The chromatographic analysis of phenolics in NADES extracts of hazelnut cuticle performed by Liquid chromatography-mass spectrometry (LC-MS) demonstrate the presence of gallic acid, caffeic acid, p-coumaric acid, ferulic acid, quercetin-3-O-rutinoside, catechin, epigallocatechin, kaempferol-3-O-glucoside, quercetin, and apigenin. The antioxidant properties of the extract obtained under optimal conditions, total antioxidant capacity (TAC), free radical scavenging capacity (FRC), total phenolic content (TPC), and ABTS radical scavenging capacity (ARC) were measured and compared with the results of the extracts obtained with the most used classical solvent mixtures (80% ethanol in water, ethanol or water).

The results showed that NADES extract had potential antioxidant activity which was higher than that of traditional solvents. Ready-to-use NADEs extract was added to hazelnut-derived vegetable milk to obtain a fortified beverage. The resulting milk demonstrated a smooth, creamy texture without undesirable separation, reflecting successful emulsification. Organoleptic evaluations confirmed the NADES-based milk was well-received, with no significant differences in taste or texture compared to a commercial-like hazelnut milk formulation. Fortification of beverage with NADES extract enhanced antioxidant efficiency, increasing total antioxidant capacity and free radical scavenging capacity between during both the initial and the in vitro gastrointestinal digestion stages compared to plant-based beverage without fortification used as control making a positive contribution in terms of the antioxidant activity of the final product. Furthermore, the fortified milk demonstrated significantly improved bioaccessibility through an in vitro investigation imitating gastrointestinal digestion. The enhanced bioaccessibility positions this fortified milk as a promising functional food capable of delivering higher concentrations of bioactive compounds, offering substantial health benefits such as antioxidant protection and anti-inflammatory effects.

Elemental profiling of PM10 in Yerevan: *F. excelsior* and *P. orientalis* leaves as bioindicators

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***Keywords**: Urban environment, Atmospheric pollution; Nature-based solutions; Research infrastructure*

Atmospheric pollution is one of the leading causes of several diseases, and particulate matter (PM) causes millions of premature deaths every year. The risk increases in urban environments, and over the past twenty years, there has been a steady increase in the studies of ecosystem services provided by urban trees. A considerable volume of literature has been published on the adsorption of PM by tree leaves, used both as filters and to evaluate the particle capture capability of different species. The selected area in this study is the city of Yerevan, where there is no structured air pollution monitoring network. In the city, in addition to pollution from urban sources of PM (transportation, household heating), there is also a large industrial area. The main purpose of this study is to gain an understanding of PM10 pollution in Yerevan by analyzing urban tree leaves. Samples were gathered from the most widespread tree species in the area (*F. excelsior* and *P. orientalis*) and used to assess the different levels of PM10, PM2.5, and PM1 pollution through Scanning Electron Microscopy analysis (SEM/EDX). Four districts and three different micro-environments (park, street, industrial) were chosen to represent different conditions. There are two central objectives of this study: 1. To describe the differential distribution of PM10 in the four districts and the two species; 2. To determine an exhaustive characterization of PM10. Results were obtained in terms of PM mass (µg/cm²), PM density (#PM/mm²), and PM quality (elemental composition), and evidenced different levels of PM pollution among the districts and a major adsorption capability for *F. excelsior*. The PM analysis evidenced high levels of potentially toxic elements like Fe, Mo, Ba, and Pb, and trace amounts of Cu, Zn, and Zr. Furthermore, the elemental analysis highlighted the relationships between the PM elemental composition and: a) the sampling site; b) the dimensional distribution; and c) the sampled species. Finally, to address the lack of local environmental monitoring stations and enhance data accessibility, the research data will be shared on domain specific open-access platform of the environmental domain, in full alignment with FAIR principles.

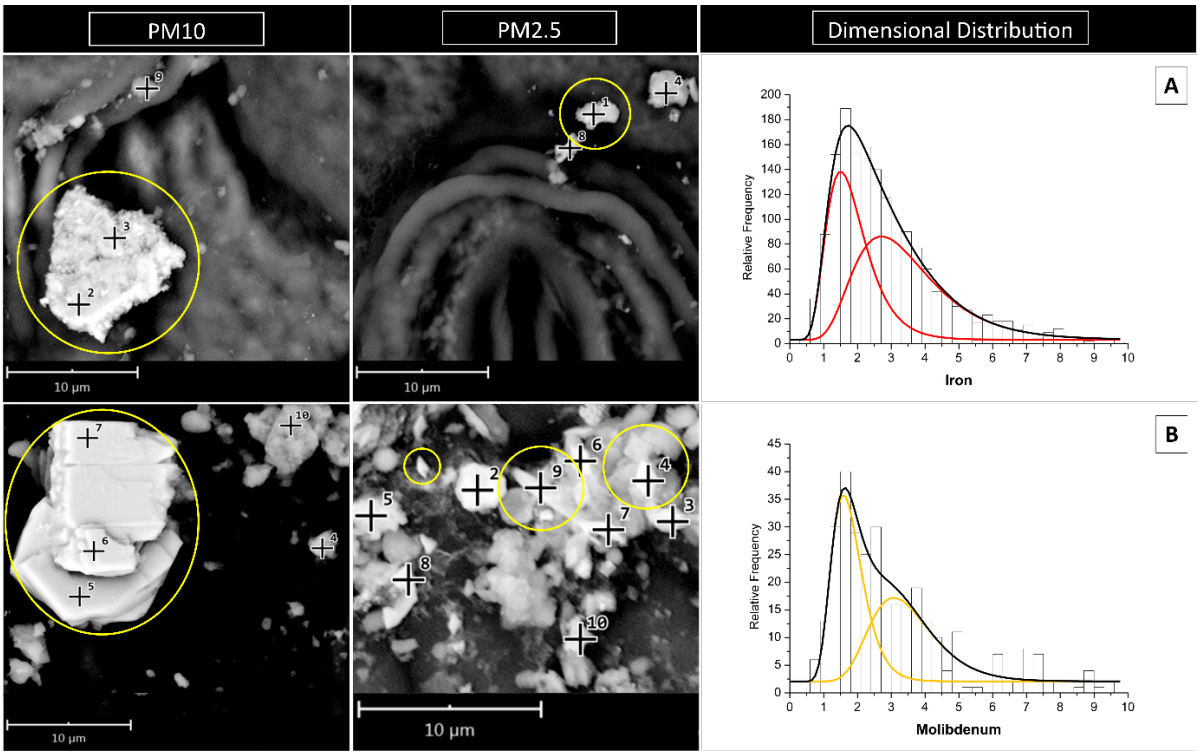

Figure 1. Detailed morphological characteristics of PM. Relationships between elemental composition (main W%) and the dimensional distribution of particles described by the lognormal curve (Xc values). A) Fe-based (Xc = 1.73; Xc_1 = 3.21); B) Mo-based (Xc = 1.71; Xc_1 = 3.35).

***Acknowledgments**: This work was supported by the EUFORICC–PRIN Project (Establishing Urban Forest-based solutions In Changing Cities), grant number PRIN 20173RRN2S, and the ProGIreg Project, Grant Agreement no. 776528. We also extend our gratitude to the Institute of Botany, Yerevan (Armenia), and the Center for Ecological-Noosphere Studies NAS RA (CENS - Armenia) for their valuable collaboration and support.*

Selenium nanoparticles: improving tomato quality and protecting ecosystems.

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***Keywords**: Selenium, Biofortification, Metabolomics, Nanoparticles, Nutraceuticals*

Selenium (Se) deficiency is one of the most difficult-to-diagnose types of malnutrition touching about one billion people globally. As an "essential poison" selenium has a narrow range of dietary uptake, making it challenging to design an effective though safe food supplement. Biofortification is the process of increasing the nutrient content of crops. Selenium biofortification traditionally relied on using selenium salts, sodium selenate and sodium selenite, as enriching agents.

However, the Se-enriched products currently available on the market often require heat processing, causing a degradation of the more bioavailable Se organic forms. Se4SAFE project is dedicated to designing a technology allowing to provide consumers with intact organic Se, using tomato as a model crop and Se carrier. Unlike onion and garlic, which are typically consumed cooked, tomato is mainly consumed fresh. Additionally, the application of bio- and chemically produced Se-Nanoparticles (SeNPs), formed from elementary Se, has shown to mitigate the risk of toxicity for plants and ecosystems.

Based on the available literature, Se was proved to positively impact plants physiology and increase stress resilience. Our previous project showed that selenium increased the accumulation of naringenin and chlorogenic acid and decreased the coumaric acid level in tomato fruit. Selenium also affected the volatile organic compound profile by increasing the production of terpenoids and alcohols. Observed delays in carotenoid biosynthesis, as well as RNA-seq results previously published by our team, reinforce the hypothesis that Se might postpone tomato ripening.

Tomato plants (*Solanum lycopersicum* cv. 'Ciliegino Ceruzzo' and cv. 'MicroTom') were hydroponically grown and sprayed with Selenium solutions at the Immature Green fruit stage. The effectiveness of 7 treatments was compared: sodium selenate 10 mg L^{-1}, sodium selenate 20 mg L^{-1}, bio-SeNPs 10 mg L^{-1}, bio-SeNPs 20 mg L^{-1}, chem-SeNPs, 10 mg L^{-1}, chem-SeNPs 20 mg L^{-1}, and control (tap water).

Sodium selenate sprayed at 20 mg L^{-1} induced the highest Se accumulation in fruit. However, despite lower uptake from SeNPs, their effect was sufficient to change the mineral composition of the fruit, including a significant increase in Se content and improved qualitative parameters. These results reinforce our previous findings, indicating that Se nanoparticles improve tomato fruit quality and biochemical composition. Eventually we suggest that selenium treatment may prolong tomato shelf-life, and improve its nutritional quality directly, by increasing the content of bioavailable Se organic forms, and indirectly, by increasing the content of antioxidants.

The upcoming steps of the project aim to investigate the effects of different selenium forms on tomatoes and carry out a more detailed analysis of fruit biochemistry under selenium treatments, including carotenoids, polyphenols, volatile organic compounds (VOCs), and amino acids.

***Acknowledgments**: This work was conducted within the frame of the project PRIN 2022 "Se4SAFE - Biogenic nano-selenium fortified tomatoes: quality and safety in a green perspective", CUP: B53D23017140006, funded by the Ministry of Education, Universities and Research (Italy) and the European Union-Next Generation Unit.*

Biohydrogen production by immobilized photosynthetic microorganisms

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***Keywords**: Hydrogen production; Photosynthetic microorganisms; Photobioreactor; Immobilization; Calcium alginate*

One of the most important solutions for overcoming energy and environmental problems and replacing the fossil fuel-based economy could be the use of photosynthesis. Photosynthetic microorganisms are currently being investigated as potential sources of biomaterials and biofuels such as hydrogen (H_2). These microorganisms have advantages over chemical synthesis as they grow on renewable energy sources and operate at ambient temperature and pressure. The improvement and stabilization of photosynthetic H_2 production could be achieved by immobilization. Cell immobilization has several advantages over suspension cell cultures that reduce the overall cost of the system. Compared to suspension cultures, the reuse of the immobilized cells over a longer period is a major advantage. The immobilization of cells in alginate hydrogels is a gentle process that results in the production of a transparent, permeable substance that increases cell density and protects the cells from contamination and shear or mechanical stress. Alginate is translucent, permeable, non-toxic, and allows the flow of gasses and metabolites in and out of the cells. In this work, the biological H_2 production by immobilized photosynthetic microorganisms such as *Chlorella vulgaris* (microalgae) and *Rhodopseudomonas* sp. (photosynthetic bacterium) is investigated.

Two different photobioreactors (PBR) were used, a cylindrical 0.2 L (C-PBR) and a flat 0.6 L Roux type (FRT-PBR); both PBRs with 4 cm light path. The ability of immobilized *Chlorella vulgaris* cells (534 mg dry cell weight in 2% v/v calcium alginate) to produce H_2 was investigated in the FRT-PBR using a K3 growth medium containing 25 mg/L KH_2PO_4 (Fig. 1A). The gas produced by the culture was analyzed using a PerkinElmer Clarus 500 gas chromatograph. The culture produced 214 mL of H_2 with an average productivity of 1.53 mL(H_2)/h) (Fig. 1B). The ability of the immobilized *Rhodopseudomonas* cells (54.1 mg dry cell weight in 2% v/v calcium alginate) to produce H_2 was examined using the C-PBR and a modified Van Niel growth medium containing butyrate (6 g/L) and 50 mg/L KH_2PO_4 (Fig. 1C). The immobilized cells produced 868 mL of H_2 with an average H_2 production rate of 2.68 mL/h (Fig. 1D).

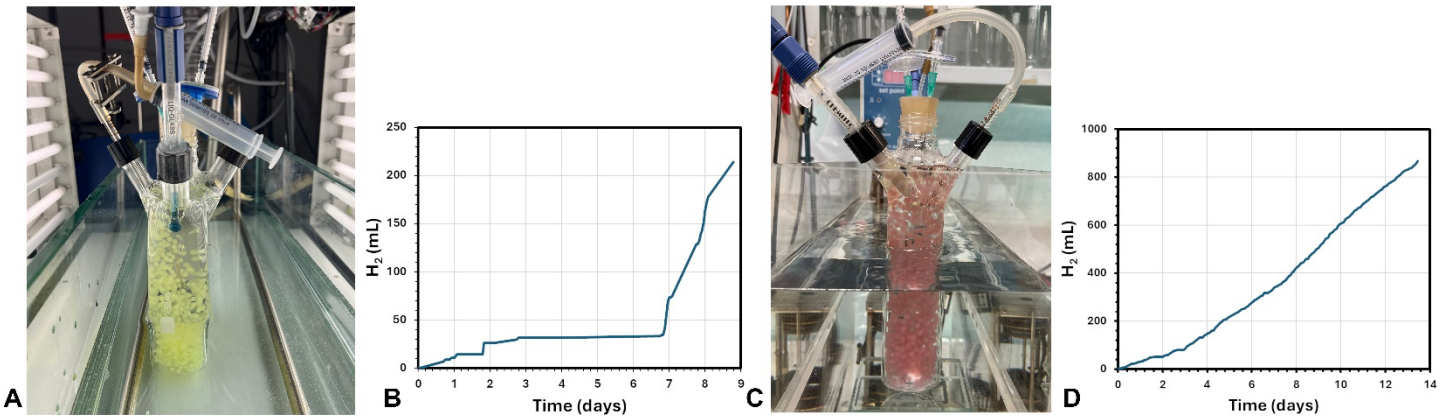

Figure 1. Image of the FRT-PBR with the immobilized *Chlorella vulgaris* cells (A) and the H_2 production over time (B). The immobilized *Rhodopseudomonas* cells in the C-PBR (C) and (D) H_2 production.

In this study, the calcium alginate immobilization technique was used to evaluate the responses of the immobilized *Rhodopseudomona*s and *Chlorella vulgaris* cells in terms of H_2 production. The ability of the immobilized cells to produce H_2 in two different PBRs was effectively tested. The results suggest that although immobilization appears to be promising, further research is required.

***Acknowledgments**: This work was supported by European Union's Horizon Europe – the Framework Programme for Research and Innovation [grant number 101093150], project LIBRA (Light Based Multisensing Device for Screening of Pathogens and Nutrients in Bioreactors).*

Sap flow methods for assessing stomatal ozone uptake in Italian forest ecosystems

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***Keywords**: Ozone; Tree Talker; PODy; Visible Foliar Injury*

Climate change intensifies air pollution, posing a significant threat to forest ecosystems. Tropospheric ozone, a harmful pollutant, affects plant growth and productivity. While the Accumulated Ozone Exposure over a threshold of 40 ppb (AOT40) index is widely used to assess ozone impacts, it may not fully include the actual ozone uptake by plants. We integrated sap flow measurements with established ozone flux indices to provide a more accurate assessment of ozone impacts on Mediterranean forests. Sap flow data were collected from four Italian forest sites including different plant species (*Fagus sylvatica*, *Quercus* sp.) and environmental conditions. These data were used to estimate maximum stomatal conductance (gmax), a pivotal parameter for calculating the Phytotoxic Ozone Dose (PODy), which considers the actual ozone flux entering the plant. We compared PODy estimates derived from sap flow-based gmax (PODy_sap) with those calculated using European standard Deposition of Ozone for Stomatal Exchange (DO3SE) model parameters (PODy_do3se).

Analysis revealed significant variability in sap flow and environmental conditions across study sites. Preliminary analyses showed a stronger correlation between PODy_sap and visible foliar injury (VFI) compared to PODy_do3se, suggesting that sap flow-based estimates of stomatal conductance more accurately reflects the actual ozone uptake and its impact on forest health. The study also observed species-specific responses to ozone exposure, indicating that beech trees grown under humid environments have shown a significant amount of VFI due to high gmax and thus stomatal ozone uptake. Furthermore, environmental factors, such as temperature, air humidity, and soil moisture, significantly influenced stomatal conductance and ozone uptake, highlighting the importance of considering these factors in ozone risk assessments especially in Mediterranean Europe where hot and dry summer is frequently observed.

This research contributes significantly to understand ozone impacts on forest ecosystems by improving ozone exposure assessments, enhancing forest monitoring, and assessing Italian forest resilience. By integrating sap flow measurements, this study provides a more accurate and biologically relevant assessment of ozone uptake by plants. This approach can be valuable for developing effective forest monitoring strategies and informing policy decisions related to air quality and climate change mitigation. Furthermore, by understanding the physiological impacts of ozone on trees, we can better assess forest resilience to environmental stressors and develop effective management strategies.

Future research will focus on expanding this approach to a wide range of forest types and geographical locations, investigating the long-term impacts of ozone exposure on forest growth, productivity, and ecosystem services, integrating other environmental stressors into ozone impact assessments, and developing predictive models to forecast future ozone impacts on forest ecosystems under different climate change scenarios.

Plant-derived extracellular vesicles: an innovative delivery system and a source of natural bioactive compounds

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Keywords: Opuntia*, Extracellular vesicles, Phenols, Wounds and injuries, Cellular responses, Antioxidant.*

Emerging threats to human health demand focused efforts to develop both preventive and therapeutic strategies, placing natural products as essential resources able to improve public health. In line with these natural solutions, plant-derived extracellular vesicles (P-EVs) have recently emerged as promising agents, offering new avenues as both carriers and cargo. P-EVs play a crucial role in intercellular communication thanks to their content. They can effectively deliver a wide range of cargoes, including proteins, lipids, nucleic acids (noncoding RNAs, DNA, mRNA), and other bioactive compounds, depending on their origin.

The health benefits of plants have been demonstrated in various human diseases, such as wound healing and tissue repair, relying on their powerful antioxidant and anti-inflammatory effects. Plant-derived EVs enable interkingdom communication by delivering bioactive molecules, making them potential tools for therapeutic strategies in various diseases. In this context *Opuntia ficus-indica* (OFI) is a rich source of bioactive compounds such as kaempferol and isorhamnetin, offering nutraceutical benefits as antioxidant, anticancer, neuroprotective, hepatoprotective, and antiproliferative properties. Based on the OFI health benefits, the present study investigates the antioxidant and anti-inflammatory effects of OFI-derived EVs (OFI-EVs) on chronic wound healing using in vitro experimental models. It is hypothesized that these therapeutic effects result from the direct delivery of bioactive molecules encapsulated within the EVs.

In this study, OFI-EVs were isolated from the whole OFI fruit using ultracentrifugation and characterized for their particle size distribution, concentration, and bioactive molecule composition, which was rich in diverse polyphenolic compounds. Firstly, in vitro cytotoxicity of OFI-EVs was evaluated on human dermal fibroblasts (HDFs), human umbilical vein endothelial cells (HUVECs), and the THP-1 cell line, indicating no significant cytotoxic effects. The analysis of OFI-EVs antioxidant capacity highlighted their effectiveness in restoring and enhancing cellular antioxidant defense mechanisms in H_2O_2-stimulated HDFs. Beyond scavenging free radicals, OFI-EVs demonstrated the ability to strengthen the endogenous defense system by modulating antioxidant and detoxifying enzyme activity. Furthermore, the results showed that pre-treatment with OFI-EVs significantly reduced the mRNA expression of pro-inflammatory cytokines (IL-6, IL-8, and TNF-α), revealing the protective effects of OFI-EVs. Finally, quantitative analysis of scratch closure and trans-well migration assays indicated the stimulatory effect of OFI-EVs on HDF migration, highlighting their effectiveness in accelerating normal wound healing processes.

In conclusion, this study underlines the relevant role of fruit-derived EVs in modulating crucial biological processes including inflammation and oxidation related to chronic skin wounds. This evidence suggests that OFI-EVs could be a valuable natural option for supporting the healing of chronic skin wounds.

Mostra d'Oltremare urban park and its botanical heritage

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***Keywords**: Biodiversity, Knowledge*

The aim of this study is to investigate and retrace the stages of the significant and progressive transformations of the botanical heritage in Naples' Mostra d'Oltremare. In 1940 the "I Mostra Triennale delle Terre Italiane d'Oltremare" was inaugurated, for the will of the fascist government. The exhibition grounds, an urbanism and architectural pride, represent today an excellent union of architecture, urbanism and biodiversity protection, and it was built according to the city plan written by architect Marcello Canino (1895 - 1970). The Exposition represent one of the most significant green interventions of the twentieth century in Naples was made. The executive design of the green spaces was entrusted to the architects L. Piccinato (1899 - 1983) and C. Cocchia (1903 - 1993). The executive design of the green spaces was entrusted to the architects L. Piccinato (1899 - 1983) and C. Cocchia (1903 - 1993). In the 600000 sq.mt. ground, which were originally allocated for the exhibition complex, more than 30000 tall trees were planted and about one million shrubs and herbaceous plants. The main green mass was made of a varied and massive collection of palm trees which gave to the environment the mediterran tropical appearance, with several specimens of eucaliptus, acacia, pine and magnolia. There's a suggestive presence of individually planted ornamental trees, such as citrus and olive trees, and numerous flourishing bushes and exotic and rare species such as rhizomatous, bulbous, tuberous, creepers etc.The executive design of the green areas, developed by L. Piccinato and C. Cocchia, was made in order to assure that the green area could have a major connecting role for the whole architectural and urban complex. The numerous exposition pavilions resulted to be immersed in suggestive green areas filled with exotic plants and that were often imported by their original lands, and which reproposed habitat and flor of the whole overseas colonies. The conspicuous green heritage, in spite of its modifications and decay over the years, is still one of the few green resources on the urban scale of Naples' wester area. It is fundamental today to work on the knowledge, enhancement and preservation this important environmental and cultural heritage.

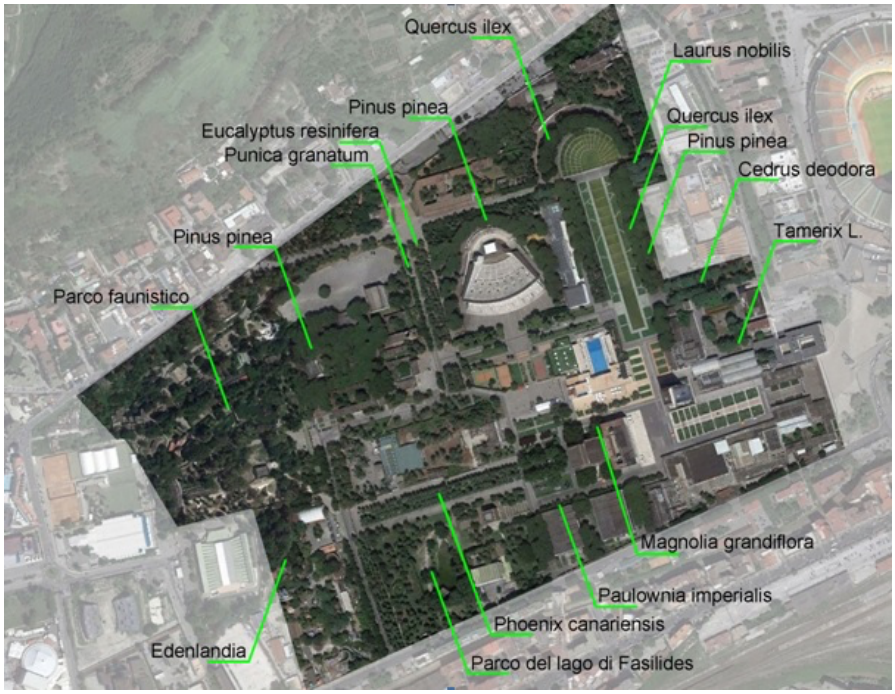

Figure 1. Mostra d'Oltremare, identification of the main clusters of trees.

The KasTrack project: chestnut biodiversity and distribution in Campania

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***Keywords**: Chestnut cultivar identification, Geographical distribution*

In Campania, the rich varietal heritage of sweet chestnut (*Castanea sativa* Mill.) has contributed to providing the region with a notable agrobiodiversity so increasing its environmental value. The peculiar soil and climate conditions of the territory favored the natural diversification of ecotypes adaptable to local environmental contexts, allowing the Campania chestnut germplasm to be among the richest and most differentiated in Italy. The selection activity carried out by chestnut growers has generated further diversity in the choice of varieties, selecting the genotypes with the best production and quality characteristics. The contribution of hybrids and non-native varieties has further enriched the local germplasm. The increasing interest in chestnut cultivation for production purposes requires greater clarity and protection in the field of varietal recognition. Varietal identification is in fact an essential element for rational management of chestnut horchards. Chestnut growers, to date, do not have a suitable tool to ascertain the genetic nature of the propagation material they use, because the morphological characteristics, as it is well known, present limitations of various kinds and can be misleading. The KasTrack project aims to provide chestnut growers with an innovative system for varietal identification through the release of suitable protocols and bioinformatics tools to the control laboratories interested in providing the service. To achieve this goal, an interactive and open access database is being developed where the detected genetic fingerprints of chestnut cultivars will be deposited. The main national cultivars are being collected in an ex-situ collection, publicly accessible for both sampling and morphological observation, to be used as reference standard for the interpretation of the results of the genetic analysis. Furthermore, geospatial data of chestnut tree positions are being acquired in chestnut farms (mainly PGI and PDO varieties) together with their genetic and molecular characterization (Fig. 1). This information will be used to define the presence and diffusion of the main chestnut species and their hybrids by creating a genetic-spatial mapping, so contributing to enriching the KasTrack database. Finally, knowledge and expertise on the effective use of the developed service for varietal recognition will be disseminated to all potential stakeholders (chestnut farms, nurseries, processing companies, and public institutions), so allowing to seize future opportunities, also in terms of sustainability, and taking into consideration the protection of chestnut agro-biodiversity.

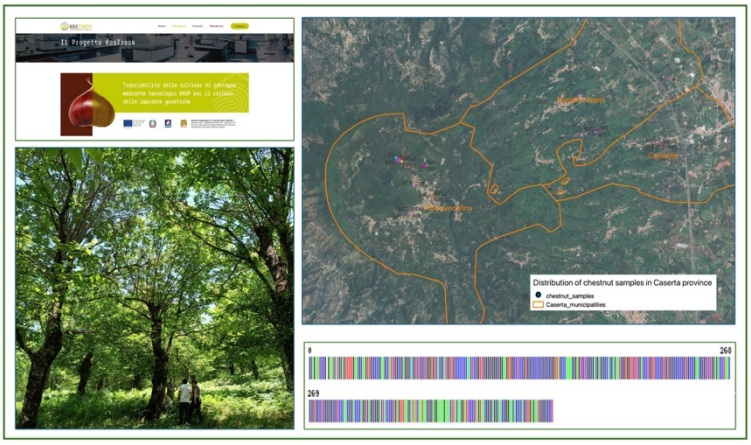

Figure 1. The images show the KASTRACK website and the preliminary results of the sampling campaign in the Caserta province.

***Acknowledgments**: This research was funded by Campania Region through a grant to the project "KASTRACK" CUP: B59H23000040006 (PSR Campania 2014–2020, M16, T.I. 16.1.2).*

Fostering the knowledge uptake on Nature-based Solutions: a review of digital tools and platforms

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***Keywords**: Biodiversity, Sustainability, Societal challenges, Ecosystem services*

Nature-based Solutions (NbS) address environmental challenges while providing co-benefits for society and biodiversity. However, their successful adoption and implementation require accessible, digital, and interactive resources to guide stakeholders through the planning, implementation, and monitoring phases. Comprehensive catalogues offer to practitioners curated lists of NbS practices, while interactive tools facilitate spatially explicit planning, co-design, and adaptive management. Over the last decade, the European Community's effort in funding NbS research and implementation projects has been very significant. The results obtained in such projects have shown that digital resources can empower policymakers, practitioners, and communities in upscaling NbS by bridging knowledge gaps and providing practical guidance. Nevertheless, information on NbS remains fragmented, dispersed across multiple platforms, or overly focused on one or a few specific ecosystems (e.g., urban). Furthermore, the lack of standardization and consistent monitoring protocols for measuring NbS effectiveness, continues to impede the interaction among disciplines and the widespread adoption of NbS across diverse ecosystems. This work provides 1) an overview of NbS platforms, tools and repositories, showing their crucial role, as well as the barriers and gaps that hinder their effective use for NbS uptake, for example the lack of monitoring- and business-oriented platforms; 2) guidance and suggestions to upload and update NbS case studies into relevant repositories, according to the project outputs and the societal challenges addressed. A total of 115 platforms and digital tools were identified and further categorised according to the kind of data stored: a) Case Studies, namely implemented solutions in projects or research studies (23%); b) Categorical Solutions, also known as measures or units not necessarily related to a specific case study (10%); c) Data, such as tabular data and databases (31%); d) Knowledge Exchange, namely publications, reports, webinars, etc. (77%); e) Decision Supporting Tools, interactive web-based or stand-alone software, as well as methodologies (30%); and f) Simulation Tools (17%). The 27 platforms storing case studies were further analysed to identify, first, the platforms enabling the users to upload or update data and, second, the following parameters assessed by simulating the upload of case studies: (i) the upload modality; (ii) the type of outcomes, namely Actions and Initiatives, Strategies including Policies, or Data generated from Monitoring activities through Key Performance Indicators; (iii) the relevancy to the 12 societal challenges following the NetworkNature's European Roadmap to 2030 for Research and Innovation on Nature-based Solutions; (iv) the focus on biodiversity and/or business data. Finally, the 12 platforms enabling the upload and update of case studies were included in a *flowchart* and described synthetically in *NbS case study repository profiles*, to guide users in selecting the most suitable online repositories, based on the characteristics of the case study they wish to share.

***Acknowledgments**: The authors would like to acknowledge Biodiversa+ partnership who promoted the work and in particular Mariem El Harrak and Cloé Durieux for their fruitful suggestions.*
Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, Project title "National Biodiversity Future Center - NBFC".

HIC SUNT LUPI Project: Monitoring and Management of Wolves in Salento

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***Keywords**: Biodiversity, Conservation*

The Salento area, in the southern part of the Puglia region, is experiencing a significant re-colonization of the wolf (Canis lupus) after centuries of absence, generating considerable ecological and economic interest. Frequent sightings and livestock attacks indicate a stable wolf presence, challenging the hypothesis of sporadic occurrences. Salento is a densely populated area where agro-ecosystems and urban settlements leave natural areas in a residual position. This creates a peculiar context, where the presence of a large predator like the wolf requires careful management and communication strategies to minimize negative effects and maximize positive interactions with the local community and ecosystem.

The “HIC SUNT LUPI” project aims to implement a management approach based on sound scientific knowledge. The focus of the project is on monitoring the wolf population and studying its diet, which are essential for understanding predator-prey interactions and ensuring effective species management. The re-colonization of Salento, in the context of the post-Xylella fastidiosa epidemic, adds an intriguing scientific element, integrating with territorial rehabilitation and habitat protection strategies.

Launched in February 2018, the “HIC SUNT LUPI” project has concentrated its efforts in the first 10 months on two main areas: stakeholder engagement and field activities.

Stakeholder Engagement: Significant progress has been made in engaging local stakeholders. A total of 28 awareness-raising meetings were held, along with 4 technical workshops involving environmental guides, local health authorities (ASL), the Institute of Zooprophylactic Studies (IZS), hunters, farmers, and their respective associations. As part of the engagement, three decalogues have been produced: one providing information about the wolf species, another for visitors of natural areas in collaboration with regional park authorities, and a third for farmers and breeders, in collaboration with ASL and sector associations. Additionally, two memorandums of understanding have been signed: one with ASL and IZS, and another with the Order of Veterinarians.

Field Activities: On the field, surveys and sampling have been conducted over approximately 120 hectares, with over 100 samples collected for dietary analysis. A total of 55 monitoring points have been established with camera traps, resulting in 97 videos. These monitoring efforts have led to the identification of 6 wolf packs and 4 reproductive events.

The second phase of the “HIC SUNT LUPI” project, which is now underway, includes dietary analysis using two different methods: genomic and macroscopic. Genomic analysis will involve extracting DNA from feces or prey remains to identify the wolf’s diet at the molecular level, providing insights into the specific species consumed. The macroscopic analysis will focus on examining the feces to identify undigested remains of prey, primarily hair and bones, in order to determine the composition of the wolf’s diet. The combination of these methods will provide essential information on the wolf’s diet, which is critical for the management and conservation of the species.

The ultimate goal is to establish participatory management guidelines tailored to the Salento context, balancing negative interactions with livestock while maximizing the ecological benefits of the wolf’s presence in the region.

***Acknowledgements:** We would like to express our sincere gratitude to the Environmental Department of the Puglia Region for funding this project. Their support has been instrumental in enabling our research and activities, contributing to the advancement of our work.*

Ex-situ collections of *Castanea sativa* provenances as genetic sources of resistance to abiotic and biotic stress

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Keywords: Castanea sativa*; Asian chestnut gall wasp; Fersa; Genetic resources; Resistance*

Emerging pests associated to global change cause important economic and ecological damages. In the last century several parasites from exotic origin have caused a severe decline of *Castanea sativa* Mill. (sweet chestnut) in Europe. Sources of resistance to these pests have been usually searched in Asian *Castanea* species and interspecific hybrids. On the other hand, intraspecific genetic resources of *C. sativa* have been under-evaluated. The ex-situ collections and common garden field trials of *C. sativa* are continuosly exposed to emerging pests and represent valuable resources to study and understand the genetic basis of resistance to biotic stressors.

Here we present two case studies carried out in the experimental field of CNR IRET, Porano (TR), which hosts a common garden field trial including six *C. sativa* provenances from Spain, Italy and Greece. The first study, focussed on the resistance to Asian chestnut gall wasp, was carried out by coupling infestation measurements and genomic analyses and identified an unique genomic region associated to resistance in a provenance from Greece. The second study is focussed on leaf necrotic spots caused by a fungal pathogen commonly named fersa. Two years of visual observations highlighted a high variability in leaf symptoms among the six provenances and a low susceptibility of a Spanish provenance. A quantitative symptom evaluations has started in 2024 to monitor the disease and verify the previous observations. Ten trees wee randomly selected from each provenance and 12 leaves were collected in the high, middle and bottom layer of the crown, from opposite sides of each tree. Leaf images were taken by a scanner and image analyses are in progress to measure the ratio of infected leaf area. The quantitative analyses will be repeated in next years to obtain representative results. This study provides characterized plant material suitable for more specialized research on plant-pathogen interaction and plant resistance mechanisms.

These case studies confirm the relevance of experimental fields and tree collecions as long-term living laboratory to study the adaptive diversity of plant genetic resources and the mecchanisms of stress tolerance.

Improving productivity in *Tanacetum balsamita* L. and vineyards through sustainable soil cropping management strategies

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***Keywords**:* Tanacetum balsamita *L, Vineyard, Soil cropping, Sustainable cultivation*

This study, conducted within the EU PRIMA project ReCROP, focuses on enhancing soil health and crop resilience in Mediterranean agroecosystems through the combined use of microbial inoculants, biofertilizers, and sustainable farming practices. The collaborative effort of Mediterranean countries involved aims to reduce the dependence on chemical fertilizers and improve soil fertility and water use efficiency.

Experimental fields of *Tanacetum balsamita* L. in Florence and vineyard in Calci, Tuscany (Italy) were established. The experimental design consisted of 12 plots each with 3 replicates. Treatments included microbial inoculants with PGPR and MF + N nutrition; high-quality compost, combination of both upper cited treatments, and the conservation of traditional management to act as control. Soil and plants were collected over two seasons (2022 - 2023) to compare various treatments and control in terms of the main soil properties and plant performances. In addition, the essential oil compounds of *Tanacetum balsamita* L. were identified by gas chromatography-mass spectrometry (GC-MS) and the stability of aqueous plant extracts was assessed by polyphenol contents, antioxidant and antiradical activities.

For Balsamita, significant differences were found in the number of leaves, average plant weight, chlorophyll *a*, and carotenoids content. These parameters contribute to enhance photosynthetic efficiency and plant health, resulting in improved growth and biomass accumulation. In the essential oils, only borneol showed significant differences among treatments. In the aqueous extracts, the total phenol content of bioactive compounds in samples stored at 4°C showed a decrease. Furthermore, antioxidant and anti-radical activities were present in all treatments of samples stored at 4°C. In contrast, at room temperature, only samples of some treatments exhibited both activities.

In vineyard the inoculum + compost treatment showed an improvement of several nutrients' availability in the soil and various elements concentration in the berries from the unhealthy plants. These results indicate that the treatment positively influenced grapes' nutrition, but the yield was not significantly affected, and treatments did not induce plant resistance to overcome the pathogen attack in 2023.

***Acknowledgments:** This study was carried out in the frame of Project: ReCROP—Bioinocula and CROPping systems. Authors wish to thank "Officina Profumo Farmaceutica di Santa Maria Novella"(Florence, Italy) for providing experimental fields.*

Vertical biodiversity: a study of tree crown microhabitats through tree climbing and drones

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***Keywords**: Vertical biodiversity, Tall trees, Microhabitats, Tree climbing, Drones*

Tall trees act as vertical ecosystems, hosting a diversity of microhabitats that significantly contribute to forest biodiversity. However, direct access to these canopy structures is often challenging, as traditional ground-based observations are limited by the observer's field of view, leading to an incomplete and potentially biased understanding of the tree's full ecological picture. This study aims to bridge this gap by investigating the relationship between ground-observed biodiversity and the broader vertical biodiversity within tree crowns. By analyzing these connections, we explore the potential to develop predictive models or coefficients that estimate whole-tree biodiversity from ground-level observations. This approach could significantly enhance forest biodiversity assessments by providing a more comprehensive and efficient methodology for evaluating vertical ecosystem complexity. Furthermore, this research assesses the effectiveness of complementary methods, such as tree climbing and drone technology, to improve accuracy in vertical biodiversity studies and enhance integration with ground-based approaches.

At this stage, the tree-climbing technique is employed to document and classify microhabitats within tree canopies, analyzing their spatial distribution and ecological functions. The study focuses on a selection of tall trees within the mature forests of Pian del Cansiglio, utilizing standardized protocols based on the "Field Key for Tree-Related Microhabitats." A trained tree climber conducts systematic explorations of the canopies, identifying and recording microhabitats — such as cavities, fungi, epiphytes, loose bark, deadwood, and organic material accumulations — using standardized data sheets, photographic documentation, and selective sampling.

Preliminary findings reveal significant vertical heterogeneity in microhabitat distribution, with a high density of cavities, deadwood, and bird nests concentrated in the upper canopy. These results highlight the indispensability of vertical observations to fully capture forest biodiversity. Tree climbing allows for close-range, detailed observations with minimal disturbance to surrounding ecosystems. However, its physical demands limit the number of trees that can be sampled, and scaling up requires more climbers, leading to increased costs. To overcome these limitations, the study is exploring drone technology as the next step for assessing whole-tree biodiversity. Drones offer the ability to ascend along tree trunks at close range, capturing high-resolution imagery and video for detailed analysis. This method promises significant savings in both time and costs, while enabling broader and more representative sampling.

This research highlights the critical role of tall trees as pillars of vertical biodiversity and emphasizes the value of innovative methods, such as treeclimbing and drone technology, in accessing and studying these complex ecosystems. Comprehensive understanding and assessment of vertical biodiversity are essential for the conservation and sustainable management of forest ecosystems.

***Acknowledgments**: This study was conducted with the support of NBFC funded by the Italian Ministry of University and Research, PNRR, Missione 4 Componente 2, "Dalla ricerca all'impresa", Investimento 1.4, Project CN00000033.*

Modelling transitional water phytoplankton communities by functional traits in climate change scenario: an ecological niche-based approach

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***Keywords**: Climate change; Phytoplankton; Transitional waters; Species Distribution Model; Ecological Niche Model*

Phytoplankton guilds have already been shown as a critical component of the aquatic ecosystems that are likely to be strongly affected by climate change. Here, we present an approach to phytoplankton ecological responses to climate change linking species functional traits and ecological niche analyses. The study is focused on transitional water ecosystems, which due to their physiographic and geomorphological characteristics are among the most strongly impacted aquatic ecosystems, particularly by global warming.

The study is based on a meta-analysis of phytoplankton shape-based occurrences data from 24 lagoons in five biogeographical regions of the world. The dataset is available on the LifeWatch Data Portal. We used a maximum entropy model to identify suitable areas for phytoplankton guilds in current and future scenarios of environmental variables, based on the GMED datasets. Furthermore, we investigated phytoplankton ecological niche breadths and overlaps to model their changes in relation to expected environmental changes. The analysis mainly shows a relationship with “depth” and “wind speed” in identifying phytoplankton suitable areas, with a general increase for spherical species. Species with rounded body shapes are influenced by warmer and less nutritious waters, as predicted in the future scenario, than the elongated ones. Finally, in the future scenario, the results showed a general increase of niche breadth that may suggest an adaptation of phytoplankton species to new conditions, as well as higher overlap values indicating an increasing competition among them for space and resources.

The application developed in this study can be useful in supporting actions to maintain ecosystem balance in a changing climate scenario.

***Acknowledgements:** project IR0000032 – ITINERIS, Italian Integrated Environmental Research Infrastructures System (D.D. n. 130/2022 - CUP B53C22002150006) funded by EU - Next Generation EU PNRR- Mission 4 “Education and Research” - Component 2: “From research to business” - Investment 3.1: “Fund for the realization of an integrated system of research and innovation infrastructures”.*

Genetic diversity and structure of *Alnus cordata* and *Quercus trojana* populations in the native range of southern Italy: an essential step towards the definition of management and conservation strategies

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***Keywords**: Genetic diversity, Endemic species, Conservation genetics*

Trees are generally long-lived sedentary organisms and their ability to adapt to environmental changes and biotic stress depends on genetic variation within and among populations. Under future climate change scenarios, several Mediterranean tree species are predicted to undergo range reduction and increased fragmentation. A conservation strategy is urgently needed for minimizing the potential loss of genetic diversity. For these resons, studying the genetic diversity and structure of current forest populations is essential for evaluating their capacity to response to future environmental changes and planning conservation strategies. In the context of the National Biodivesity Future Center (spoke 4.3.1), we performed a comprehensive analysis of within- and between-populations genetic diversity of *Alnus cordata* and *Quercus trojana*. Little information are available of genetic diversity and adaptive potential of these two Italian endemic tree species with fragmented distribution range in southern Italy. We genotyped 23 populations of *A. cordata* and 30 populations *of Q. trojana* using polymorphic nuclear microsatellite markers (SSRs). Moderate genetic diversity characterizes *A. cordata* populations. This diversity decreases towards the peninsula's southern latitudinal margins. Despite low genetic differentiation, STRUCTURE identified two genetic clusters weakly correlated with the elevation. Similarly for *Q. trojana,* the results obtained with Principal Coordinate (PCoA) and STRUCTURE analysis revealed the presence of two main gene pools, highlighting a sharp genetic divergence between two populations in the core of distribution and the remaining germplasm. Moreover only for *A. cordata*, the habitat suitability was modeled under current and future climate scenarios using an ensemble forecasting approach. We carried out a genetically informed conservation prioritization using the Reserve Selection analysis implemented in DIVA-GIS. In conclusion, our study highlights the importance of evaluate the genetic diversity and integrating genetic analyses, habitat suitability modeling, and spatial prioritization techniques for effective conservation planning of *A. cordata* and *Q. trojana.*

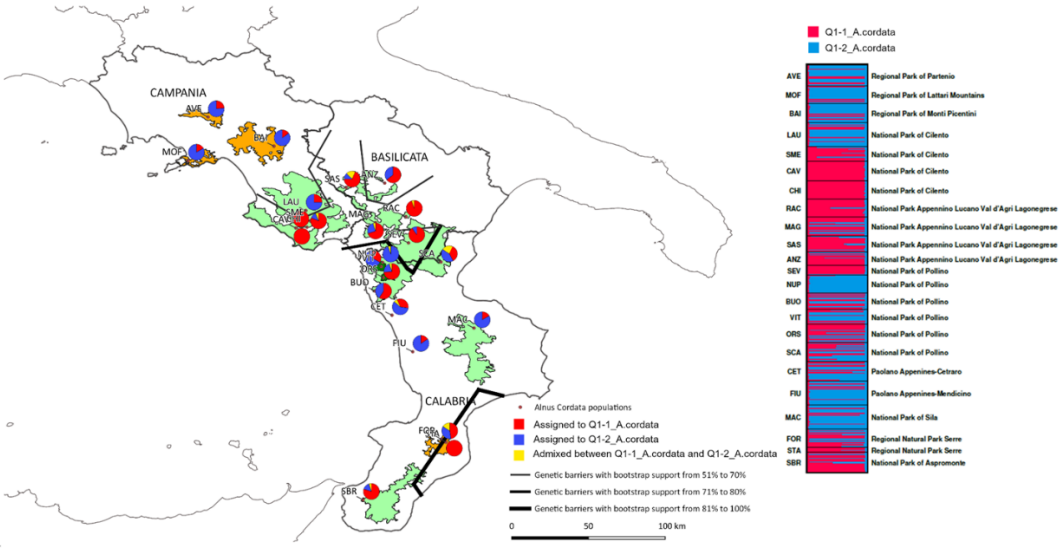

Figure 1. Population structure inference for 23 Italian alder samples by Bayesian assignment using STRUCTURE (Pritchard et al. 2000) for K=2 clusters

Integrating species traits and environmental dynamics to predict spatial behaviour and climate change impacts on vertebrates

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***Keywords**: Biodiversity; Climate change; Conservation; Home range; Research infrastructure*

A comprehensive understanding of how species traits and external factors interact to shape dynamics of space use behaviour could help clarify key factors driving the strategies of energy requirements, fitness and life expectancy. In an era of rapid climate and environmental changes, predicting biological responses and consequences to such changes become critical to addressing current challenges and ensuring species' long-term preservation.

To address these points, we followed a two-steps approach: first, we assembled a global dataset with a representative selection of aquatic and terrestrial vertebrate behavioural and life-history traits, to investigate relationship between home range size and body size, as well as the relative influence of various biological and ecological factors including habitat type, thermoregulation, trophic behaviour and mobility, in home range and maximum longevity variation across different taxa. The resulting dataset includes 1164 species spanning fish, reptiles, mammals and birds from around the globe that will be harmonized using LifeWatch data services following Findable, Accessible, Interoperable and Reusable (FAIR) principles.

Secondly, part of the Aquatic Biomass VRE workflow was adapted and applied to analyse the variation in mean temperatures, as a proxy for global warming, across species' ranges (via IUCN source) and building future scenario of how these changes may evolve. Using remotely sensed images of sea surface temperature and land surface temperature from MODIS products, we built a temperature time series for each species from 2003 to 2024 and projected them into the future to predict potential changes likely to occur in the species' range. This twice, not-mutually exclusive approach, will facilitate comprehensive estimates of home range relationships with both intrinsic and extrinsic factors and would allow us to understand the impact of climate change to biodiversity organization and conservation.

***Acknowledgements:** project IR0000032 – ITINERIS, Italian Integrated Environmental Research Infrastructures System (D.D. n. 130/2022 - CUP B53C22002150006) funded by EU - Next Generation EU PNRR- Mission 4 "Education and Research" - Component 2: "From research to business" - Investment 3.1: "Fund for the realization of an integrated system of research and innovation infrastructures".*

Jackal-howling in urban and rural areas: status of a protected species in Tuscany

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Keywords: Canis aureus*; Urban areas; Habitats directive; Urban wildlife*

The golden jackal *Canis aureus*, a medium-sized canid native to Asia and Eastern Europe, has experienced a significant range expansion since the 20th century. In Italy, this species was first recorded in the early 1980s, but its presence has been primarily confined to north-eastern regions, in spatial continuity with Balkan populations, until recent years. In 2021, the first record of a golden jackal was documented in Tuscany, marking a substantial southward expansion. In the framework of the activities of the National Biodiversity Future Center, we assessed the current distribution of the golden jackal in Tuscany, by conducting a comprehensive review of published records and performed acoustic surveys in urban and rural areas with presumed occurrence based on the available data. Additionally, mitochondrial analysis was conducted on a road-killed individual to determine its potential origin.

Our findings suggest that the golden jackal presence in Tuscany is still relatively scarce, with two individuals detected via opportunistic camera trapping between the provinces of Prato and Florence, a single road-kill near Empoli (i.e., in the central part of the region) and one individual camera-trapped in the Maremma Regional Park (southern part of the region). Acoustic surveys yielded positive results only in the northern outskirts of Florence urban area (municipality of Calenzano), indicating a territorial settlement in the area. However, the reproduction of this group was not confirmed yet. Molecular analysis revealed a strong genetic affinity between the Tuscan individual and populations from the Balkan Peninsula, confirming the southward expansion of the Italian golden jackal population amidst environmental and anthropogenic pressure. Further research is needed to fully understand the ecological implications of this range expansion, which might be hindered by the presence of a large grey wolf *Canis lupus* population in central Italy.

Meta-Genomics: Exploring Every Surface

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***Keywords**: Microbiome diversity, Pollution, Urban, Invasive species*

Over the last four years, the CNR-Research Institute on Terrestrial Ecosystems (IRET, Porano, Terni) has focused its attention on (1) assessing the biodiversity of microbial communities in relation to pollution levels in urban environments and (2) shedding light on the role of soil microbiomes in the plant invasion process, thought high-throughput amplicon sequencing of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) regions. This approach has been applied to environmental DNA extracted from various substrates, including PM10 filters, leaf surfaces, paved road surfaces, wall surfaces and soil samples (Figure 1).

In collaboration with Sapienza University of Rome, the Russian Science Foundation (project MicroAir), and the University of Salento (project PRIN2022 BIOMASTER), we studied the composition and structure of PM10-associated bacterial and fungal communities across different seasons and urban sites in Rome, as well as in cities with varying pollution levels. Our findings suggest that in Rome, the absence of precipitation, combined with the resuspension of dust caused by vehicular traffic, contributes to the peak abundance of soil-associated microbes during winter and summer. Additionally, elevated PM10 concentrations, influenced by climatic conditions, domestic heating, and dust advection events from the African desert, further shaped microbial communities in winter. Over the seasons, extremotolerant microbes and opportunistic pathogenic fungi showed a progressive increase in response to rising pollution levels. Similarly, we also investigated the impact of environmental pollution on the bacterial and fungal community structures of the ancient walls of Villa Farnesina in Rome, highlighting how urban pollution and site-specific conditions influence microbial diversity and abundance. Finally, in collaboration with CNR-IRET of Florence, we are currently testing the effects of ozone on the microbial community in the phyllosphere of common grape vine.

In the framework of UseIt, a project focusing on Invasive Alien Species (IAS), funded by the National Research Council, coordinated by CNR-IRBIM (Ernesto Azzurro) and involving scientists from three headquarters of CNR IRET (Lecce: Cristina Di Muri, Ilaria Rosati, Sassari: Laura Loru; and Porano), we combined a high-throughput amplicon sequencing of ITS regions, with stable isotopes analysis of soil samples to investigate the community compositions and structures of soil-associated fungi across *Ailanthus. altissima* density gradient (Absence, Front and Infested) in three pilot experimental sites (Urban Park, Rural and Natural) of central Italy. The tree of heaven (*A. altissima*) is in fact one of the worst invasive plant species in Europe and North America. We are currently investigating the putative role of symbiotic relationships between *A. altissima* and associated Arbuscular Mycorrhizae Fungi (AMF) in the Evolution of Increased Competitive Capacity (EICA) of *A. altissima*.

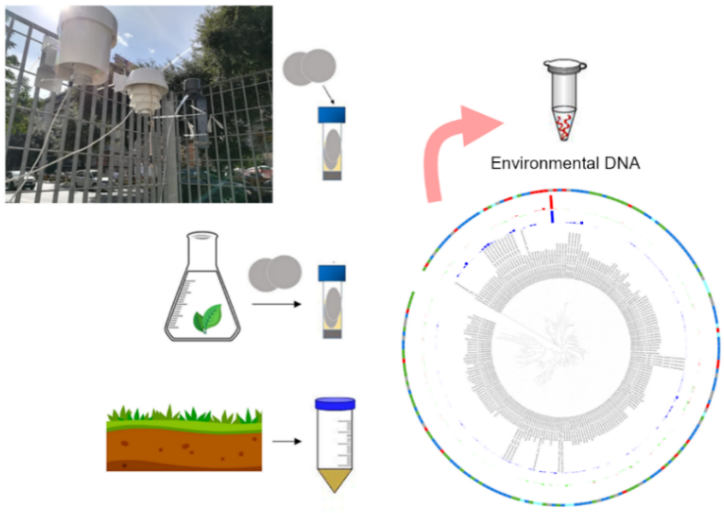

Figure 1. High-throughput amplicon sequencing of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) regions from environmental DNA extracted from various substrates, including PM10 filters, leaf surfaces, paved road surfaces, wall surfaces and soil samples.

***Acknowledgments**: The work was realized with the financial support of UseIT: Utilizzo di Sinergie operative per la gestione integrata specie aliene Invasive in ITalia, Programma: Progetti di Ricerca @CNR and of Italian Ministry of University and Research (European Union, Next Generation EU), project PRIN 2022 n. 202249BMWR*

Application of the QBS-ar index as a new indicator for the NEC network in Italy

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***Keywords**: QBS-ar; Microarthropods; Soil biological quality*

The QBS-ar index (Qualità Biologica del Suolo) is based on the following concept: the higher is the soil quality, the higher is the number of microarthropod groups morphologically well adapted to this soil habitat. The fluctuation of the soil quality can be related to direct human inputs (including land management practices) or to long-term processes such as climate change. QBS-ar has been developed by an Italian team (Parisi et al. 2005) and it has been applied to several ecosystems, including agricultural lands, grasslands, urban soils, woods at different level of wilderness, and degraded soils. It does not require identifying the fauna to the species level. It is applied to the soil microarthropod community, separated according to the biological form approach with the intention of evaluating the microarthropods' level of adaptation to the soil environment life, and overcoming the well-known difficulties of taxonomic analysis to species level for edaphic mesofauna. In addition, this index is rather inexpensive, both in terms of equipment required and time/energy needed in the sampling activity and the analysis of the samples.

The high number of applications in Italy, European and non-European countries signal the potential of QBS-ar. It is a good candidate index for continuous biomonitoring of soil communities to describe patterns and processes in the microarthropod biodiversity across the landscape. A deeper knowledge of soil biodiversity in response to landscape use will provide guidance in effective management planning for sustainable renewable resource use and nature conservation.

QBS-ar index was considered to be a standard protocol for measuring soil fauna across Europe LTER sites ExpeEr Ecosystem Research Program (Experimentation in Ecosystem Research), and it is reported in the European Commission - DG ENV 2010 - Soil biodiversity: functions, threats and tools for policy makers.

The QBS-ar index has been applied in 10 sites of the NEC Directive thanks to the LIFE MODERn (NEC) project with the aim of testing the indicator for use in a long-term monitoring program. The sites are widely distributed throughout the Italian peninsula, from Passo Brocon in the province of Bolzano to the Piani di Limina in the province of Reggio Calabria, and also include a site in southern Sardinia, in the Marganai forest. For the most part, they are beech forests of the mountain belt, but mixed oak forests, holm oak forests, and a spruce forest are also present.

***Acknowledgments**: LIFE20 GIE/IT/000091 LIFE MODERn (NEC) – new Monitoring system to Detect the Effects of Reduced pollutants emissions from NEC Directive adoption*

Determinants of vascular species diversity on poplar natural and semi-natural woodlands: a stand scale approach

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***Keywords**: Plant diversity, poplar plantations; stand structure; soil properties; soil enzymatic activities*

Restoring biodiversity in riverine landscapes is pivotal to enhance ecological processes that are characteristic of river and wetland ecosystems. This is particularly relevant in floodplain areas traditionally characterised by intensive human transformation as the Po plain. In this framework, improving the functionality of the river environment through new plantations is a powerful NBS. The impact of poplar (*Populus* spp.) plantation on biodiversity has been little studied, even more considering it along different environmental gradient. This study was conducted in three sites of Piedmont region, Italy (Fig. 1), where restoration actions have been started in the year 2004. They included forest plantations to improve biodiversity and ecosystem services. Analysis focuses on the main determinants (soil properties and stand structural parameters) of vascular species diversity within natural (i.e., derived from spontaneous recovery of vegetation), semi-natural (i.e., planted woodland for biodiversity restoration purposes) and productive woodland stands that have been affected by different environmental – site-specific – dynamics. Data were collected at stand and plot level, with an integrated approach that consider the joint effect of mero-biotic and stand structure characters on biodiversity (Fig. 1). Specifically, our main hypotheses were that: a) semi-natural stands host higher vascular species diversity with respect to the natural ones; b) structural attributes, coupled with soil properties, influence herbaceous layer richness and composition as well as its functional traits; c) natural stands are more homogeneous in terms of species composition but with less presence of non-native species; and d) poplar plantations host lower biodiversity levels in all sites. The final model indicated that quadratic mean diameter affected the total species richness ($p < 0.05$) and the native species richness ($p < 0.01$). Total Ca has a statistically significant negative effect ($p < 0.001$) on exotic species richness, while a positive effect was found for microbial N limitation ($p < 0.01$). Our work tested the effectiveness of a novel and replicable methodological approach that allows the ecological and functional assessment of riverine poplar stands with different gradients of naturalness. Results will allow the selection of river ecosystem management strategies that meets the requirements of nature restoration law (Regulation EU 2024/1991).

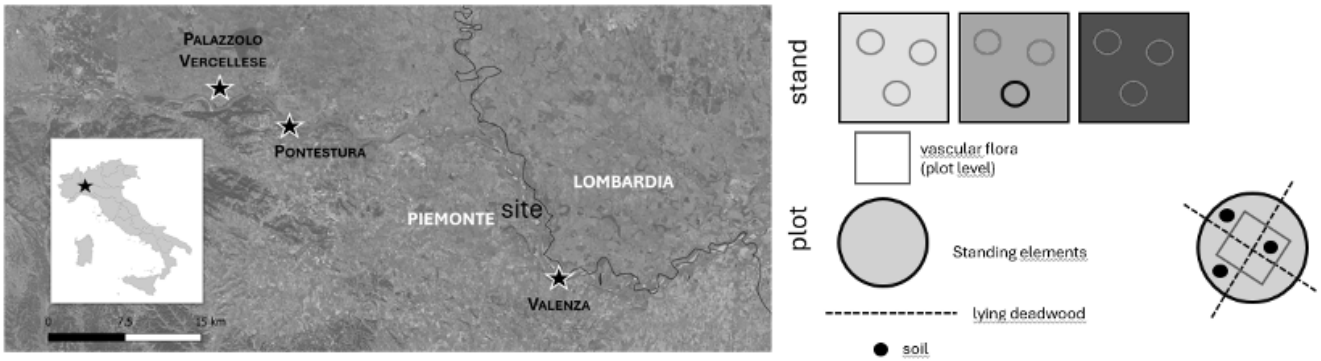

Figure 1. Study area and sampling design from site to plot level used in the field data collection.

Acknowledgments: *This work was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC".*

Manipulation experiments in Alpine ecosystems: Exploring climate change impacts and carbon dynamics

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***Keywords**: Climate change, Microcosms, Transplantation, Alpine ecosystem, Carbon fluxes*

During the 21st century, alpine areas are experiencing warming above the global average, making them more sensitive to climate change with potential significant release of CO_2. Indeed, an expected impact of climate change is the alteration of the balance between carbon assimilation and its storage in vegetation and soils, and its release to the atmosphere via respiration. The field of experimental global change ecology has expanded rapidly in recent decades, shifting from observational studies to manipulation experiments. These experiments increasingly employ space-for-time substitution methods, such as transplanting plants across elevations, to test acclimation and adaptation strategies. Simultaneously, advancements in technology have facilitated the use of controlled systems (e.g., micro-, meso-, and macrocosms) to simulate future climate scenarios. In this context, the ***PRIN MICROPLANTALP* project** provides valuable insights into how alpine grassland ecosystems respond to climate change, by integrating the study of carbon flux dynamics, plants, and soil microorganisms. The project employs two complementary experimental approaches: ***transplantation*** and ***microcosm experiments***. In the transplantation approach, soil monoliths were transplanted from 2500m to 1500m altitude in Val Veny, Courmayeur, Italy, simulating a 5°C temperature increase as projected for 2100 by the IPCC's RCP 8.5 scenario. Two transplantations were conducted: in 2022 (old plots) and in 2023 (new plots), allowing the study of carbon fluxes in different acclimation stages. Continuous chamber systems were installed at both altitudes to measure CO2 fluxes every 15 minutes in undisturbed control plots and transplanted plots, using transparent and opaque chambers. In the microcosm experiments, soil monoliths from the alpine ecosystem (2500 m a.s.l.) were transferred to the Montpellier European Ecotron (CNRS, France) for climate manipulation experiments. Monoliths were exposed to current (~ 420ppm CO_2, Control), and two future climate scenarios (~ 550ppm CO_2 and ~ 800ppm CO_2, according to RCP 4.5 and RCP 8.5, respectively) forecasted for 2070. The Ecotron's experimental chambers allowed to manipulate different climate variables, such as temperature, precipitation, relative humidity, radiation, and CO_2 concentration, simultaneously. The combined results from transplantation and microcosm experiments highlight the contrasting trajectories of alpine ecosystems under climate change. Transplantation experiments underscore the vulnerability of carbon sequestration functions to warming and drying, particularly in the context of extreme events like heatwaves, where reduced leaf area and shifts in species composition significantly impair carbon uptake. Conversely, microcosm studies reveal that under high-emission scenarios (RCP 8.5), increased CO_2 levels and elevated temperatures enhance canopy growth sustaining carbon sink functions despite higher respiration rates. These findings demonstrate that while alpine ecosystems exhibit a degree of short-term resilience under elevated CO_2 conditions, their long-term capacity to maintain carbon sequestration under combined stressors of warming, drying, and species loss remains uncertain. Together, these insights emphasize the importance of both field-based and controlled experimental approaches in understanding the complex dynamics of alpine ecosystems under future climate scenarios.

***Acknowledgments**: This study was conducted within the framework of the Italian PRIN project n. 20204KF4RW, entitled "MICROorganism-PLANT Interactions in the Forefield of Glaciers: a Hotspot for Studying the Impact of Climate Change in ALPine Habitats" (MICROPLANTALP), under the scientific coordination of Dr. Angela Augusti, whose memory and contributions continue to inspire this research.*

Revitalizing urban landscapes by desealing: the REUSES project for soil restoration and sustainable community development

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***Keywords**: De-sealed soil, Urban areas, Land consumption, Soil ecosystem services, Urban/community gardening*

Urban development across European countries continues to grow in response to social and economic demands, often leading to significant soil loss through increased land uptake. Among the adverse effects of this trend, soil sealing—where soil is irreversibly covered by impermeable materials—poses the most serious threat. This practice impairs essential soil ecosystem services included soil productivity, carbon sequestration, regulation of water and gas fluxes. As a result, soil sealing profoundly affects environmental quality and the life of all living organisms. The REUSES (Restore Urban Sealed Soil for Alternative Ecosystem Services; Fig. 1) project seeks to study and eventually understand how to restore soil functionality of areas long sealed by impermeable materials (such as concrete and asphalt) while establishing green spaces for citizens in the form of community gardens. The project focuses on two case studies in Ancona, where two abandoned parking areas have been de-sealed. This process involves removing the impervious surfaces, followed by soil ploughing, hoeing, and amending with suitable compost at varying concentrations to reduce compaction and promote plant growth. To further evaluate the impact of de-sealing besides soil chemical, physical, and biochemical parameters, two crop cycles of seasonal vegetables have been cultivating on the rehabilitated soils. Analyses on soil parameters and the edible parts of the plants have been performed to assess both crop productivity and food safety. Additionally, a local survey has been conducted to gather citizens' perceptions and interests regarding urban gardens, aiming to understand their needs and promote active involvement. In this way, the REUSES project not only addresses soil rehabilitation but also evaluates the broader environmental, social, and economic benefits of de-sealing sealed surfaces by highlighting soil as a critical resource while addressing citizens' growing interest in green initiatives. These insights contribute to the wider goal of fostering sustainable urban landscapes, aligning with the EU's 'No Net Land Take' policy by 2050 and promoting soil care as an essential pillar of environmental quality and urban resilience. Here first year results are discussed.

Figure 1. REUSES project phases.

***Acknowledgments**: The REUSES project has been funded by the European Union-Next Generation EU–PNRR (Mission 4 • Component 2 • Investment 1.1 PRIN 2022–Project code: P2022M3ENS, CUP: I53D23007390001). The municipality of Ancona is acknowledged for the free loan of the two locations and the assistance in the cover removal. Ms. Lucia Vigoroso is acknowledged for her contribution to drafting the survey.*

Holistic assessment of ecosystem restoration strategies after a natural disaster

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***Keywords**: European Research Infrastructure, Ecosystem Restauration, Carbon Fluxes, Nature Based Solution*

In the last six years the combined attack of two pathogens (*Toumeyella parvicornis* and *Tomicus destruens*) has been fatal to all the stone pine (*Pinus pinea* L.) trees present in the Castelporziano Presidential Natural Reserve in Italy. The pines were over 100 years old and covered more than 250 ha in mostly monospecific stands. The removal of the dead trees left large open areas where different ecosystem restoration strategies can be applied, from reforestation to natural recolonization and different options for grazing control. The natural reserve already hosts an ICOS station and now, thanks to the collaboration between three European Research Infrastructures - ICOS, eLTER and LifeWatch - and boosted by the ITINERIS and NBFC Italian projects, five additional monitoring plots will be established. Three station plots have been already implemented and started to measure in mid-August 2024, while the other two are under implementation with the start of the measurements planned for spring 2025. The stations are all equipped with an eddy covariance system for CO_2, water and energy continuous exchange measurement. The different plots, covering each a different post-pine option, will also be the focus of additional monitoring activities on vegetation and soil characteristics, biodiversity evolution and hyperspectral and SIF local measurements. In this presentation the first preliminary results will be illustrated, together with the plan and the activities on going exploring the possible contribution from the scientific community. The data, collected in the context of the European Research Infrastructures, are open access and FAIR and will be fundamental for better evaluating and understanding different restoration options from a holistic point of view including carbon storage, water balance, plants and animal biodiversity, with a link to the remote sensing for their possible upscaling. All these will make the cluster site of Castelporziano a fundamental hub for Nature Based Solution strategies evaluation and selection.

FlorTree model: optimal tree species selection considering air pollution removal capacity in urban ecosystems

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***Keywords**: Air quality; Ecosystem services; Ornamental trees; Tree selection; Urban greening*

Atmospheric pollution, mainly caused by urbanization, is a threatening problem around the world especially in industrialized countries such as Europe and Asia. Among atmospheric pollutants, tropospheric ozone (O_3), nitrogen dioxide (NO_2) and particulate matter (PM2.5 and PM10), are the most dangerous affecting citizens' health. Urban trees can reduce the air concentrations of these pollutants thanks to stomatal uptaking and allowing dry deposition on their canopies. On the other hand, some species emit hydrocarbons (VOCs) such as isoprene and monoterpene that are O_3-precursors leading to air quality deterioration. For this reason, within AIRFRESH project (LIFE19 ENV/FR/000086), we developed FlorTree an innovative single-tree model to estimate the flux of air pollutants and select the best species for urban greening. FlorTree considers species-specific parameters such as tree morphology (height and crown leaf area), leaf/shoot structure, leaf habit (deciduous or evergreen) and physiological responses (stomatal conductance and VOCs emissions) to environmental factors. Hourly concentration data for air pollutants (O_3, NO2, and PM10) and meteorological parameters (temperature, solar radiation, relative humidity and wind speed) were recorded during one-year of observations and used as model input. In this study we compared the urban trees' removal capability in cities characterized by different pollution situations: two European (Florence, Italy and Bucharest, Romania) and an Asian one (Tokyo, Japan).

Predicting Ecosystem Functional Properties at ICOS sites with hyperspectral PRISMA data using machine learning: a comparison between random forest and extreme gradient boosting

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***Keywords**: Ecosystem Functional Properties; Carbon Fluxes; Vegetation Indices; Hyperspectral; Ensemble Learning*

A set of Ecosystem Functional Properties (EFPs), calculated from measured data collected at Eddy Covariance (EC) flux tower stations of the ICOS network, were related to Vegetation Indices (VIs) computed on hyperspectral PRISMA satellite data, with the general objective of testing the predictive capacity of VIs on EFPs, to provide a solution for the upscaling of ICOS tower information to larger spatial extents. To this purpose, a total of fifteen European ICOS sites belonging to one of five ecosystem types (including three forest types, grassland and wetland) was considered, yielding a total of 71 PRISMA images over the period 2020-2023.

Because EC techniques measure fluxes of carbon, water and energy, which are related to the physiological and hydroecological processes that influence the biogeochemical cycles, quantifying EFPs with EC measures allows for the biophysical assessment of ecosystem functions, thus providing relevant information about the state and behaviour of ecosystems. Five EFPs were obtained from measured carbon flux data at ICOS stations: gross primary productivity (GPP), net ecosystem exchange (NEE), light use efficiency (LUE), water use efficiency (WUE), and Bowen ratio (BW). A total of 29 VIs representative of vegetation properties relevant for the selected EFPs were identified based on a literature review. Prior to VI computation, some pre-processing steps were performed on PRISMA data to remove or improve noisy bands. Following calculation of the 29 VIs for each image, a 90 m radius buffer polygon built around the tower station – after checking for land cover homogeneity – was used to select VI pixels that fell within the buffer by at least 70% of their area, which were then averaged to obtain single average VI values for each image.

The predictive capacity of the VIs was tested using two ensemble learning approaches, namely random forests (RF) and XGBoost, which is the short name for eXtream Gradient Boosting, a novel learning algorithm proposed in 2016 that achieved state-of-the-art results on many machine learning tasks and data mining competitions. Through bagging and random feature sampling, RF reduces model variance typical of single or bootstrapped decision trees, while maintaining low bias and high predictive capacity. XGBoost is an implementation of a gradient boosted decision tree, or shorter gradient boosting, which could yield high accuracy in both classification and regression tasks. Additionally, XGBoost incorporates a regularized term in the objective function to reduce model complexity and prevent overfitting, which is very common for small datasets. Specifically, XGBoost can perform three major gradient boosting techniques, that is *gbtree* that uses tree-based models for each boosting iteration, *gblinear* that employs linear models, and *DART* (Dropouts meet Multiple Additive Regression Trees), which helps prevent overfitting by employing a dropout approach during training. In both approaches, modelling was optimized through hyperparameter tuning, which was evaluated applying cross-validation to the dataset. In RF modelling, to reduce redundancy among features, and favour model interpretability, a step-wise selection was performed on the set of predictors using R package VSURF, and then for each EFP an RF model was fitted using the selected predictors and optimized with hyperparameter tuning using R package caret. Results for the RF models show good predictive capacity of the VIs for GPP, NEE, LUE and BW, with R^2 varying between 0.61 (LUE) and 0.75 (GPP). WUE displays a considerably lower R^2 (0.31) and only two relevant VIs, which highlights a poor predictive capacity of the VIs for WUE. Considering all five RF models, only 15 out of 29 VIs have been identified as relevant predictors, underlining a high degree of correlation among most VIs. On the other hand, GPP, NEE, LUE and BW share five recurrent VIs (VOG, IRECI, CAI, OSAVI and NIRv), showing that combinations of few VIs of different types (broadband, narrowband, leaf pigment, dry carbon) provides a way to accurately predict EFP patterns along a spatial-temporal gradient and, most importantly, across different ecosystems types. For XGBoost, an emerging hyperparameter optimization framework was adopted, called OPTUNA, using a tree-structured Parzen Estimator; the tuned hyperparameters were then used for performance analysis. Results for XGBoost model, with *gbtree* booster, show an R^2 varying between 0.61 (GPP) and 0.33 (NEE), with the lowest value of -1.02 given by WUE. In the XGBoost model and DART booster, the highest R^2 value was achieved by VIs for GPP (0.62), followed by NEE (0.57), while WUE showed a value of -0.18, confirming the poor predictive capability of the VIs.

***Acknowledgements:** LN and DP acknowledge the project PRIN 2020 "MULTIFOR - Multi-scale observations to predict Forest response to pollution and climate change_PRIN_2020_LS9". GVL and DP acknowledge the PNRR, Missione 4, Componente 2, Avviso 3264/2021, IR0000032—ITINERIS—Italian Integrated Environmental Research Infrastructures System CUP B53C22002150006.*

The BRISMIC project: Interactions among landform, soil, vegetation, and microbiome during initial colonization stages in High Arctic patterned grounds

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***Keywords**: Patterned ground; High Arctic; Colonization; Microbiome; Vegetation; Soil*

Patterned grounds like sorted circles, frost boils and polygons are widely diffused in the High Arctic, where they are connected to the presence of permafrost. Their structure, development, and pedology have been thoroughly studied, while associated colonizing plants and microbiota are far less characterized, and their distribution in relationship to the developmental stages of the landforms not well known.

The BRISMIC project addresses these research needs by evaluating how plants and soil microbiota affect the properties of patterned ground soil and the development of permafrost-driven morphologies in two High Arctic ecosystems along a N-S gradient using latitude and exposure to the North-Atlantic Current as a proxy for climate/temperature change. To reach this goal, the sites at Brøggerhalvøya in Svalbard (78° 58' N, 11° 30' E) and Villum in Greenland (81º 36' N, 16º 39' W), have been selected. In summer 2024 we went to the study site at Brøggerhalvøya, on the southern coast of Kongsfjorden where the international research settlement of Ny-Ålesund is located, and characterized the presence of patterned ground systems with different degrees of plant and biocrust colonization: slightly, moderately and highly colonized. Hence, the study is aimed to assess the different level of plant colonization within a large study area covering the coastal plan of the Brøggerhalvøya along the Kongsfjorden and up to the Kongsfjordneset and to the westernmost point of the peninsula, the Kvadehuken. There, we started a sampling and on site measurement campaign targeting 1) plant species; 2) soil morphology; 3) soil physical properties (bulk density and structure); 4) soil organic matter content and its pools; 5) microbial community structure and activity in bulk and rhizosphere soil. In the next summers, we will complete the large surveying and sampling at Brøggerhalvøya and replicate the study at Villum, where the patterned system is less diverse. At present, we are still waiting for the delivery at controlled temperature of samples collected during the last summer field trip.

Finally, after completing field activities, linking soil physical, chemical and biochemical properties and functioning to the presence and activities of microbiota and plants in the two targeted sites will allow to identify the potential drivers of the early stages of colonization and soil development of patterned grounds in the High Arctic, where cyclic burial and exhumation of material is believed to play an important role in the soil carbon cycle.

Another objective is linked to the observation that sorted circles and frost boils are sites where initial colonization stages take place in undisturbed conditions. As a consequence of the rapid retreat of glacial fronts in the terrestrial High Arctic, increasingly large areas of new ice-cleared land are subject to colonization by specialized associations such as biocrusts and pioneer plants. This phenomenon, widespread throughout the Brøggerhalvøya, is amplified by the increase in temperature which makes the spread of the colonizing organisms faster and their metabolic activity and interaction with the mineral component more effective. On the front of retreating glaciers, such a colonization process is disturbed by the high instability of the terrain and hence it is practically impossible to understand in detail the colonization mechanism and distinguish its development stages. The patterned grounds that form in lowlands and other flat areas can therefore represent a model system to study the mechanisms of primary colonization and succession of the microbial and plant components in response to High Arctic warming.

***Acknowledgments**: The authors gratefully acknowledge the support of NBFC, Italian National Biodiversity Future Center (MUR-PNRR, Mission 4.2. Investment 1.4, Project CN00000033).*

The ICOS ecosystem site of Real Bosco di Capodimonte

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***Keywords**: NBS, ICOS CITIES, Climate, Greenhouse Gas measurements*

The experimental site of "Real Bosco di Capodimonte" is located within the city of Naples and cover an area of about 134 ha; the site is part of the monitoring network of ICOS (Integrated Carbon Observation System) infrastructure: continuous monitoring of greenhouse gases, various environmental and soil parameters are collected continuously to investigate the temporal dynamics of gas exchanges typical of urban vegetation:

The new eddy covariance tower reaches a total height of 30 meters and consists of a telescopic mast capable of lifting the instrumentation above the vegetation. The activities were made possible thanks to the new ten-year agreement between the CNR-IRET and the Management of the Real Bosco di Capodimonte. The purpose of the agreement is the development and maintenance of collaboration and the development of research projects, aimed at expanding knowledge, promoting and enhancing the results of the activities carried out in an urban environment.

The Capodimonte site, stands on a windy hill that dominates the entire Gulf of Naples and has an important historical, cultural and ecological value. The study area is characterized by a Mediterranean climate and vegetation mainly composed of *Quercus ilex* L., which occupies about 80% of the upper canopy layer. The other species that characterize the urban park are *Tilia platyphyllus*, *Quercus pubescens*, *Acer opalus* and *Ostrya carpinifolia,*

TECHNOLGIES FOR ECOSYSTEM RESEARCH: FO_3X – FREE – AIR O_3 EXPOSURE

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***Keywords**: Ozone; FACE; Infrastructure*

FO_3X is an advanced FACE (Free Air Controlled Exposure) system developed to investigate the effects of ozone (O_3) pollution on vegetation in realistic environmental conditions. Unlike experiments conducted in laboratories or closed chambers, FO_3X realizes the treatment of O_3 enrichment with minimizing effects on meteorological factors inside experimental plots where plants grow. This is particularly critical for studying stomatal flux, the primary pathway through which plants absorb ozone, as it is influenced by environmental conditions such as temperature, humidity, wind speed and solar radiation. The FO_3X facility incorporates state-of-the-art technology to ensure accurate data collection and controlled ozone exposure. A Campbell Scientific CR1000 data-logger, coupled with a multiplexer, manages a meteorological station equipped with multisensory instruments positioned at a 3 m height. These instruments continuously measure essential meteorological parameters, including wind speed and direction, air temperature, relative humidity, solar radiation, and atmospheric pressure. Spatial ozone concentrations within the experimental plots were assessed using diffusive passive samplers, ensuring homogeneity with a concentration variability below 20%. Such precision ensures the reliability of results and the accurate replication of field-like conditions for plant research. Thanks to a collaboration with CNR - IBE, the facility will be expanding its capabilities with the integration of Temperature Free Air Controlled Enhancement (T-FACE) technology. This new system will allow researchers to study the combined effects of ozone exposure and elevated temperatures on plant ecophysiology, addressing a critical gap in understanding how plants respond to simultaneous environmental stressors. By simulating these conditions in an open-air environment, FO_3X aims to provide comprehensive insights into the impacts of ozone pollution and climate change on vegetation, contributing to the development of mitigation strategies and more resilient ecosystems. This innovative approach positions FO_3X as a cutting-edge tool for ecosystem research, offering a unique platform to study plant-environment interactions under realistic conditions and advancing our understanding of the complex interplay between atmospheric pollutants and global climate change.

A FAIR and User-Friendly Web Application for Democratizing Research on Zoosporic Parasites in Aquatic Systems.

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***Keywords**: Parasite, Fairness, Database, Web Application, Interoperability*

The ParAqua web application is an innovative platform developed through COST Action CA20125, in collaboration with CNR IRET Lecce and LifeWatch Italy. It is dedicated to advancing the understanding and management of zoosporic parasites in aquatic ecosystems. These aquatic microorganisms are critical ecological players that influence host populations, aquatic food webs, and community diversity. They also pose significant challenges to the algal biotechnology industry, causing substantial economic losses in the production of food ingredients, biofuels, and nutraceuticals. The application serves as a bridge connecting academia, industry, and water management authorities to collaboratively address these challenges.

The user-friendly web application makes accessible the data coming from Working Group 1 (WG1) and Working Group 2 (WG2), focusing respectively on the occurrence of zoosporic parasites and the environmental drivers influencing their dynamics. ParAqua database integrates in situ observations, genetic data from NCBI, and environmental parameters from literature.

Key features of the web interface include advanced query functionalities, data visualization, and export options, designed to meet the diverse needs of researchers, industry professionals, and policymakers. The application was built to align with the requirements of the ParAqua community and ensure usability.

Challenges addressed include data mobilization from heterogeneous sources, standardization of metadata, and ensuring compliance with FAIR data principles using controlled vocabularies to standardize data and enhance semantics.

The resulting web application embodies the principles of open science by promoting transparency, reusability, and interoperability through an open interface and RESTful API services. By overcoming these challenges, the application facilitates robust meta-analyses, fostering insights into host-parasite dynamics and their implications for ecosystem management and industrial algae production.

This web application not only serves as an important resource for the ParAqua community but also establishes a framework for broader application in biodiversity and ecological informatics. It exemplifies the role of open science in advancing knowledge exchange, supporting interdisciplinary research, and enhancing the impact of scientific discoveries through accessible and reusable data infrastructure.

***Acknowledgments**: This publication is based upon work from COST Action 20125 - Applications for zoosporic parasites in aquatic systems (ParAqua), supported by COST (European Cooperation in Science and Technology).*

Analytical Workflow to Study Ocean Production Response to Global Warming

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***Keywords**: Phytoplankton Biomass, Net Primary Production, Sea Surface Temperature, Climate Change*

Ocean primary production is a critical ecosystem function that maintains energy flow and biomass production in aquatic ecosystems and plays a key role in climate change mitigation. This process is primarily driven by the concentration and growth of phytoplankton, which form the base of the ocean food chain in aquatic ecosystems. However, phytoplankton photosynthesis is highly sensitive to temperature, making it vulnerable to the effects of global warming. This can have a major impact on biomass and primary production and on the ability of the ocean to act as a sink for CO2. The aim of this research was to investigate the variation of phytoplankton biomass and processes, using Chl-a as a proxy, and Net Primary Production (NPP), related to sea surface temperature (SST). The study covered 20 years and focused on the equatorial zone, where ocean warming has a major impact. The study followed an analytical workflow integrating field measurements of SST, Chl-a and NPP, available in online repositories and acquired by WP6 of the ITINERIS project, with remote sensing imagery. Firstly, different machine learning algorithms were trained and tested using field data of SST, Chl-a and NPP, showing that random forest was the best model to capture the patterns of variation between the variables used. Secondly, the Random Forest model, trained with field measurements, was applied to MODIS SST imagery to generate Chl-a and NPP time series from 2003 to 2023. The moving average applied to the SST time series showed an increasing trend, while the moving average applied to the Chl-a and NPP time series showed decreasing trends, with strong temporary peaks of decreasing values. Kendall's test showed that the trends in SST, Chl-a and NPP were statistically significant. The recurrence plots of the Chl-a and NPP time series showed that the strong temporary peaks correspond to perturbations in the system evolution, possibly related to the El Niño-Southern Oscillation (ENSO). Therefore, the constructed analysis model allows us to highlight long-term changes in Chl-a and NPP caused by global temperature increases and short-term temperature change effects caused by single perturbation events such as ENSO. Finally, the "Seasonal Autoregressive Integrated Moving Average" applied to the time series allows the production of new forecast scenarios. The analytical workflow could be a useful tool for estimating the potential impact of temperature changes on phytoplankton biomass and processes at long and short temporal scales. The methodology thus allows researchers working with field or laboratory measurements to extend their studies to broader spatial and temporal scales, providing economists and planners with insights that can be incorporated into future scenario building and reused and adapted for other applications and case studies.

***Acknowledgements:** project IR0000032 – ITINERIS, Italian Integrated Environmental Research Infrastructures System (D.D. n. 130/2022 - CUP B53C22002150006) funded by EU - Next Generation EU PNRR- Mission 4 "Education and Research" - Component 2: "From research to business" - Investment 3.1: "Fund for the realization of an integrated system of research and innovation infrastructures".*

Infrastructure improvement at IRET-CNR Institute of Montelibretti financed by ITINERIS project

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***Keywords**: Instruments; VOC; Gas Exchange; Climate Change*

Keeping pace with the new frontiers of research in the field of environmental sciences is a challenging task that the Research Institute on Terrestrial Ecosystems has been addressing for years. Recently, the opportunity to apply for PNRR fundings has enabled IRET to make significant investments in local infrastructures, enhancing both the quality and impact of ongoing scientific research activities. The acquisition of state-of-the-art instruments will facilitate deeper investigation into plant responses to both biogenic and anthropogenic disturbances, critical factors in the climate change era we are currently navigating. Gas exchange parameters, including net photosynthesis, will be analyzed via Li-COR LI-6800, which replaces the older LI-6400XT model. THE LI-6800 offers faster data acquisition, improved portability and extended configuration options. Its accessories also allow monitoring of soil respiration, the respiration rates of small organisms (e.g., insects), and the photosynthetic rates of both evergreen and broadleaf species.

Stress indices in higher plants will be assessed through the new High Resolution Spectroradiometer ASD FieldSpec 4 which is designed for faster, more precise spectral data measurements across a wide range of remote sensing applications. Its spectral performance spans the full solar irradiance spectrum (350–2500 nm), enabling the evaluation of NDVI, PRI, WI, NDWI, ARI, and CRI indices. These indices provide insights into functional traits of photosynthetic pigments (e.g., chlorophyll, carotenoids and anthocyanins), as well as leaf structure and water content. Data collected directly from plants using both LI-6800 and the spectroradiometer offer valuable clues about plant health.

To gain a more comprehensive understanding of climate change, monitoring the emission of the major greenhouse gases (GHGs) such as CO_2, CH_4 and H_2O is crucial. For this purpose, the LICOR LI-7810 has been added to our equipment portfolio. This instrument enables high-precision, long-term monitoring of CO_2, CH_4 and H_2O emissions and can be mounted on a backpack for real-time field data acquisition in both static and dynamic configurations. Additionally, the LI-7810 supports data acquisition in soil experiment designs. Up to 16 LI-7810 units can be interconnected to continuously monitor CO_2, CH_4 and H_2O emission rates over larger areas.

Another fundamental aspect to study in higher plants involves the emission of Organic Volatile Compounds (VOCs). These compounds are emitted by plants either as results of ordinary metabolic process, in response to stress conditions or as signaling mechanisms directed toward other plants or animals. In all cases, VOCs play crucial roles in terrestrial ecosystems. To support this line of research, the latest investment in instrumentation includes the installation of Agilent Gas Chromatography/Mass Spectrometry (GC/MS) system, equipped with a hydrogen gas generator for *in-situ* hydrogen carrier gas production. The instrument is also fitted with a Markes autosampler capable of handling up to 100 tubes for continuous analysis. This new setup allows for the analysis of numerous samples within a few hours, including the possibility of overnight programming, significantly speeding up the process. Compared to the older GC/MS system, this system eliminates the need for helium, which is neither cost-effective nor environmentally sustainable. Liquid-phase injections are also supported, enabling the analysis of lab-derived extracts.

In conclusion, the substantial investment made to acquire the aforementioned instruments will enable IRET-CNR to play a pivotal role on the international stage in the field of the Environmental Sciences.

***Acknowledgements**: PNRR, Missione 4, Componente 2, Avviso 3264/2021, IR0000032—ITINERIS—Italian Integrated Environmental Research Infrastructures System CUP B53C22002150006*

FAIRer, Better, Faster, Stronger

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***Keywords**: Research Infrastructure; e-Science; data; Open Science; FAIR*

LifeWatch Italy is the Italian node of the European Research Infrastructure Consortium, LifeWatch ERIC, the distributed e-Science Infrastructure for biodiversity and ecosystem research. Its primary aim is to develop and maintain digital solutions for the FAIR and open management of digital research products. Such products come from our scientific community in various types, ranging from research data, to scripts, training materials, and others. Thanks to the PON-IR "LifeWatchPLUS" project, LifeWatch Italy has significantly enhanced its infrastructure to effectively managed this diverse set of products, now offering a comprehensive toolkit to support scientists throughout the entire research cycle (Figure 1).

The first step of the cycle involves the review and validation of (meta)data, verifying syntax and taxonomy, assured by the alignment against curated taxonomic backbones (Italian checklists, Catalogue of Life, WoRMS, GBIF). The Semantic Platform allows annotating metadata, variables, and datasets, employing semantic technologies to enable data discovery and reuse. The Data Portal and Metadata Catalogue provide access to data and associated resources, while the integration with DataLabs facilitates collaborative code creation for biodiversity and ecosystem data analysis. Furthermore, a dedicated platform has been developed to store and provide access to citizen science projects and their outputs. The integration with a helpdesk and the e-training platform ensures user support and training, enhancing the accessibility and usability of LifeWatch Italy's digital tools and services.

The development of such an infrastructure is not a process with a defined start and end, but rather a continuous endeavour. Indeed, efforts are already underway to keep it aligned with emerging technologies and initiatives. Current activities include restructuring the model behind the semantic platform, and mapping metadata profiles to ensure compliance with the European Open Science Cloud (EOSC). In the meanwhile, we actively supported the scientific community in mobilising their data. The next challenge will be to ensure the continuous evolution and functionality of LifeWatch Italy. This requires not only technological updates but also sustained community engagement and dedicated personnel to provide long-term support and foster adoption within the research community.

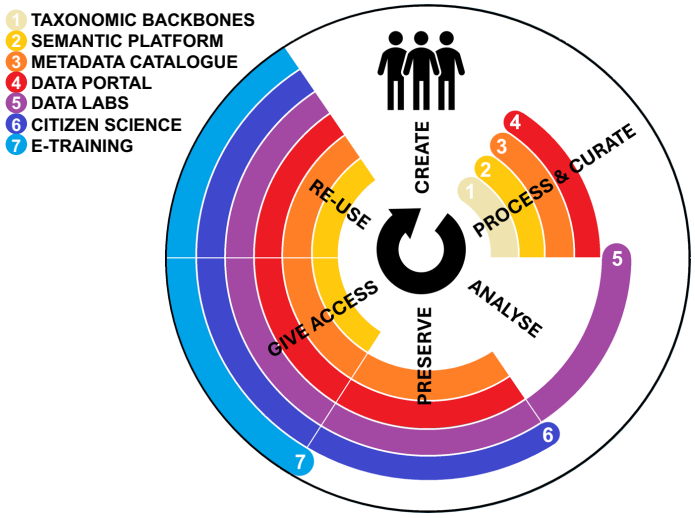

Figure 1. Exemplification of how the components of the LifeWatch Italy infrastructure fulfil the data lifecycle management steps.

***Acknowledgements**: This work has been supported by the PON-IR "LifeWatchPLUS" (CIR01_00028; PIR01_00028) and Next Generation EU Mission 4 "Education and Research" – Component 2: "From research to business" – Investment 3.1: "Fund for the realisation of an integrated system of research and innovation infrastructures" – Project IR0000032 – ITINERIS – Italian Integrated Environmental Research Infrastructures System – CUP B53C22002150006.*

Enhancing Ecosystem Recovery: The Role of Digital Plant Phenotyping in supporting Nature-based Solutions

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***Keywords**: Digital Plant Phenotyping, Plant stress, Ecosystems services, Real time monitoring*

The structural, physiological and biochemical characteristics of vegetation are crucial for understanding the biodiversity, functioning, and overall health of ecosystems. As the foundation of terrestrial ecosystems, plants play a central role in maintaining ecological balance, often acting as the most visible indicators of local biodiversity and its associated benefits. They contribute significantly to ecosystem services (ES), including the production of food and energy, the sequestration of atmospheric carbon dioxide, and the mitigation of environmental pollutants in air, soil, and water through processes like phytoremediation and ecosystem restoration. However, the growing issue of soil and water salinity, along with widespread salinization, have become a critical global challenge. This is especially problematic in arid and semi-arid regions, such as the Mediterranean, where factors like rising sea levels, the use of poor-quality irrigation water, increasing temperatures, and climate change exacerbate the problem.

Integrating the Digital Plant Phenotyping (DPP) technology into Nature-based Solution (NbS) enhances their effectiveness by identifying key biological traits in plants or organisms that can optimize ecosystem restoration. This tool is a high-end non-destructive acquisition system especially useful in projects like phytoremediation, where specific plants are chosen to clean contaminated soils or water by absorbing harmful substances. By matching species to specific ecological tasks, DPP ensures that NbS are targeted and efficient, leading to more successful outcomes in restoring and protecting ecosystems. Digital phenotyping, where technology and agriculture merge to monitor crop health and optimize growth through data-driven approaches like drones, sensors, and real-time analysis, can be a key resolution tool.

The DPP technology has been applied to evaluate salinity stress on Mediterranean maquis plants This research provides fundamental insights into the increasing salinity resulting from sea level rise, a contemporary issue arising from climate change. This study presents methods and preliminary findings on plant responses and functions under saline stress, focusing on Mediterranean scrub and environments (Fig.1). Specifically, gymnosperms (e.g., *Pinus pinaster*, *P. halepensis*, *Cupressus sempervirens*) and evergreen angiosperms (e.g., *Myrtus communis*, *Arbutus unedo*) were assessed for salt resistance during a six-month experiment conducted under semi-controlled greenhouse conditions.

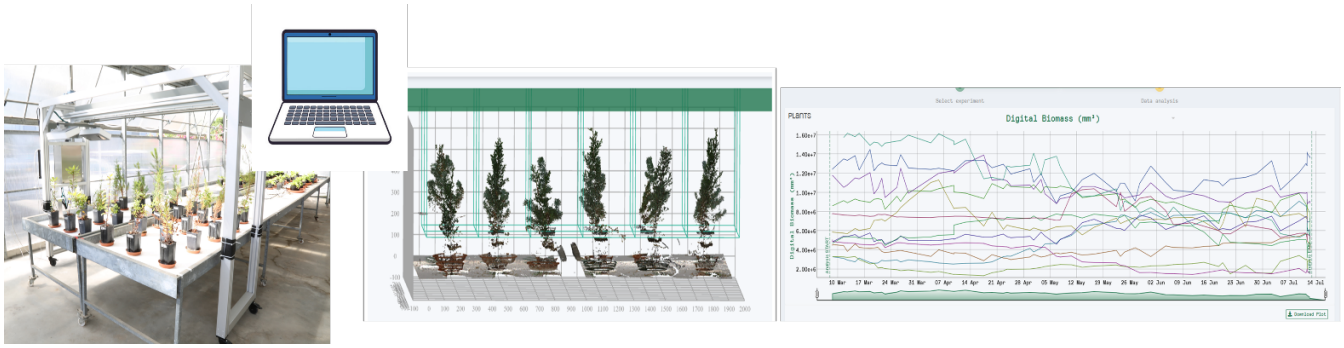

Figure 1. Experimental set up and Digital Plant Phenotyping: 3D imaging, data acquisition and elaboration

***Acknowledgments**: This work is part of Activity 5, 'Conceptual Framework and Methodological Tools of Nature-based Solutions and Restoration Ecology,' within Spoke 4, which focuses on ecosystem function, services, and solutions as part of the National Biodiversity Future Center (Piano Nazionale di Ripresa e Resilienza, PNRR project).*

Morpho-physiological and gene expression analyses in *Lepidium sativum* plants exposed to drought stress, heat stress and their combination

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***Keywords**: Heat stress, drought stress, transcriptomics, plant physiology, photosynthesis*

Global warming and extreme events associated with climate change are causing temperatures to rise steadily, altering seasonal rainfall and exposing ecosystems to the combined effects of drought and heat waves. Previous studies have investigated the impact of drought and heat stress on the development of different plant species revealing the activation of specific physiological responses and detailed signaling pathways to cope with these stresses; however, several aspects remain still unclear such as how plants face to the combined drought and heat stress, which often occur simultaneously.

The garden cress (*Lepidium sativum* L.) is a little fast growing Brassicaceae, cultivated for nutritional and medicinal purposes, and used as a reference plant species for ecotoxicological assays. L. sativum has been extensively studied to evaluate the effect of organic and inorganic pollutants on plant development; however, little is known about the impact of climate change-related stresses such as drought, heat, cold and salinity, on this plant. To date, a few authors described the effect of drought stress or heat stress on L. sativum development, while studies on combined drought and heat stress are still missing.

To fill this gap, we monitored overtime morphological, physiological and molecular responses of L. sativum plants exposed to water shortage and high temperatures, both individually and in combination. Our results showed that after 6 and 9 days of water shortage L. sativum plants had lower stomatal conductance and impaired biometric traits, such as fresh weight, leaf area and leaf length, compared to controls. After 24 hours of heat stress treatment at 35°C and 40°C, stomatal conductance was higher while the photosynthetic efficiency was decreased in L. sativum plants due to the repression of photosystem II (PS II) activity. The combination of both stresses increased the severity of all morpho-physiological effects. Interestingly, RNA sequencing revealed 4656 drought stress de-regulated genes (DEGs) compared to 5506 heat stress DEGs. The most enriched GeneOntology (GO) terms for drought stress were "response to water", "response to water deprivation", and "response to abscisic acid (ABA)", whereas for heat stress were "response to heat", "response to temperature stimulus" and "response to jasmonic acid (JA)". We also found common DEGs for both drought and heat stress, such as genes involved in the regulation of the ROS-induced molecular stress response. Similarly to Arabidopsis thaliana L. and *Solanum lycopersicum* L, we found the activation of specific pathways, such as the ABA signalling pathway, mainly during drought stress responses, and the engagement of the blue light-induced components mostly during heat stress responses. Interestingly, these pathways are involved in mechanisms regulating stomata closure and opening, in agreement with physiological data.

Altogether, our findings provide a detailed analysis of morpho-physiological effects of drought stress, heat stress and their combination on L. sativum development. Results from transcriptomic analysis showed that, like in A. thaliana, drought and heat stress elicit similar but not overlapping pathways. The identification of morpho-physiological traits and stress-responsive regulatory factors, triggered by the combination of drought and heat stress is critical for the selection of stress tolerant plant varieties, which are pivotal for adaptation to climate changes.

***Acknowledgments**: Supported by Comune di Viterbo in the framework of "Programma di interventi per l'adattamento ai cambiamenti climatici in ambito urbano" con avviso pubblicato su GU n. 135 del 08/06/2021, con DD n. 117 del 15 aprile 2021" – CUP: D81B21004740001 Funded by Ministro dell'Ambiente e della Sicurezza Energetica (ex MiTE).*

New indicators for the selection of plants adapted to saline environments: combining High-Throughput digital Plant Phenotyping with morpho-physiological and biochemical analyses in sustainable water management

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***Keywords**: Extreme environments, Saline waters, Plant phenotyping, Functional biodiversity, Stress indicators*

The global area of salt-affected soils is more than 833 million hectares, and more than two thirds of this is found in arid and semi-arid climatic zones, including the Mediterranean. There, the salinization and sodification of soils and waters in cultivable lands is increasing by 10% and annually inhibits plant growth and productivity worldwide. In such conditions, most of soils and waters is reaching a saturated hydraulic conductivity exceeding 4.0 dS m−1 (≈40 mM NaCl), which significantly impacts plant growth and development. This phenomenon is aggravated by the effects of climate change (high temperature and increased evapotranspiration rate) leading to water scarcity (< 500 m3 per year per capita). In this scenario, possible solutions to mitigate or solve the increasing global salinization and water scarcity are: i) the identification of renewable water sources; ii) the selection of tolerant plants to drought and salinity.

In this work, we used a model crop (*Lactuga sativa* L.) to test the effects of poor quality waters available for irrigation in agroecosystems of arid and semi-arid regions. With this aim, we used waters having the same characteristics of non-conventional waters (NCW) used for agriculture, that is 1) treated wastewaters following its use for domestic, municipal and industrial purposes or 2) saline waters from groundwater, drainage and surface sources. They were "reconstituted" waters, highly saline (EC> 25 dS m-1) and containing metals such as copper and zinc, simulating those typically widespread in intensive agriculture systems. The main morpho-physiological parameters (growth, photosynthesis) were monitored in vivo thanks to an automated plant phenotyping system (MicroScan-PlantEye F600 system, Phenospex, Heerlen, The Netherlands) and validated with non-destructive and destructive measurements during and at the end of the growing season (about four weeks). Destructive chemical determinations on mature plants (mineral composition, pigments, antioxidants) and soil (physical-chemical properties) completed this investigation. Results were a series of new and easy-handling indicators for the selection of plants/crops adapted to be grown/cultivated in adverse environments (some examples in Fig. 1), while maintaining important water-dependent ecosystems and ecosystem services.

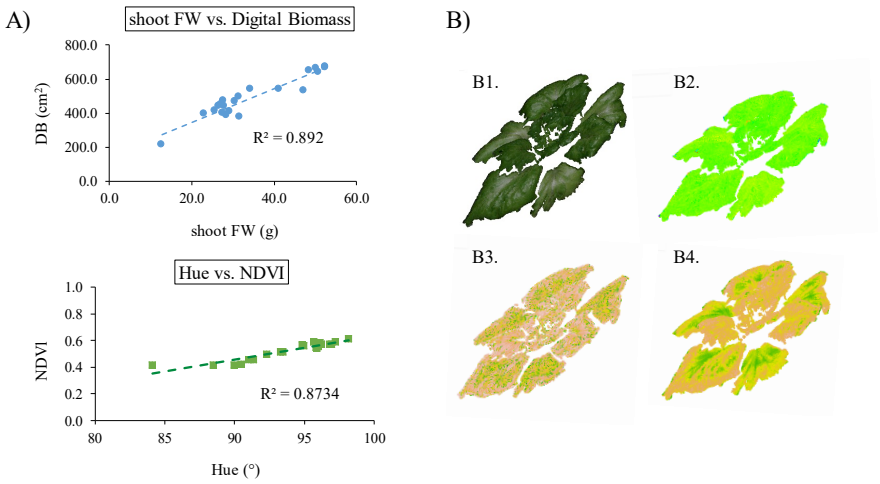

Figure 1. A- Relationships between some measured plant traits (e. g.: fresh weight, FW) and morpho-structural and physiological parameters in *Lactuga sativa* L., var. *Canasta*, irrigated with saline waters. B- Spectral 3D images (point clouds) generated by a single scan and processed with the software HortControl (Phenospex, Heerlen, The Netherlands). Each point within the cloud contains information on the position (X, Y, Z coordinates) as well as the reflection of Red (620-645 nm), Green (530-540 nm), Blue (460-485 nm) and near-infrared (820-850 nm) wavelengths. Based on this information, RGB colour (B1), Hue (B2), NDVI (B3) and GLI (B4) are visualized. Hue=visible colour dependent on the light wavelength being reflected, NDVI= Normalized Difference Vegetation Index, GLI= Green Leaf Index

Eco-physiological and growth characters of woody plant species in determining the potential of traditional agroforestry practices as nature-based carbon sinks in Mediterranean area

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***Keywords**: Biodiversity, Agroforestry, Carbon sink, Ecophysiology*

Carbon farming is a capable strategy for more sustainable production of food and other related products. According to the food and agriculture organization (FAO), agriculture, forestry, and other land-use practices account for 24% of global greenhouse gas (GHG) emissions and total global livestock emissions of 7.1 gigatons of CO2-equivalent per year, representing 14.5% of total anthropogenic GHG emissions. An agroforestry system that deliberately well integrates trees and crops with livestock in agricultural production can increase carbon sequestration and decrease GHG emissions from terrestrial ecosystems, thus helping to mitigate global climatic change. There is growing interest in Climate smart land uses as integrate solutions to rising GHG emissions, given their potential to store additional carbon in terrestrial ecosystems, while also enhancing livelihoods and biodiversity. However, substantial high uncertainty remains around how much carbon can be captured into the soil and standing vegetation, and how that varies by site conditions (climate and soil) and by practice. The tree components in agroforestry systems can be important sinks of atmospheric carbon due to their fast growth and high productivity. It has the ability to enhance the flexibility of the system for coping with the unfavorable impacts of climate change. These systems suggest important opportunities for creating synergies between both adaptation and mitigation behavior. Thus, although agroforestry systems seem to have high potential for C sequestration, significant effort is essential in realistically assessing the extent of this potential. The responsibility of agroforestry practices in climate change mitigation and adaptation is required to realize the potential next to overcoming various components of current and future international climate mitigation policies. In order to study the potential of the agroforestry system as a “nature-based carbon sink,” we have established the planting of an agroforestry system consisting of Olea europaea L. (var. leccino and canino) and Cupressus sempervirens L. (var. stricta and horizontalis), interspersed with the presence of vicia faba L. (as an herbaceous crop), at the Santa Paolina experimental farm, located in Follonica (GR). This agroforestry system, established as part of the project “Nature-Based Business Model and Emerging Innovations to enhance Carbon Farming Initiatives (CFIs) while preserving Biodiversity, Water Security and Soil Health” (INNO4CFIs), will be subjected to water and salt stress. This study will allow us to observe how the growth and ecophysiological characteristics of the forest species determine the potential of agroforestry systems as carbon sinks, under abiotic stress.

Low $\delta^{13}C$ variability and high vessel density reveal anisohydric olive cultivar as most drought-resilient

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***Keywords**: Drought resilience, olive cultivars, dendrochronology, carbon stable isotope, xylem anatomy*

The olive tree (*Olea europaea* L.), widely cultivated in Mediterranean climates, is increasingly impacted by climate variability. This study examined the adaptive responses of three widely cultivated olive cultivars, Arbequina, Arbosana, and Koroneiki, to seasonal climate variations between 2020 and 2023 in central Italy. We employed dendrochronological methods, wood anatomical analyses, and high-resolution $\delta^{13}C$ profiling of tree rings (Figure 1) to investigate how these cultivars respond to changes in temperature and precipitation, and how these responses affect growth and yield.

Results revealed clear differences in climate sensitivity and physiological strategies among the cultivars. Arbequina and Arbosana exhibited isohydric behavior, characterized by strong stomatal control in response to water deficits. Both cultivars showed reduced growth in drier years, with Arbequina's growth and $\delta^{13}C$ values correlated to spring precipitation and summer temperatures. Arbosana showed the highest $\delta^{13}C$ variability, indicating marked sensitivity to both precipitation and temperature fluctuations. In contrast, Koroneiki displayed a more anisohydric strategy, maintaining stable growth and hydraulic efficiency under fluctuating climatic conditions. Its $\delta^{13}C$ values were significantly influenced by the previous year's winter precipitation, suggesting a reliance on carbon reserves for spring growth, a trait that may offer an advantage in less predictable climates.

The wood anatomical data supported these findings, with Koroneiki exhibiting higher vessel density and a greater proportion of lumen area, enhancing its water transport capacity. This, coupled with its consistent productivity, makes Koroneiki the most resilient cultivar under the variable Mediterranean climate. The integration of tree-ring, anatomical, and isotopic data underscores the importance of selecting cultivars with differing water-use strategies to ensure sustainable olive production in the face of climate change.

Figure 1. Cross-section of an olive tree branch from the cultivar Arbosana. The shots (100 µm in size and spaced 300 µm apart) were created by laser ablation coupled with an isotope ratio mass spectrometry system for $\delta^{13}C$ pattern analysis.

***Acknowledgments**: This work was supported by the CNR-Short Term Mobility Programm (STM) 2023 "Dendro*ecological analyses for the characterization of olive cultivars" (SAC.AD002.045) and MUR-PRIN 2022 project "Varietal selection and biostimulant use in olive growing to face climate change" (DTA.PN011.007 20227MNHKN_LS9).

Urban vegetation and air pollution: dealing with particulate matter deposition, physiological and molecular responses in plants grown in a green wall in Rome. The study case of Villa Leopardi

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***Keywords**: Green wall, Green infrastructure, Air pollution, Gene expression, Plant physiology*

Air pollution is steadily increasing due to rapid urbanisation and industrialisation and is one of the major causes of mortality related to cardiovascular and respiratory diseases. Reducing urban air pollution has become one of the main challenges faced by policymakers worldwide. Green Infrastructures (GI), such as the green walls, are considered as a win-win solution to address air pollution, improving the health and well-being of urban populations. However, previous studies on the impact of GI on air quality have led to conflicting results due to the large number of variables encountered in urban environments. Vice versa, there is limited knowledge regarding the responses induced in plants of GI exposed to urban air pollution.

In this framework, the focus of our work was to evaluate the physiological and molecular responses of plants grown nearby urban traffic-congested road to particulate matter (PM) mainly released by vehicular traffic (exhaust and non-exhaust emissions such as vehicle brake abrasion) and soil resuspension.

To this end, a green wall containing 12 different plant species was assembled along one of the most trafficked streets in Rome (Italy), near via Nomentana. Leaves were collected monthly (from July 2023 to January 2024) and analysed for several elements including those tracers of brake and soil dust in the PM deposited on the leaves and accumulated in the plant tissues, to evaluate the capacity of plants to retain PM. Almost all plant species in the green wall retained PM from non-exhaust traffic and soil dust on their leaves. Among plants, *Photinia x fraseri* and *Pteris vittata* showed interesting and different deposition patterns onto leaves surface for each element. Their deposition exhibited a "seasonal" trend for *Pteris vittata*, with higher values recorded between July 2023 and October 2023, followed by lower values in the winter months. On the other hand, *Photinia x fraseri* showed a steady state trend for all elements throughout the sampling period. These two plant species were further analysed to get insights into the physiological changes and gene expression variation correlated with the deposition of PM.

The analysis of photochemical indices, such as maximum quantum yield of PSII (Fv/Fm), Quantum Yield (QY), photochemical reflectance index (PRI), carotenoid reflectance index (CRI1), and anthocyanin reflectance index (ARI), revealed that both plants experienced mild stress, but their photosynthetic apparatus remained functional all over. Furthermore, *Photinia x fraseri* exhibited the lowest values for carotenoid content, while *Pteris vittata* revealed the highest anthocyanin production rate in autumn, in agreement with the "seasonal" deposition trend of some elements in PM.

Genes analysis was focused on the expression of abiotic stress-marker genes, including superoxide dismutase (*SOD*) and ascorbate peroxidase (*APX*), and cellulose synthase (*CeS*) and beta-galactosidase (*βGal*). In both plant species, *SOD* and *APX* gene expression decreased during the winter months compared to the summer, except for *Pteris vittata*, which showed an upregulation peak for both genes in January 2024, when less PM deposition was detected. On the other hand, *CeS* and *βGal* expression was mainly similar in both plant species for the whole sampling period, with up-or downregulation peaks between March 2024 and May 2024 depending on the species. These results lead us to speculate the presence of an acclimation process, but further studies are required to understand how much the deposited PM affects plant development, in comparison to the season and temperature conditions.

Our results suggest that both *Photinia x fraseri* and *Pteris vittata* could be used as a biomonitoring plant of PM from urban sources, in particular *Photinia x fraseri* showed more resilience to seasonal stresses revealing a more constant ability to retain metals on its leaf surface without showing signs of suffering.

***Acknowledgments**: The project was funded under the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.4-Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union- NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022, adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center-NBFC".*

New vegetables rennet development for vegetarian cheeses production

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***Keywords**: Cheese making, vegetables rennet, flavour development, vegetarian cheeses*

Cheese making is an ancient practice to preserve perishable food such as milk for a long time. The first phase of cheese making involves the addition of rennet of animal origin, which contains the enzymes necessary for the hydrolysis and coagulation of milk caseins, and for cheese ripening (mainly lipase/esterase).

The proposed technology concerns the production of cheeses using rennet from vegetables sources, i.e. replacing the enzymes involved in milk caseins coagulation and cheeses ripening, with enzymes of vegetable origin. In this way, vegetarian cheeses are obtained. Various vegetables have been selected and tested for this purpose, in particular: cardoon (*Cynara cardunculus*) , artichoke (*Cynara cardunculus* var. *scolymus*), papaya (*Carica papaya* L.) , pineapple (*Ananas comosus* (L.) Merr.), mushrooms (*Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm.) and fig milky sap (*Ficus carica* L.).

In all cases, cheeses were obtained, and as observed by the analysis of some profiles of volatile substances released, they showed interesting characteristics. In particular, the artichoke, cardoon, and thistle mushroom extracts showed high proteolytic activity compared to calf rennet, while the level of esterase activity appeared to be similar for all the extracts. The papaya extract showed the lowest proteolytic and esterase activity. Although the pH, moisture, fat, and protein contents were very similar to those of cheese made with calf rennet, the medium- and long-chain FFAs broadly differed among produced cheeses, with variations in the lipid quality indices.

***Acknowledgments:** Prof. Severina Pacifico and Simona Piccolella for the sensorial analysis (Department for Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100 Caserta, Italy).*

Tavole rotonde tematiche “Interazione e integrazione in IRET”

The Role of Advanced Technologies in Ecosystem and Biodiversity Monitoring

Chairs: Alessandro Mei[1] (CNR-IIA); Alessio Collalti[2] (CNR-ISAFOM)

[1]National Research Council, Institute on Atmospheric Pollution, [2]National Research Council, Institute for Agriculture and Forestry Systems in the Mediterranean

In recent years, the application of advanced technologies has increasingly enabled the effective and innovative monitoring of ecosystems and biodiversity. The quality of research and the networking opportunities among institutions can be enhance through a multidisciplinary and cross-sectoral approach. Emerging technologies provide innovative tools for analyzing marine and terrestrial ecosystems and studying the relationships between humans and their habitats.

The roundtable on advanced technologies for environmental monitoring brought together approximately thirty IRET personnel working in different parts of Italy, each with highly diverse technological approaches to various research topics. Among the main priorities of the discussion were fostering interaction among IRET researchers, technologists, and technicians with overlapping research interests but limited collaboration, stimulating active participation, and laying the groundwork for long-term partnerships. The goal was to develop a broad vision that extends beyond the Institute's boundaries, leveraging collective expertise and experiences.

During the initial phase of the roundtable, an exploratory survey was conducted on the innovative technologies used by the attendees. Participants described their backgrounds, location of their IRET branch, research interests, and methodologies employed in their work. The key application domains identified included "forests", "urban environment", "habitats", "soil", "marine ecosystems", "terrestrial ecosystems", "biodiversity", and "grasslands". As for the most widely used technologies, the following emerged as the most relevant: "satellites", "sensor technologies", "molecular analysis", "spectroscopy", "physical analysis", "data transmission", "modeling", "isotopic analysis", "chemical analysis", "GIS", and "drones/aircraft".

While several emerging technologies were highlighted, particular emphasis was placed on sensor technologies, satellites, modeling, drones, and artificial intelligence. However, there was a general acknowledgment of the importance of integrating multiple approaches, whether advanced or traditional, to maximize effectiveness. Earth observation, forest and climate management, habitat conservation, as well as ocean and pollution monitoring, are just some of the areas where the Institute can benefit from a multidisciplinary technological integration.

Nevertheless, the implementation of innovative technologies raises several challenges. These include limited access due to high costs, the need for cross-disciplinary collaborations to compensate for technical shortcomings, and the necessity of data standardization to ensure interoperability between different systems. Establishing partnerships that focus on key topics should facilitate the integration of cross-sectoral expertise, thereby renewing established approaches and addressing future challenges with increasing efficiency.

Ecosystem conservation and restoration (best practices, applications, and health impacts)

Chairs: Diego Fontaneto[1], Gianfranco Peluso[2]

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The meeting began with a presentation prepared by the moderators. They briefly introduced their expertise and the history of their research activity. Subsequently, an overview of the general changes occurred in the National Research Council (CNR) over the past decade was provided, with a particular focus on the post-COVID period and the implementation of PNRR projects, e.g., NBFC and ITINERIS. The discussion highlighted the distinctive features of these projects, which have undeniably fostered greater interaction among colleagues from different CNR Institutes.

Following this, additional national and European activities and projects were introduced, with the aim of creating critical mass on specific research topics. Before concluding the introductory phase, the activities of several working groups (GdL) within the Department of Earth System Sciences and Environmental Technologies (DSSTTA, https://dta.cnr.it/gruppi-di-lavoro/) were described, with particular attention to the Biodiversity GdL and the Nature, Research, and Society GdL, as well as the newly established interdepartmental GdL on Environment and Health.

Subsequebtly, the discussion started with interventions of participants, focusing primarily on topics such as One Health, research ethics, the current global geopolitical landscape, the role of researchers in society, and the public perception of science. The debate then shifted towards the "third mission" and the role of researchers in fostering cultural development within society. Part of the discussion also addressed the dissemination of scientific knowledge to the general public and the role of the Nature, Research, and Society GdL in this regard.

Climate adaptation in cities: making urban ecosystems resilient

Chairs: Giulia Capotorti[1], Maria Chiara Pastore[2]

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Climate change, characterized by increasing global temperatures, higher frequency of extreme weather events, and sea-level rise, has significant implications on ecosystem functioning. Urban areas, with high concentrations of people, infrastructure, and economic activities, are particularly vulnerable to these impacts. Despite the ability of urban systems to withstand and recover from shocks and stresses is a key aspect in addressing these challenges. Nature-Based Solutions (NBSs) are increasingly recognized as possible solutions to mitigate these pressures due to their potential to provide multiple ecosystem services supporting societies in achieving more resilient, liveable, and sustainable cities.

The objective of this roundtable was to foster an exchange of knowledge and experiences among people working on urban ecosystems, paving the way for future collaboration and initiatives that will advance our understanding of urban environments' response to climate change.

The roundtable, which involved about 30 participants, began with a short presentation by the moderators on their main research activities. Then, the participants described their research experience in urban context. The discussion highlighted a wide range of activities undertaken by IRET personnel on this topic, often in the framework of national and international projects. The studies cover soil and air contamination, ecosystem services, animal and plant biodiversity, plant physiology, soil quality indicators, data management, environment (pollution), and health correlation with a special focus on the role of green infrastructures in mitigating climate change. These studies take place in several Italian urban areas located throughout the country, as well as in some international sites.

In conclusion, the roundtable has allowed an active exchange of knowledge and experiences between IRET personnel working in urban context, laying the foundations for future collaborations and initiatives that will allow a deeper understanding of urban ecosystems and the identification of best strategies to face climate change.

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