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Project LIFE PRIMED: Integrating interdisciplinary and ecosystem-based approaches in restoring endangered mediterranean coastal forests and freshwater ecosystems

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Abstract

Transitional freshwater wetlands and coastal forests, particularly in the EU, face severe degradation and threats, leading to unfavourable conservation statuses for many habitats and species. The EU project LIFE PRIMED, operating in the 'Bosco di Palo Laziale' and 'Nestos Delta', Natura 2000 sites in Italy and Greece, aimed to bolster conservation efforts in the Mediterranean region by adopting direct restoration and management practices. These practices included, inter alia, introducing novel carbon–neutral water harvesting systems and invasive alien species control, specifically designed to mitigate the impacts of climate change and address challenges in forest management. The project outcomes demonstrated that the collaborative development of innovative, evidence-based and need-oriented restoration solutions, coupled with adaptive management of natural resources, has the potential to enhance ecological resilience in degraded ecosystems. This highlights the importance of adopting interdisciplinary approaches and focusing on ecosystem-based restoration measures in nature conservation. Such ambition is essential for achieving the European Union's biodiversity objectives and underscores the potential for broader application of innovative Nature-Based Solutions in the Mediterranean region.

Keywords Restoration ecology · Nature-based solutions · Natura 2000 · Life programme · Habitats Directive · Declined mediterranean forests · Mediterranean temporary ponds · EU nature restoration law

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Introduction

Transitional freshwater wetlands and coastal forests are among the most degraded and threatened ecosystems in the European Union, with significant alteration and loss occurring over the past century due to human activities (Čížková et al. 2013; Newton et al. 2014; Verhoeven 2014; Stojanović et al. 2015). This has resulted in many habitats and species being turned to'unfavourable','vulnerable'or'near threatened'conservation status according to the EU Habitats Directive (92/43/EEC) (Janssen et al. 2016). In the Mediterranean region, these ecosystems serve as critical habitats for a wide range of species, including endemic and endangered species, and provide essential ecosystem services such as water purification, flood control, and carbon sequestration (Scarascia-Mugnozza et al. 2000; Nocentini et al. 2022; Bagella 2023). Nevertheless, indicators of current negative trends in biodiversity and associated ecosystem functions and services provision are projected to continue in response

to direct and indirect drivers, as well as the impacts of climate change (Grillas et al. 2004; De Filippo et al. 2010; Peñuelas et al. 2010; Camarero et al. 2016; Touhami et al. 2020; Conte et al. 2022). Disturbance factors, including severe human pressure, urbanisation and agriculture, and climate change, exacerbate the current, already negative trajectories (Bagella et al. 2016; Thomas et al. 2002).

Wetlands in the Mediterranean biogeographic region encompass various ecosystems, such as brackish lagoons, estuaries, deltas, freshwater marshes, and riparian woodlands (Britton and Crivelli 1993; Grillas et al. 2004; Basset et al. 2006), with many of them listed in Annex I of the Habitat Directives as priority habitats (e.g. 1150*, 1510*, 3170*) (Biondi et al. 2012; Janssen et al. 2016). These habitats lie at the interface between land and water, making them highly diverse and productive areas (Deil 2005; Verhoeven 2014). They support high concentrations of birds, mammals, reptiles, amphibians, fish, and invertebrate species unique to the region (Médail et al. 1998; Quézel 1998). Despite their ecological value, these habitats, in particular transitional freshwater habitats,have faced considerable threats, including water extraction, agricultural conversion, urban expansion, and the impacts of climate change, leading to significant habitat loss and degradation (Schwartz and Jenkins 2000; Bagella et al. 2016; Caria et al. 2021). Furthermore, the lack of comprehensive definitions for many EU-designated freshwater habitat types complicates their field detection, geographical distribution assessment, and future conservation trend estimation. This hampers proper habitat identification and the development of appropriate restoration and conservation measures at the biogeographical scale (Fois et al. 2021; Spampinato et al 2023; Bagella 2023).

Mediterranean coastal forests are unique ecosystems that thrive under the region's distinct climate, marked by wet winters and hot, dry summers. These forests are biodiverse, hosting a variety of plant and animal genera and species, some of which are endemic and cannot be found anywhere else, such as *Phillyrea* spp., *Laurus nobilis* L., *Quercus ilex* L. and *Q. coccifera* L. for plants and *Lacerta* spp. and *Testudo hermannii* Gmelin, 1789 for animals (Blondel and Aronson 1999; Cox et al. 2006). These forests stabilize coastlines, prevent erosion, and act as significant carbon sinks, combating climate change (Vayreda et al. 2012; Anaya-Romero et al. 2016; Sferlazza et al. 2017; Roces-Díaz et al. 2021). They bridge terrestrial and marine habitats, supporting numerous life cycles, although this function has been less explored in the Mediterranean area. Yet, urban expansion, land abandonment, inappropriate management, and climate change threaten their long-term conservation (Quézel and Médail 2003; Blondel 2006; Sardans and Peñuelas 2013; Alessandri et al. 2014; Doblas-Miranda et al. 2017; Peñuelas and Sardans 2021; Conte et al. 2022).

These ecosystems establish intertwined ecological relationships, supporting various life cycles and biological patterns, providing habitat connectivity, and enhancing biodiversity (Verhoeven 2014). Coastal forests act as buffers, reducing erosion, filtering sediments and improving water quality for adjacent wetlands, while freshwater habitats hydrate, nourish, and enrich coastal forests, enhancing biodiversity and stability.

Various international and regional initiatives have supported efforts to conserve and restore freshwater habitats and coastal ecosystems (Verhoeven 2014). For example, the Ramsar Convention on Wetlands and the Mediterranean Wetlands Initiative aim to promote the wise use and conservation of wetlands through international cooperation and capacity building. Moreover, projects focused on the conservation of wetland and coastal ecosystems in the region, such as those supported by the Global Environment Facility (GEF) (Camacho González et al. 2019) and LIFE programme (EU 2007), emphasise the importance of urgent actions at threatened sites to preserve globally significant species diversity and ensure the sustainability of these critical habitats.

In Europe, such delicate ecosystems are protected by the EU Biodiversity Strategy for 2030 (EC 2021), and partially by the Water Framework Directive (EU 2000). The EU Biodiversity Strategy for 2030 builds on the Habitats Directive and Natura 2000 by aiming to expand protected areas and restore ecosystems, enhancing the network's role in preserving Europe's natural heritage and biodiversity. The LIFE Programme is the key EU financing instrument to finance and support projects that contribute to implementing this process. Since 1996, it has increasingly unlocked ideas and solutions aimed at restoring degraded landscapes, habitat types, and associated species, with more than EUR 1 billion lent to support restoration activities (LIFE public database https://webgate.ec.europa.eu/life/publicWebsite/search accessed on 22 March 2024). This commitment is expected to be strengthened with the entry of the new EU Nature Restoration Law into force.

Traditional and innovative ecological restoration solutions have been designed and applied from 2018 by the EU-funded project ‘LIFE PRIMED’ (LIFE17 NAT/GR/000511) in two such areas along the Greek and Italian coasts, ‘Bosco di Palo Laziale’ and ‘Delta Nestou’, to enhance the conservation status and resilience of'Pannonian-Balkanic turkey oak-sessile oak forests'(habitat 91M0), ‘Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior*’ (habitat 91E0), and'Mediterranean temporary ponds'(*3170) that have been increasingly exposed to climate change and inappropriate forest and water management.

The primary objective of this paper is to showcase the restoration, conservation, management, and monitoring efforts towards the project's targeted habitats and associated species. It aims to offer guidance and practical solutions for managing similar natural areas in the Mediterranean region. The actions undertaken, their results, and the challenges encountered are meant to provide valuable insights and inspiration for future initiatives in this field.

Project areas

Bosco di Palo Laziale

The Palo Laziale Wood is one of the last remaining patches of an ancient planitial forest that once covered the shoreline of the Lazio region (central Italy) from the Tiber mouth to Capo Linaro (La Montagna et al. 2023). It covers about 50 ha within the largest SAC IT6030022 'Bosco di Palo Laziale', a flat area of about 130 hectares, with an altitude between 3 and 10 m above sea level, located about 40 km northwest of Rome, directly facing the Tyrrhenian Sea, designated as a Natura 2000 site (Fig. 1), featuring unique oak forests (e.g.'Pannonian-Balkanic turkey oak-sessile oak forests', habitat 91M0) and rich tapestry of biodiversity ('Mediterranean temporary ponds', habitat *3170, Hermann's tortoise, European pond turtle, etc.). The site is embedded in the Municipality of Ladispoli (Rome), a small-medium town of about 40,000 residents settled in a very developed urban area with high population density and few green areas. The community is characterised by low wages and low employment levels, mainly from tourist activities, even if seasonal (summer), and secondly from agriculture.

The predominant vegetation of Palo Laziale is represented by a mixed forest of deciduous oaks, with a prevalence of *Quercus cerris* and rare individuals of *Q. petraea* and *Q. frainetto*. During rainy periods, the forest becomes completely swamped, with ponds in the depressed areas persisting until late summer and plant assemblages with species adapted to cope with such drastic changes (e.g. *Fraxinus angustifolia* subsp. *oxycarpa*) (La Montagna et al. 2023). Nevertheless, over the last three decades, the area of Palo Laziale was affected by an increase in aridity due to a decrease in rainfall and a rise in temperatures, accompanied by extreme drought events (e.g. summer of 2003, 2007, 2020, 2022). This has limited the soil water content, also due to excessive plant evapotranspiration. Such abiotic stress has induced endophytic fungal agents hosted in the oak plants (e.g. *Biscogniauxia mediterranea, Discula quercina, Diplodia corticola*) to turn into a pathogenic stage, causing severe forest dieback with about 40% of the adult trees found dead and the forest canopy reduced by about 80% in 2004 (Scarnati and Attorre 2014). Unsustainable forestry practices and ineffective water management have caused severe forest dieback cases on the site, exacerbating climate change effects, shrub encroachment, inter and intra-specific competition among trees, pathogen vulnerability, and topsoil aridity.

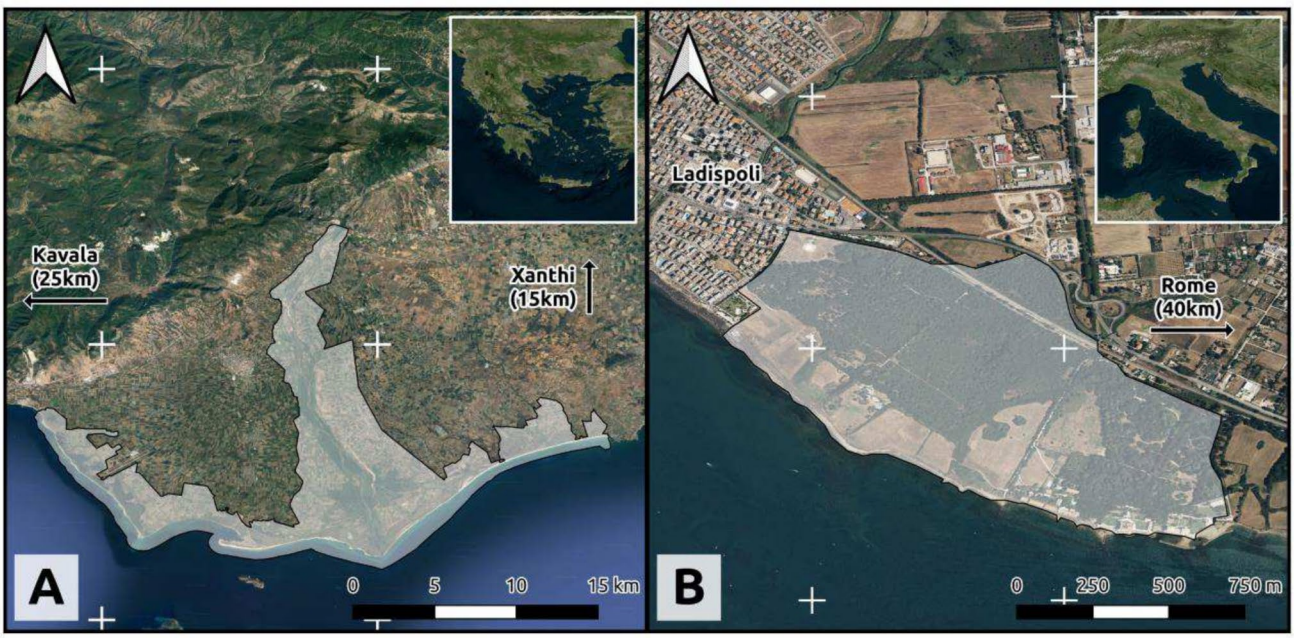

Fig. 1 On the left side (**A**), the 'Nestos Delta' and on the right side (**B**), the 'Bosco di Palo Laziale'—both Natura 2000 sites targeted by the project

Delta Nestou

The Nestos Delta, created by the alluvial deposits of the river that have extended the land into the sea (Fig. 1), covers an area of about 55,000 ha and is of high ornithological value, hosting 307 different bird species, 34 of which are endangered (Samaras and Koutitas 2008). Because of its size and the variety of its habitats, the Nestos Delta is considered among the most important wetlands of Greece and Europe, protected within the National Park of Eastern Macedonia and Thrace. Its ecological significance is reflected by the establishment of two NATURA 2000 sites: The Birds Directive site (SPA) GR1150001 "Delta Nestou Kai Limnothalasses Keramotis Kai Nisos Thasopoula" (14,783.79 ha) and the Habitats Directive Site (SCI) GR1150010 "Delta Nestou Kai Limnothalasses Keramotis—Evryteri Periochi Kai Paraktia Zoni" (23,043.86 ha).

The latter hosts the largest remaining riparian forest in the Mediterranean region, the so-called "Kotza Orman" (Great Forest), which has been severely reduced in size and functionality in past decades. Today, along with the 155 ha of the remains of the ancient forest, a dense secondary forest has grown along the artificial course, covering 1700 ha (Avramidou et al. 2023). The reduction was mainly due to tree cutting, land reclamation, and dam construction that forced the river into one central riverbed, altering the water discharge regime and reducing the sediment load (Xeidakis and Delimani 2002; Mallinis et al. 2011). Habitats and species in both Natura 2000 sites face several serious threats, including shrub expansion and invasive species encroachment, eutrophication, expansion of agricultural activities and inappropriate forest and water management. These pressures are related to direct or indirect human actions that expose forest ecosystems to multiple stress factors.

Previous nature conservation efforts in the area, include project LIFE NESTOS (LIFE02 NAT/GR/008489), implemented in the 2000 s to address reforestation activities in public lands along the river with indigenous and exotic forest species to increase the size of the forest. In total, 60 ha of new natural riparian forests on former agricultural land on both riverbanks were created, converting large patches of grasslands to areas covered by broad-leaves deciduous (12,790 seedlings of willows, poplars, alder, ash, oak and *Robinia pseudocacia*). Irregular planting schemes and mixed plots with clearings in between were adapted to the local conditions and natural plant communities (especially the EU-priority habitats of alluvial and gallery forests). Parts of the reforested areas were fenced to prevent access, and vegetation has grown abundantly. The project also implemented hydraulic interventions to reconstitute four old branches with the mainstream of Nestos River to recover freshwater marshes and riparian forests and prevent saltwater intrusions into ground and surface waters.

Materials and methods

Preparatory actions

The project's restoration efforts were planned and executed after assessing the biotic and abiotic factors contributing to the decline of the sites' ecosystems. This initial goal was to tailor the interventions to the specific conditions of each habitat type, including geological, climatic, pedological, and biological characteristics. A team of experts from different fields cooperated to quantify these factors and ensure that the proposed interventions were appropriate and scalable (Fig. 2). As an output of this collaborative work, a comprehensive collection of documents detailing the project's deliverables has been made available at https://www.lifeprimed.eu/en/download.

Several field visits were conducted in the two project areas to design and calibrate the project's hydraulic systems. Topographic surveys were carried out to assess ground slopes and understand how gravity could influence water distribution in terms of direction, flow, and speed. These surveys employed GPS markers (benchmarks) and total station equipment in Real-Time Kinematic mode for precise ground measurements. Additionally, airborne LiDAR topographical surveys were used to develop high-resolution Digital Terrain Models (DTMs) in areas with standing freshwater habitats.

Soil analyses explored the relationship between edaphic structure and vegetation dynamics, sampling relevant parameters such as electrical conductivity (salinity), exchangeable sodium percentage (sodicity), pH, and granulometry, following USDA (1975) and IUSS Working Group WRB (2007). Additionally, soil permeability was evaluated to predict the drainage network's capacity to channel water to plant roots. Twenty soil pits were performed in Palo Laziale in the area occupied by the declining woodland, the temporary ponds and the meadow. In Nestos Delta, pits were dug in three different areas; eight were conducted on the western bank of the Nestos river (control site), and nineteen on the river's eastern bank, i.e."Voice of America"and"Lazaros", respectively.

Climate data series were built and analysed to determine the local effects of climate change on the Palo Laziale and Nestos areas. Various weather stations and climate databases near these areas were used to retrieve data, which were then used to depict the climate patterns in these regions and quantify the water stress accumulated by the forests over time. In Italy, data were sourced from the Maccarese and Fiumicino stations belonging to the Regional Agro-Meteorological monitoring network of ARSIAL (Regional Agency for Development and Innovation of Agriculture), located approximately 4 km from

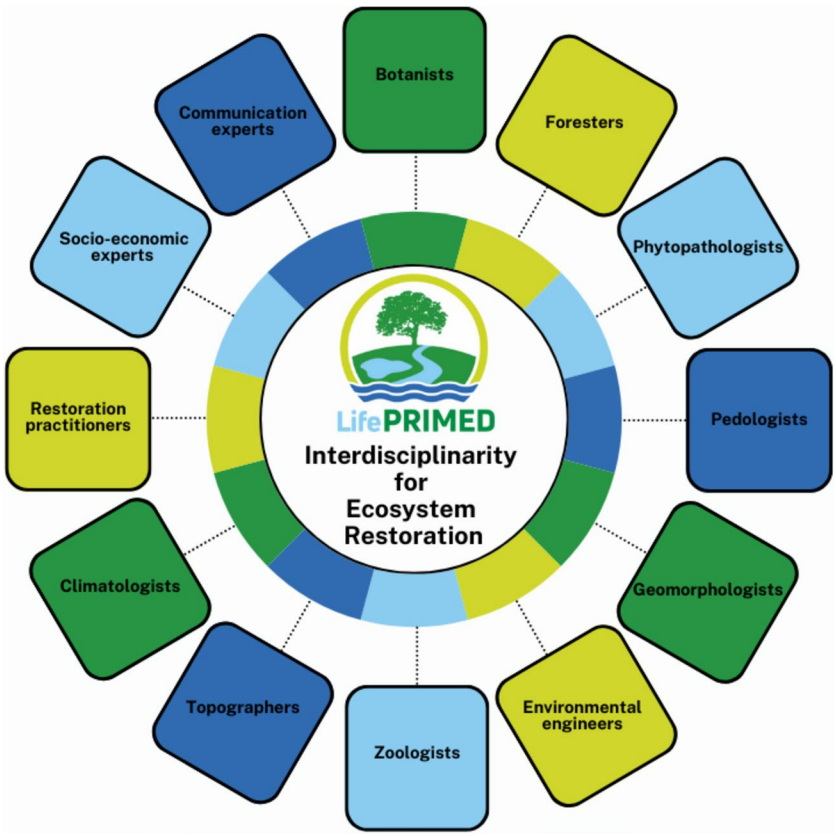

Fig. 2 Summary of the interdisciplinary expertise involved in the LifePRIMED project, working together for the ecosystem restoration of both sites

Palo Laziale. In Greece, data were retrieved from the Hellenic National Meteorological Service (HNMS) and the Public Power Corporation S.A. – Hellas (DEH). The collection points included Kavala (2 stations), Xanthi, and Chrysoupoli, positioned roughly 25 km west, 16 km east, and 14 km north of Nestos, respectively. The gathered data encompassed several key variables, crucial for assessing climate change impacts, including precipitation (P), average temperature (Tmed), maximum (Tmax) and minimum temperatures (Tmin), humidity (H), wind speed (Ws) and direction (Wd, and solar radiation (PAR). We calculated the Thornthwaite Global Moisture Index based on the potential evapotranspiration of the target vegetation to identify annual water deficit trends and, thus, the soil water balance within the study areas. Finally, the climatic water balance (CWB) was determined by subtracting the potential evapotranspiration depth from the precipitation depth at each site over a specific period.

The botanical surveys finalised the assessment of the target ecosystems, addressing a floristic diachronic comparison and a homogeneous vegetation census in 37 plots of 100 m^2 in Palo Laziale following the indications of Lucchese (1990) for floristic census, and the methodology of Bullock (2006) and Fratarcangeli et al. (2022) for establishing 1 ha plots. Vegetation transects of 2×2 and 1×1 m plots were furthermore carried out according to Delipetrou et al. (2015) to specifically address 'Mediterranean temporary ponds' (habitat 3170*), which included a sub-selection of 20×20 cm plots to enable fine-scale comparisons between Palo Laziale and Nestos Delta (Lazaros site)(Fig. 3).

Forestry surveys involved sampling of 22 circular georeferenced plots in Palo Laziale and 15 in Nestos Delta to study the composition, structure, and regeneration of the deciduous and evergreen forests (habitat 91M0, 9340 and 5230*) and alluvial forests (habitat 91E0*), respectively. In Nestos, plots were evenly distributed along the river's eastern and western banks, with each plot's central tree marked for reference, while in Palo Laziale they were randomly scattered within the forest canopy. Measurements in both sites included tree height and diameter at breast height (DBH) for trees ≥ 2.5 cm, using a calliper and a NIKON Forestry 550 Laser Rangefinder, and were categorised into 10 cm diameter classes to evaluate forest composition, density, distribution, and basal area. Additional observations included climber strain, insect infestation, and tree damage. Forest regeneration was assessed by calculating the Index of Regeneration (IR) (density of regeneration [m^2] times the average regeneration height [cm]) based on seedling density and average height for seedlings less than 2.5 cm in diameter and over 20 cm tall (Bianchi & Paci 2008; Avramidou et al. 2023).

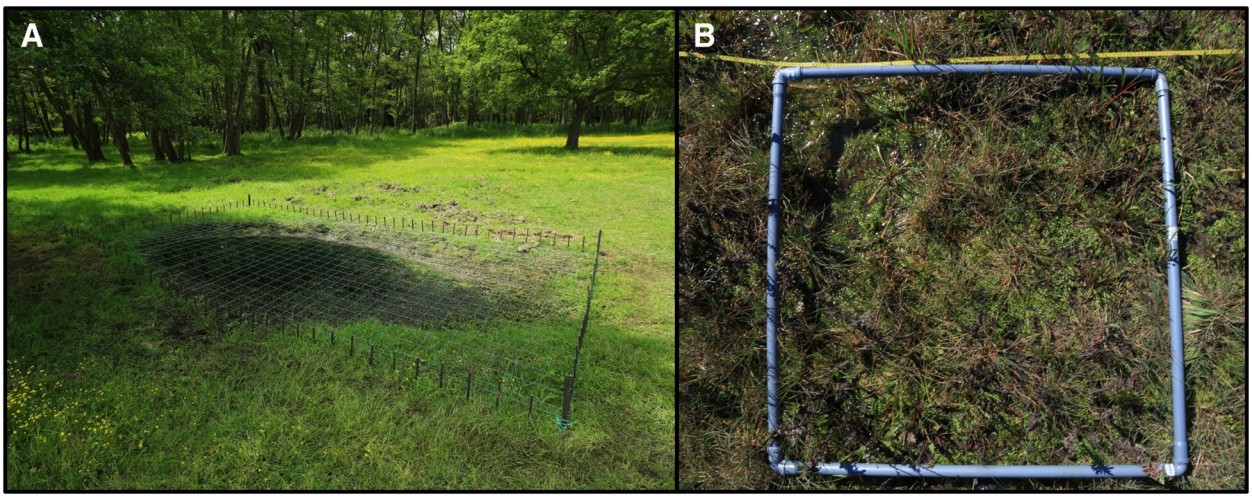

Fig. 3 Grids used for monitoring the vegetation of Mediterranean temporary ponds in both project sites

Conservation actions

LIFE PRIMED addressed climate and human impacts on coastal forests and freshwater habitats, including extreme weather and biological invasions, through a holistic, evidence-based, transnational approach. The project team designed and delivered nature-based solutions to restore dysfunctional, poorly managed coastal ecosystems across the Mediterranean, offering replicable and transferable strategies for regional recovery and long-term management. To this aim, the project combined traditional practices with innovative techniques to recreate suitable environmental conditions for rejuvenating degraded habitats. Traditional methods include reforestation, trimming of encroaching shrub vegetation to manage undergrowth and reintroduction of native species. Innovations involved using technology for precision monitoring and interventions, such as drone mapping for vegetation analysis, in-field IAS control trials, a free-pathogen forestry nursery and automated hydraulic systems for controlled watering in water-depleted areas. Biomolecular techniques advanced the ex-situ capacity to detect fungal pathogen agents in tree individuals and seeds, enabling in-situ genetic resilience in reinforced plant communities facing climate change. Collaborative projects harness community involvement for sustained conservation efforts, integrating local knowledge with scientific research. These practices aimed to restore biodiversity, enhance ecosystem services, and mitigate climate impacts, promoting an interdisciplinary approach and environmental stewardship.

1- Cleaning and site preparation

In Palo Laziale, site preparation actions involved vegetation clearance, soil excavation, and contouring with a crawler tractor for optimal rainwater retention. Selective clearance was conducted over two phases with both mechanical and manual tools to remove encroaching shrubs, mainly *Rubus ulmifolius*, from 40 hectares of habitat 91M0. All removed vegetation was chipped and left on-site. Existing roads for anti-fire vehicle access and in-situ forest operations were also re-established. After the clearing, three sites were selected for creating proper ecological conditions for establishing new temporary ponds (habitat 3170*), following existing guidelines like the"Million Ponds Project"(Pizzuti Piccoli 2022). In Nestos, aerial drone photography combined with in-field surveying was employed to map the expansion rate of the invasive species *Amorpha fruticosa* (Avramidou et al. 2023) and select suitable locations and methods for controlling its spread. Traditional chemical control methods being unsuitable, the forestry team implemented various experimental treatments applying different control treatments in three experimental plots for invasive alien species, including a) artificial vegetation shading with the placement of a net at 3 m height, complete removal of *A. fruticosa* and use of geotextile as a ground cover, b) trimming of *A. fruticosa* and simultaneous new plantings of *Alnus glutinosa* and *Populus alba* to enhance the abundance of native tree individuals, c) repeated cutting of *A. fruticosa* to reduce its vigour and control its spread by depleting resources and preventing seed production, and d) experimental periodical goat grazing treatment in designated fenced areas. These approaches were determined in consultation with the Kavala Forest Service and the Management Body and monitored for two years to identify the most effective strategies for mitigating *A. fruticosa*'s impact. The spread of the alien species *A. fruticosa* and *Acer negundo* was also addressed by completely removing

adult trees and seedlings from 10 out of 15 circular georeferenced plots and monitoring the potential recovery of the natural regeneration of the native species.

2- Reforestation and pathogens monitoring

The project established a forestry nursery for the Italian site at ARSIAL's Cerveteri Research Farm, located 4 km from Palo Laziale. Starting in 2018, nursery operators conducted several successful seed harvesting campaigns for keystone forest species such as *Quercus cerris, Q. ilex, Q. pubescens, Q. suber, and Fraxinus angustifolia* subsp. *oxycarpa*. Seeds were then subjected to phytopathological screenings using metagenomic techniques to check for fungal agents, as described by Beccaccioli et al. (2021). Only seeds free from fungal signs were propagated. Resultant seedlings underwent annual monitoring, which included identifying fungal communities through in vitro growth in potato dextrose broth (HiMedia) for 5 days under dark conditions and then the DNA was extracted from fungal tissues with the cetyltrimethylammonium bromide (CTAB) method (Punelli et al. 2009). The DNA extracted therefore it was sequenced with Nanopore metabarcoding in the ITS region. The growth of the saplings was also monitored, with ARSIAL staff recording allometric data (height and diameter) to compare the growth of different tree species.

3- Ex-situ conservation of habitats' keystone species

At the Botanical Garden of Rome's Germplasm Bank, measurements and germination trials were carried out for *Ranunculus sardous*, *Lythrum tribracteaum*, *Mentha pulegium*, *Juncus bufonius, Isoetes histrix* and *Isoetes durieui*, all characteristic species of Palo Laziale's temporary ponds. Seeds and fruits were collected from donor sites, cleaned, and stored in petri dishes. To determine each species'germination requirements, we measured seed/fruit size and moisture content and performed germination tests under varying temperatures (25/15 °C, 20/10 °C, 15/6 °C) and two light conditions (12 h light coinciding with the highest temperature, and continuous darkness), simulating natural conditions. They were finally dehydrated for 45 days at 15 °C and 15% relative humidity, then stored at −20 °C for long-term conservation (Bacchetta et al. 2006). The keystone species of Nestos' Mediterranean temporary ponds (3170*) and Alluvial forest (91E0*), i.e. *Mentha pulegium*, *Cyperus fuscus*, *Pycreus flavescens*, *Pycreus flavidus*, *Populus alba*, *Alnus glutinosa*, were treated and stored in the seed bank of the Department of Forest and Natural Environment Sciences in Drama. Seed viability of each species were assessed through the 2,3,5-triphenyl tetrazolium chloride (TTZ) test (Jacob et al. 2023).

4- Hydraulic works

The project employed hydraulic engineering solutions to restore and conserve target ecosystems threatened by climate change. A custom hydraulic system was installed at each site to address water scarcity in specific habitats (i.e., 91M0, 9340, 3170*, 5230*), enhancing the resilience of Mediterranean coastal ecosystems. In Palo Laziale, the system relies on a drainage trench that intercepts surface water during increasingly extreme rain events. This setup reduces rapid rainfall run-off and delivers the water into an underground tank. The stored water is then gradually released throughout the forest during critical drought periods via an extensive network of micro-perforated channels (Fig. 4). In Nestos, the system consists of a network of well points that extracts groundwater from the aquifer using a photovoltaic pumping system. During extended dry seasons, this water is released through micro-sprinklers. These systems provide additional

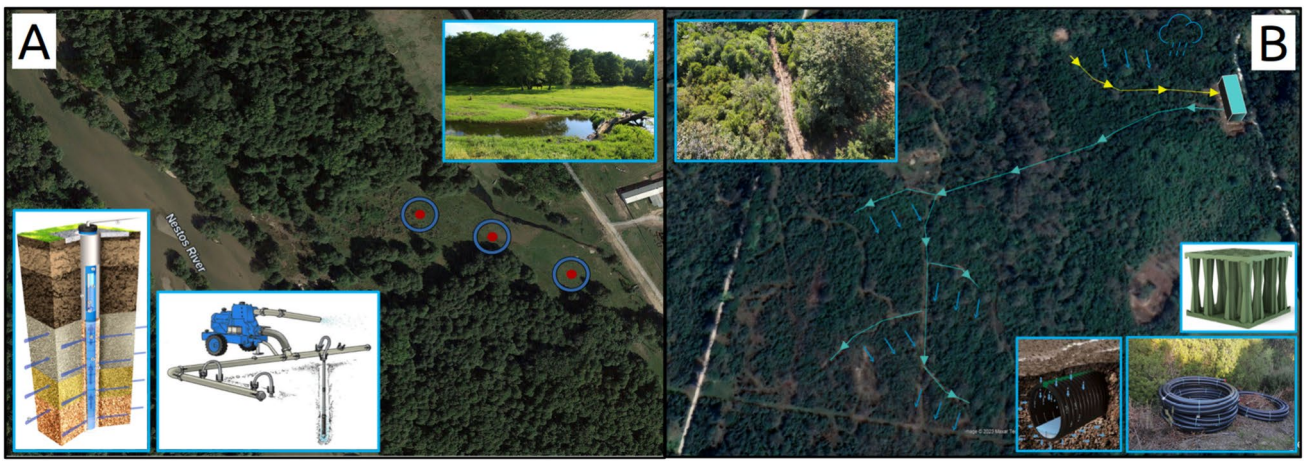

Fig. 4 The hydraulic system projects in the Natura 2000 sites (**A**) 'Delta Nestou' and (**B**) 'Bosco di Palo Laziale' showing the three dwells system in the former and the water accumulation tank in the latter

water during dry spells, ensuring a balanced water supply for the mixed deciduous forest and associated freshwater habitats, crucial for mitigating aridity and preventing excessive evapotranspiration. Expected to operate for 30 years, these interventions aim for a self-sustaining ecosystem requiring minimal human maintenance (e.g., silvicultural practices) after that.

5- Long-term management

The operation of the hydraulic systems is governed and optimised by a Water Resources Management Plan drafted to manage meteoric and surface water in each site. The plan outlines the water needs of the target ecosystems, drawing from historical and novel weather data, climate trends, soil characteristics, tree canopy structure, and the conservation status of forest habitats By analysing these factors, the plan estimates forest evaporation and evapotranspiration rates, determining the water demand and the volume of water the hydraulic system needs to deliver (Lombardi et al. 2023). Reference thresholds like precipitation, temperature, and soil moisture guide the release of water, especially during critical periods in late spring and early summer. These indicators adjust the utilisation of water stored in the underground tank to address water scarcity precisely, based on the ecosystems'specific stress conditions. The sustainability and long-term success of forest restoration efforts at each site are guaranteed through a Strategic Management Plan for Forest Sustainability (SFSMP). The plan, compliant with Natura 2000 and regional protected area regulations (e.g., L.R. 28 Oct 2002, No. 39), schedules silvicultural practices for thirty years to secure the multifaceted functions of forest ecosystems and the sustainable exploitation of associated resources.

Monitoring actions

Both in-situ and ex-situ monitoring activities were planned to verify the effectiveness of restoration actions and provide technical-scientific guidance for enabling the conservation of similar habitat types and threatened species elsewhere. Permanent ecological-based monitoring is crucial to evaluate the Action's success and enable the proper management and long-term conservation of target ecosystems. A multilevel monitoring system was designed and operationalise in the project areas to track changes in the habitat biotic and abiotic conditions and verify ecological responses to the interventions of plant and animal species, focusing a) TREE INDIVIDUALS through xylem flow measurements to evaluate gas exchange performance of trees during the growing season); b) FUNGAL TAXA through molecular characterisation of Palo Laziale's oak seedlings and acorns to detect potential pathogenic agents; c) PLANT SPECIES through leaf-based measurements of the carbon assimilation and water fluxes to quantify photosynthesis and evapotranspiration of *P. latifolia, P. lentiscus, Q. cerris*, and *F. angustifolia sub. oxycarpa*; d) ANIMAL SPECIES through in-field surveys to keep track of the conservation status of *E. orbicularis*, *T. hermanni*, *P. colchicus colchicus*, and most representative bird populations; e) PLANT COMMUNITIES through in-field census and sampling to monitor the structure and dynamics of target habitats; f) FOREST AND FRESHWATER ECOSYSTEMS through weather and soil measurements to verify responses of environmental factors to the restoration actions.

Results

Preparatory actions

Two high-resolution maps were produced for each area: one for Palo Laziale at a 1:500 scale with level 8 DTM and another for Nestos at a 1:200 scale with level 6 DTM. These maps include a synthesis of mapping, photographic, and field activities. Additionally, for Nestos, aerial photogrammetry and an orthophoto at a 1:500 scale were provided.

Soil analysis at Palo Laziale revealed relatively high salt levels in the topsoil, showing a slight decline from 2003 figures, potentially due to increased precipitation up to 2018. Despite high salinity/sodicity, it does not significantly hinder plant growth, reflecting more a permanent feature rather than a direct and exceptional consequence of an external source. The discovery of a substantial clay layer at 75–100 cm depth was crucial to recreate proper conditions for new temporary ponds, as it is essential for maintaining water in the topsoil (Table 1). On the other hand, clay can restrict plant root growth and water absorption during prolonged dry spells, impacting meteorological and climatic resilience and requiring long-term monitoring. In Nestos, all soil samples across three sampled sites originated from alluvial deposits. Despite low carbonate salt content (max 4% CaCO3), some soils exhibited high pH levels. Conductivity and exchangeable sodium percentage (ESP), indicators of salinity, were generally low, except for site B,"Voice of America,"where both were high in soils with significant cation exchange capacity (CEC). This site also recorded the highest clay concentration (30–36% in surface layers), creating an impermeable layer that facilitates the formation of temporary ponds. However, soil analysis and flora surveys suggested these conditions do not align with habitat 3170* but rather with Mediterranean salt meadows. At site C,"Lazaros,"identified with habitat type 3170*, the pond's predominant soil feature is sandy texture, observed in four out of five soil pits. This, along with the river level exceeding the land level, likely causes water to rise in the soil, forming ponds to maintain

Table 1 Results of pedological analysis across various zones within the two project sites, detailing parameters such as textural class, pH levels, and other key soil characteristics

Soil pit code	Site	Coordinates X	Coordinates Y	Depth (cm)	pH	Sand (%)	Clay (%)	Silt (%)	Soil characterization	CEC (meq/100 g)	ESP	$CaCO_3$(%)	Electrical Conductivity (µS/cm)	Exchangeable bases (cmol/kg)			
														Ca	Mg	Na	K
1	Palo Laziale	259624.8436	4647409.467	0—10	6.6	44.6	28.5	26.9	Clay loam	29.1	< 8	0.6	245	25.4	4.6	1.9	0.4
				10—30	6.5	31.4	36.8	31.8		36.8	10.2	11.3	306	24.0	6.9	2.1	0.8
				30—100	7.1	30.3	44.5	25.2		76.8	17.2	18.1	618	25.5	9.6	4.6	0.8
2	Palo Laziale	259585.8416	4647436.691	0—10	6.8	33.0	24.2	42.8	Clay loam	15.2	< 8	6.6	302	30.8	7.8	1.2	0.9
				10—30	6.2	31.1	25.8	43.1		18.1	13.3	11.2	192	18.0	5.7	1.3	0.8
				30—100	7.3	28.6	36.8	34.6		19.2	18.3	19.2	380	17.7	6.6	2.3	0.8
3	Palo Laziale	259374.3231	4647088.109	0—30	6.3	25.6	28	46.4	Clay loam	17.3	< 8	8.4	827	38.4	11.9	3.5	1.4
				30—100	7.1	16.6	40	43.4		24.4	< 8	27.2	1254	24.4	15.5	14.9	0.9
4	Palo Laziale	259353.2619	4647135.293	0—10	6.4	40.9	31.6	27.5	Clay loam	18.2	< 8	2.2	122	19.3	4.1	1.1	0.6
				10—30	6.1	44.4	29	26.6		25.7	16.0	7.9	90	17.2	4.8	1.1	0.6
				30—100	6.9	31.3	42.2	26.5		38.3	24.5	16.3	313	29.1	7.5	2.1	0.7
5	Palo Laziale	259470.8556	4647190.896	0—10	6.5	43.1	23.5	33.4	Clay loam	28.6	< 8	0.9	848	47.1	10.0	3.6	0.9
				10—30	6.2	42.0	27.1	30.9		39.2	13.4	12.7	817	21.6	5.7	2.7	0.6
				30—100	7.5	28.4	47.3	24.3		57.7	17.1	21.2	1305	28.0	10.7	7.7	0.4
6	Palo Laziale	259460.2676	4647525.102	0—30	6.2	38.8	21.2	40.0	Medium loam	13.6	< 8	3.4	208	27.3	5.7	1.5	0.6
				30—100	7.4	32.1	35.8	32.1		16.6	9.40	22.6	604	18.7	11.4	4.1	0.7
7	Palo Laziale	259477.2492	4647677.986	0—10	6.3	42.9	21.2	35.9	Clay loam	12.4	< 8	3.5	560	26.5	5.3	0.7	0.5
				10—30	6.1	46.1	36	17.9		16.7	< 8	11.1	222	11.8	3.7	1.0	0.5
				30—100	7.7	32.8	48	19.2		19.2	10.5	19.6	589	30.1	7.8	4.6	0.4
8	Palo Laziale	259439.5603	467281.449	0—30	6.6	47.7	25.1	27.2	Clay loam	15.2	< 8	1.7	81	13.0	4.3	0.5	0.5
				30—100	6.9	38.3	38	23.7		21.4	< 8	19.5	167	15.5	8.0	2.4	0.6
9	Palo Laziale	259273.9731	4647602.234	0—30	6.8	43.2	31.9	24.9	Clay loam	17.5	< 8	2.3	382	43.8	4.3	1.0	0.9
				30—100	8.1	43.7	31.1	25.2		20.0	19.50	20.1	467	16.9	2.8	0.4	0.4
10	Palo Laziale	259167.8128	464777.9859	0—30	6.9	49.5	26.8	23.7	Sandy clay loam	14.7	< 8	8.3	111	35.5	6.6	1.1	0.9
				30—100	7.6	46.9	30.5	22.6		19.8	10.20	16.1	237	31.9	12.2	2.9	0.9
11	Palo Laziale	259189.9224	4647615.313	0—30	6.6	46.2	22.2	31.6	Medium loam	17.4	< 8	5.5	177	28.7	8.0	2.5	0.4
				30—100	7.5	45.8	24.1	30.1		23.9	11.70	9.2	281	19.9	9.2	2.9	0.5
12	Palo Laziale	259178.3105	464079.748	0—30	6.9	41.5	32.1	26.4	Clay loam	12.7	< 8	1.7	93	15.1	5.8	1.7	0.2
				30—100	7.6	41.1	36.7	22.2		18.7	9.20	5.2	185	16.9	3.7	3.9	0.5
13	Palo Laziale	259643.167	4647347.591	0—30	6.5	45.5	23.2	31.3	Medium loam	18.2	< 8	7.3	293	48.2	9.9	1.9	0.9
				30—100	7.0	40.9	30	29.1		25.6	21.60	7.8	519	38.9	11.4	3.3	1.6
14	Palo Laziale	259008.264	4647459.723	0—30	6.8	43.8	32.6	23.6	Sandy clay	18.9	< 8	2.4	103	15.8	6.5	0.7	1.5
				30—100	7.4	39.9	40.2	19.9		27.4	< 8	8.6	362	19.6	8.1	1.6	2.8

Table 1 (continued)

Soil pit code	Site	Coordinates X	Coordinates Y	Depth (cm)	pH	Sand (%)	Clay (%)	Silt (%)	Soil characterization	CEC (meq/100 g)	ESP	$CaCO_3$(%)	Electrical Conductivity (µS/cm)	Exchangeable bases (cmol/kg)			
														Ca	Mg	Na	K
15	Palo Laziale	259666.4006	4647386.604	0—10	6.4	44.4	28.4	27.2	Clay loam	21.7	< 8	11.5	199	33.6	11.6	3.9	0.4
				10—30	7.8	41.7	36.2	22.1		33.3	14.2	21.1	451	21.8	15.4	4.4	0.8
				30—100	8.3	30.6	50.7	18.7		38.88	0.8	24.4	821	19.7	13.3	2.3	0.7
16	Palo Laziale	259284.417	4646984.989	0—30	6.7	30.9	34	35.1	Clay	21.2	< 8	0.9	332	37.0	7.5	2.7	0.7
				30—100	7.3	25.1	47.3	27.6		46.2	17.70	3.8	644	21.4	6.4	1.9	0.9
17	Palo Laziale	259323.4405	4647113.54	0—30	6.4	42.1	31	26.9	Clay loam	18.5	< 8	0.6	195	27.7	5.8	1.9	0.3
				30—100	7.0	34.9	46.5	18.6		35.9	14.60	3.1	455	26.1	3.8	1.1	0.8
18	Palo Laziale	259295.8274	4646915.12	0—30	6.8	36.5	40.8	22.7	Clay	38.1	10.20	1.5	188	19.1	8.1	3.6	0.8
				30—100	7.4	25.2	53	21.8		67.2	24.10	5.8	203	29.2	7.5	9.2	0.9
19	Palo Laziale	259631.5825	4647199.126	0—30	6.4	44.8	37.5	17.7	Clay	24.7	< 8	0.6	301	35.1	4.2	1.9	0.5
				30—100	7.8	35.5	47.7	16.8		51.1	20.00	3.7	520	35.9	5.9	3.5	0.7
20	Palo Laziale	259061.7496	4647367.747	0—30	8.3	33.1	35.5	31.4	Clay loam	17.4	< 8	0.7	167	41.8	8.7	0.9	0.7
				30—100	7.2	34.0	38.7	27.3		22.7	19.60	3.9	381	32.9	9.3	3.5	1.0
TP1	Delta Nestou	E024 43 55	N41 01 56	0—10	7.3	85.8	5.2	9.0	Loamy sand	8.4	0.1	0.0	333	10.0	0.4	0.0	0.1
				10—30	8.4	96.8	0.2	3.0		1.2	0.8	0.4	148	1.0	0.2	0.0	0.1
				30–100	9.3	89.8	4.2	6.0		2.4	0.4	0.4	267	2.3	0.2	10.0	0.1
TP2	Delta Nestou	E024 43 53	N41 01 59	0—10	8.2	59.8	14.2	26.0	Sandy loam	15.0	0.1	0.0	467	11.0	0.9	0.0	0.2
				10—30	8.9	96.8	1.2	2.0		1.7	0.5	0.0	198	1.5	0.1	0.0	0.1
				30–100	8.8	95.8	0.2	4.0		0.8	1.2	0.2	241	0.6	0.1	0.0	0.1
TP3	Delta Nestou	E024 44 11	N41 02 27	0—10	9.0	94.8	1.2	4.0	Sand	3.3	0.4	1.3	282	3.0	0.2	0.0	0.1
				10—30	9.3	95.8	0.2	4.0		158.0	0.7	0.4	262	1.4	0.1	0.0	0.1
				30–100	9.3	98.8	0.2	1.0		0.9	1.1	0.4	243	0.7	0.1	0.0	0.1
TP4	Delta Nestou	E024 46 53	N40 54 12	0—10	6.1	55.8	13.2	31.0	Sandy loam	11.5	0.2	0.0	294	8.3	1.1	0.0	0.3
				10—30	7.3	76.5	4.84	18.6		2.6	0.5	0.0	170	5.8	0.8	0.0	0.2
				30–100	7.2	78.5	2.84	18.6		1.4	0.8	0.0	198	8.3	0.6	0.0	0.1
TPG1	Delta Nestou	E024 44 07	N41 02 18	0—10	7.7	68.5	9.84	21.6	Sandy loam	4.9	0.3	0.0	320	9.3	0.6	0.0	0.2
				10—30	6.1	75.5	6.84	17.6		3.2	0.4	0.0	347	6.8	0.4	0.0	0.1
				30–100	8.2	80.5	3.84	15.6		2.5	0.6	0.0	326	6.5	0.3	0.0	0.1
TPG2	Delta Nestou	E024 46 57	N40 54 09	0—10	8.2	50.5	11.2	38.3	Loam	2.1	1.2	0.4	736	10.3	0.8	0.0	0.3
				10—30	8.8	50.5	11.2	38.3		6.3	1.0	0.4	669	10.5	1.3	0.1	0.1
				30–100	8.8	70.5	3.2	26.3		3.1	0.6	1.7	342	13.5	0.6	0.0	0.1

Table 1 (continued)

Soil pit code	Site	Coordinates X	Coordinates Y	Depth (cm)	pH	Sand (%)	Clay (%)	Silt (%)	Soil characterization	CEC (meq/100 g)	ESP	$CaCO_3$(%)	Electrical Conductivity (µS/cm)	Exchangeable bases (cmol/kg)			
														Ca	Mg	Na	K
TP5	Delta Nestou	E024 49 011	N40 52 987	0—10	8.7	19.6	33.1	47.3	Clay loam	26.8	9.6	4.28	1350	26.2	9.3	2.6	1.7
				10—30	8.8	13.6	33.1	53.3		26.8	4.6	1.12	1152	25.5	5.0	1.2	0.3
				30–100	8.9	89.6	7.1	3.3		2.1	12.4	0.0	499	4.4	1.0	0.3	0.1
TP6	Delta Nestou	E024 49 042	N40 52 977	0—10	9.7	59.6	19.1	21.3	Sandy loam	7.4	22.8	0.82	1760	15.3	3.7	1.7	0.4
				10—30	10.1	25.6	31.1	43.3		28.7	34.8	3.98	3571	17.4	8.8	10.0	0.5
				30–100	9.7	93.8	5.2	1.0		2.5	42.6	0.25	666	4.8	1.0	1.1	0.1
TP7	Delta Nestou	E024 49 032	N40 52 960	0—10	9.9	20.3	52.4	27.3	Clay	32.5	55.9	4.39	4736	17.3	9.3	18.2	0.9
				10—30	9.9	38.3	26.4	35.3		24.2	31.4	2.55	3002	16.7	6.7	7.6	0.5
				30–100	9.9	95.2	2.2	2.6		5.4	14.4	0.41	730	4.5	1.2	0.8	0.1
TP8	Delta Nestou	E024 48 951	N40 53 039	0—10	8.0	38.3	40.4	21.3	Clay loam	15.9	3.6	0.31	1690	19.2	4.2	0.6	0.7
				10—30	9.1	19.6	29.12	51.3		31.7	11.5	2.35	1376	29.4	5.4	3.7	1.2
				30–100	9.6	80.2	10.2	9.6		13.0	6.2	1.02	800	10.8	1.7	0.8	0.2
TPG3	Delta Nestou	E024 49 004	N40 52 997	0—10	8.1	51.6	20.4	28.0	Loam	28.2	1.3	0.0	442	16.6	2.2	0.4	0.3
				10—30	7.9	79.6	6.4	14.0		5.7	2.1	0.0	122	3.2	0.5	0.1	0.1
				30–100	7.6	97.6	0.4	2.0		2.2	1.2	0.0	102	1.4	0.2	0.0	0.0
TP9	Delta Nestou	E024 45 46	N40 56 30	0—10	6.2	82.9	2.56	14.6	Sand	5.2	6.8	0.0	1811	10.1	1.2	0.4	0.2
				10—30	6.7	94.4	1.56	4.0		0.8	13.1	0.0	670	2.5	0.2	0.1	0.1
				30–100	7.1	96.4	0.56	3.0		0.4	36.7	0.0	399	2.0	0.2	0.2	0.1
TP10	Delta Nestou	E024 45 45	N40 56 31	0—10	6.7	89.4	2.56	8.0	Sand	1.9	18.0	0.0	883	6.3	1.2	0.3	0.1
				10—30	7.1	86.4	5.56	8.0		2.7	9.1	0.0	936	7.4	0.6	0.2	0.1
				30–100	6.8	97.4	0.56	2.0		0.3	54.4	0.0	167	1.5	0.2	0.1	0.1
TP11	Delta Nestou	E024 45 46	N40 56 30	0—10	7.3	78.9	7.12	14.0	Sand	5.5	10.4	0.0	700	9.9	1.0	0.6	0.1
				10—30	6.0	86.4	3.56	10.0		1.1	13.7	0.0	236	3.7	0.4	0.2	0.1
				30–100	7.5	94.4	1.56	4.0		1.0	11.7	0.0	166	3.1	0.4	0.1	0.0
TP12	Delta Nestou	E024 45 45	N40 56 31	0—10	7.0	97.4	0.56	2.0	Sand	1.4	10.5	0.0	737	2.6	0.2	0.1	0.1
				10—30	5.9	94.4	0.56	5.0		1.6	9.8	0.0	1619	3.5	0.3	0.2	0.1
				30–100	6.7	97.4	0.56	2.0		0.8	19.8	0.0	451	2.0	0.2	0.2	0.1
TP13	Delta Nestou	E024 45 41	N40 56 35	0—10	7.2	48.9	15.12	36.0	Loam	5.1	7.2	0.56	2982	13.0	1.6	0.4	1.4
				10—30	8.2	44.9	13.12	42.0		2.4	11.0	2.76	831	12.1	0.9	0.3	0.5
				30–100	8.4	78.4	3.56	18.0		2.6	8.3	1.70	534	10.1	0.8	0.2	0.3
TPG4	Delta Nestou	E024 45 40	N40 56 33	0—10	8.1	58.9	13.12	28.0	Sandy loam	3.4	32.2	2.12	2086	17.3	3.1	1.1	0.1
				10—30	8.3	60.9	11.12	28.0		3.5	14.1	0.85	881	12.4	1.3	0.5	0.1
				30–100	8.5	90.4	2.56	7.0		1.5	12.7	0.35	606	7.7	0.5	0.2	0.1

Table 1 (continued)

Soil pit code	Site	Coordinates X	Coordinates Y	Depth (cm)	pH	Sand (%)	Clay (%)	Silt (%)	Soil characterization	CEC (meq/100 g)	ESP	$CaCO_3$(%)	Electrical Conductivity (µS/cm)	Exchangeable bases (cmol/kg)			
														Ca	Mg	Na	K
RF1	Delta Nestou	E024 44 42	N40 58 15	0–30	8.7	80.5	2.2	17.3	Loamy sand	4.0	0.95	0.34	435	6.0	0.4	0.0	0.1
				30–100	8.8	88.5	2.2	9.3		0.7	245.00	0.00	320	3.5	0.2	0.0	0.1
RF2	Delta Nestou	E024 44 42	N40 57 39	0–30	8.6	34.3	8.4	57.3	Silt loam	1.9	27.57	2.13	2010	12.0	4.6	0.5	0.2
				30–100	9.4	63.5	2.84	33.6		0.4	74.30	1.94	614	4.7	1.8	0.3	0.1
RF3	Delta Nestou	E024 44 42	N40 57 39	0–30	9.0	60.5	5.84	33.6	Sandy loam	1.2	2.11	1.84	541	7.3	0.8	0.0	0.1
				30–100	9.0	65.5	4.84	29.6		1.2	1.93	1.21	436	8.0	0.8	0.0	0.1
RF4	Delta Nestou	E024 44 44	N40 57 23	0–30	9.1	55.5	5.84	38.6	Sandy loam	0.6	10.29	1.16	557	7.3	0.8	0.1	0.1
				30–100	8.3	55.5	9.8	34.6		1.5	1.98	1.89	524	9.8	1.0	0.0	0.1
RF5	Delta Nestou	E024 44 45	N40 57 19	0–30	9.0	38.3	8.4	53.3	Silt loam	2.3	3.87	2.90	787	10.9	2.6	0.1	0.1
				30–100	9.0	71.5	2.8	25.6		1.2	1.75	0.24	271	7.5	0.7	0.0	0.1
RF6	Delta Nestou	E024 47 13	N40 54 36	0–30	8.2	84.5	3.8	11.6	Loamy sand	1.8	0.98	0.58	374	6.8	0.5	0.0	0.1
				30–100	8.1	96.5	0.84	2.6		1.4	0.82	0.00	200	3.8	0.2	0.0	0.1
RF7	Delta Nestou	E024 47 10	N40 54 41	0–30	7.0	95.5	0.2	4.3	Sand	4.0	0.30	0.00	256	3.8	0.3	0.0	0.1
				30–100	7.9	97.5	0.2	2.3		5.0	0.20	0.53	315	4.8	0.2	0.0	0.0
RF8	Delta Nestou	E024 47 45	N40 52 51	0–30	8.4	43.5	13.2	43.3	Loam	17.9	3.10	1.94	3277	18.8	1.7	0.6	0.1
				30–100	8.4	27.0	12.4	60.6		19.8	0.46	2.90	890	15.1	4.5	0.1	0.1
RF9	Delta Nestou	E024 48 17	N40 51 34	0–30	8.9	83.8	0.56	15.6	Loamy sand	6.2	2.17	0.65	1043	5.5	1.5	0.1	0.1
				30–100	9.0	79.8	3.56	16.6		7.8	0.27	0.19	531	8.3	0.6	0.0	0.0
RF10	Delta Nestou	E024 47 51	N40 52 19	0–30	8.6	76.3	4.4	19.3	Loamy sand	8.0	0.16	0.68	399	7.5	0.6	0.0	0.2
				30–100	9.3	72.3	2.4	25.3		12.6	1.27	2.47	654	11.0	1.6	0.2	0.1

hydrostatic balance. The riparian forest's soil properties show great variability, highlighting the ecosystem's adaptability, with eight out of ten samples being sandy and only two classified as loam and silt loam.

Meteorological data were utilised for climate trend analyses at both project sites. From 1980–1981 to 2022, Palo Laziale experienced marked aridity stress, attributed to diminished rainfall and escalating temperatures, with severe droughts in the summers of 2003, 2007, 2020, and 2022. Since 1994, temperatures have steadily climbed across the Lazio region's coastal area, exacerbating the effects of low rainfall between 1981 and 2021. This period witnessed prolonged and intense droughts, extending beyond just the summer months, significantly contributing to forest dieback in Palo Laziale. The Climatic Water Balance (CWB) was negative from 1951 to 2022, indicating that the forest's actual water demand frequently surpassed the potential supply. Although certain isolated years (e.g. 2018) have shown a positive CWB, reflecting increased rainfall, the rising temperatures alone could prompt another dieback event in Palo Laziale. Long-term climate monitoring, as outlined in the project after-LIFE plan, will continue beyond the project's conclusion. Limited standardised data in the Nestos area resulted in inconsistent historical series, making time-series data of varied lengths that did not fully align. Analysis indicates a trend towards increasing aridity after 1987, evidenced by several aridity indices and prolonged drought stress. Kavala is identified as a high drought risk area from 1993 data. Chrysoupoli station data (1985–2017) showed no clear drying trend due to opposing rainfall and temperature trends. On the other hand, Xanthi station data (1975–2004) suggests a shift from a humid to a sub-humid climate.

According to the floristic census, Palo Laziale hosts 490 plant species, displaying a high level of biodiversity albeit with significant species turnover over time (see La Montagna et al. 2023). Palo Laziale's vegetation comprises six main groups that can be grouped into two main groups, with vegetation series divided into forest-dominated and grassland-dominated formations (see La Montagna et al. 2024), revealing a remarkable number of 12 habitat types (habitat map in S1). The forest main group is represented by a) a deciduous woodland dominated by *Quercus cerris*, with sparse *Q. petraea* and *Q. frainetto* (habitat 91M0); b) a mosaic of marsh associations dominated by *Fraxinus angustifolia* subsp. *oxycarpa* (habitat 91B0) in the wetter areas and dominated by *Q. cerris* fin the drier portions (91M0), and clusters of Mediterranean temporary ponds with *Isoetes durieui* (habitat 3170*) in the clearing areas; c) a Mediterranean xeric forest, shrub and underbrush vegetation, with *Quercus ilex*, *Pistacia lentiscus*, *Phillyrea latifolia* and *Ligustrum vulgare* (habitat 9340), and the arborescent matorral of *Laurus nobilis* (5230*). In the grassland formations can be distinguished a) ruderal Mediterranean meadows with scattered temporary ponds with *Isoetes histrix* (habitat 3170*); b) humid meadows with basiphilous dry grasslands (habitat 6220*) and; c) annual vegetation of drift lines (habitat 1210), eroded embryonal dunes (1410, 2110), and small annual acidophilous Mediterranean grasslands. Finally, from the field surveys, other plant communities were encountered in the semi-permanent pools, including Mediterranean tall humid herb grasslands of the *Molinio-Holoschoenion* (habitat 6420), Mediterranean salt meadows (*Juncetalia maritimi* -1410), *Magnopotamion* (3150) and *Paspalo-Agrostidion* communities (3290).

Trees of habitat 91M0 vary in height from 10 to 15 m, with ages ranging from 20 to 80 years and diameters between 10 and 55 cm. The sub-formation with *Q. pubescens* occurring in the drier areas of Palo Laziale was the most affected by the forest dieback, leaving behind tree cover from the aftermath. After a massive decrease in older trees, this woodland experienced a significant understory renewal, with an increase in younger age classes (Renewal Index rising from 106.45 cm to 220 cm) and overall forest biomass, and a return of good representativity of native oak species (*Q. cerris, Q. ilex, Q. pubescens*) and ash (*Fraxinus angustifolia* subsp. *oxycarpa*). On the other hand, the forest structure of habitats 9340 and 5230* has remained steadily stable over time, except for cases of natural expansion of *Phyllirea angustifolia* and *Laurus nobilis* into areas affected by previous dieback. This forest segment, untouched by logging, shows a stable and healthy plant structure with dense vegetation, average tree diameters of 10 cm, and heights up to 4 m, without signs of decay. The canopy often creates low-light conditions with limited or balanced renewal between *Phillyrea* and *Laurus*. The alluvial forest in the Nestos Delta, classified as habitat type 91E0*, spans 300 ha. Within this area, 14 tree species were identified across 15 surveyed plots. The five species most commonly found in at least four plots have their average densities detailed in the Deliverable A.5. *Alnus glutinosa* emerges as the dominant species, exhibiting significantly higher density ($P < 0.01$) than others. A*cer negundo*, *Salix alba*, and *Populus alba* also show robust presence. However, *Fraxinus angustifolia*'s regeneration is notably scarce, with few seedlings on the Nestos River's west bank. *Alnus glutinosa*, *Salix alba*, and *Populus nigra* stands, marked by diverse diameter classes, suggest structural stability. Noteworthy is the regeneration of *Populus alba* and *A. glutinosa*, particularly along the riverbanks, contrasting with the absent regeneration of *P. nigra*. The eastern bank's alluvial forest demonstrates superior regeneration compared to the west. Alarmingly, alien species *A. fruticosa* and *A. negundo* dominate regeneration (32.3% and 21.5%, respectively), as shown in Deliverable A.5, suppressing native species. Mapping of *A. fruticosa* in both Nestos river banks revealed a cover of approximately 40 ha, with densities between 15,200 and 40,500 plants/ha,

indicating that the species occupies 50% of this area, marking a 60% increase since 2015. Botanical surveys in the eastern bank site 'Lazaros' confirmed the presence of one cluster of typical plant assemblages of 'Mediterranean temporary ponds'. In this temporary pond and the surrounding areas, other plant communities belonging to the synalliance *Callitricho-Batrachion* Den Hartog & Segal 1964 (habitat 3260) were also retrieved. Six distinct habitats were identified at the Lazaros site, underscoring its high conservation value.

Conservation actions

1- Cleaning and site preparation

Selective trimming at Palo Laziale successfully removed 70% of encroaching vegetation, revealing a rejuvenation of trees aged 5 to 18 years across dominant species like *Quercus cerris*, *Q. pubescens*, *Q. ilex*, *Fraxinus angustifolia* subsp. *oxycarpa*. The second phase maintained this clearance rate. Firebreaks and access routes for firefighting and recreational purposes were reinstated, creating a circular network for ongoing forest management and firefighting access. Generated waste contributed to organic fertiliser and mulch for local use. Phytosanitary efforts removed 45 burned or disease-threatened trees, yielding about 24 m^3 of timber, with official approval from Ladispoli Municipality. Three new temporary ponds, covering 4,000 sqm, cater to diverse biological needs with varied depths up to 1 m, designed to support water invertebrates and aquatic fauna like *Emys orbicularis*, and specific habitats for herbaceous species. In Nestos, experimental goat grazing treatment shows promising potential for controlling the invasive species *A. fruticosa*, as goats effectively remove stem phloem, which can lead to tree mortality. Additionally, two years after the removal of the alien species *A. fruticosa* and *A. negundo* from the 10 plots in Nestos, the regeneration index (IR) for both alien species showed a substantial decline from 31.75 and 21.12 cm m^{-2} to 2.07 and 2.6 cm m^{-2}, respectively. Concurrently, space was created for the expansion of native tree species. Four new ponds, totalling approximately 0.1 ha, were successfully established in three locations in the Lazaros area. Wooden sticks for fencing were placed around the ponds. Monitoring these habitats'biological evolution supports their management and conservation.

2- Reforestation

Approximately 10,000 seeds from native trees (*Q. cerris, Q. pubescens, Q. ilex, Q. suber, Fraxinus angustifolia* subsp. *oxycarpa)* collected in Palo Laziale and nearby sites (e.g. Castelporziano Presidential Estate) over the first two years of the Project resulted in about 4,000 germinations, with 2,500 seedlings subsequently planted in Palo Laziale. The germination rates were notably high for *Q. cerris* (60%), *Q. ilex* (65%), and *Q. suber* (53%), but lower for *Q. pubescens* (26%). Seeds from Castelporziano exhibited even higher germination rates, with 80% for *Q. cerris* and 66% for *Q. ilex*.

Plant pathologists isolated both non-pathogenic and pathogenic fungi from the oak acorns through metabarcoding of ITS region fungal sequences. Non-pathogenic species included *Acremonium* sp., *Cladosporium cladosporioides*, and *Penicillium* sp., common endophytes in Mediterranean oaks. Pathogens such as *Biscognauxia mediterranea* which do not immediately threaten the host, tend to remain latent until the plant is stressed. The entire genome of the pathogen was sequenced to aid future identification efforts. Sequencing analysis consistently detected fungal pathogens like *Diplodia corticola* and *Discula quercina*, major contributors to forest decline in the Mediterranean. Growth measurements taken on the oak seedlings at four and eight months post-germination revealed no significant differences between the seed stocks from Palo Laziale and Castelporziano.

3- Ex-situ conservation of habitats' keystone species

In total, 37,360, 24,520, 33,450 seeds and 6,340 achenes of *Juncus bufonius*, *Lythrum tribracteatum*, *Mentha pulegium* and *Ranunculus sardous*, were collected in Palo Laziale. According to morphometric analyses, *Ranunculus sardous* and *Juncus bufonius* have the largest achenes and smallest seeds. The seeds of all selected species are classified as orthodox, allowing long-term storage conservation in germplasm banks (Seed Information Database 2023); their internal humidity supports this classification. Although *Lythrum tribracteatum* has not yet been officially classified, our experiments suggest it is also orthodox. Further studies are needed to confirm this hypothesis. Orthodox seeds are advantageous in environments such as temporary ponds, which undergo alternating periods of drought and flooding, allowing species to accumulate seeds abundantly in the soil. All examined species require intense light exposure for germination and are temperature-dependent. Despite varying temperatures and light conditions during germination tests, no germination was observed in *I. histrix* Bory and *I. durieui* Bory. In Nestos, the collected seeds of both *Populus alba* and *Mentha pulegium* were found to be non-viable. The harsh weather conditions during both collection years, particularly the prolonged hot and dry summer, likely impacted the moisture content of the seeds, thereby severely affecting the viability of the seed embryos. *Alnus glutinosa* exhibited a high percentage of fertile seeds (95%), but the viability of the seed embryos was relatively low (25%). In contrast, *Cyperus fuscus, Pycreus flavescens*, and *Pycreus flavidus* presented a high percentage of fertile seeds (more than 90%) with satisfactory viability of the seed embryos (50% after the TTZ test). Regarding the germination test,

ten weeks of stratification proved to be sufficient to overcome seed dormancy for *Alnus glutinosa, Cyperus fuscus, Pycreus flavescens* and *Pycreus flavidus*. The germination percentages of all examined species were recorded to be satisfactory, with all or nearly all viable embryos successfully germinating (*Alnus glutinosa:* 20%; *Cyperus fuscus:* 50%; *Pycreus flavescens:* 50%; *Pycreus flavidus:* 50%).

4- Hydraulic systems

In Palo Laziale, the hydraulic system took inspiration from agricultural irrigation and rainwater harvesting techniques, transferring for the first time such a solution to restore Mediterranean coastal ecosystems impacted by climate change. It comprises three main elements: a) a drainage trench to capture surface water, reducing rapid rainfall run-off towards the sea and loss of valuable water, feeding b) an underground tank that stores water in wet periods and releases it during dry spells, and c) a network of drainage trenches that distribute the stored water throughout the forest during peaks of drought periods. The system minimises construction impact in ecologically sensitive areas, operating independently of fossil fuels (eliminating the need for pumps and water transport), and maintains a low visual profile with the underground tank. Solar-powered, remotely controlled pumps release the stored water, and local excavation material covers the channels to avoid concrete use. Most of the excavation material is reused on-site, minimising landfill waste. This intervention, alongside other conservation efforts, mitigates increasing aridity from climate change, restoring ecological conditions over 40 hectares of woodland. In Nestos, three well points were constructed around the temporary ponds at a depth of 4 m with an exploitable yield of 5 m^3 h^{-1} and a pump intake depth of 2 m. The pumps will be powered by solar panels. The uptake system is connected with a water nebulisation network (tubes, micro-sprinklers, water pumps, etc.) to provide an additional water source to the temporary ponds in case of need.

Project dissemination

LIFE PRIMED leveraged a broad community of local and international stakeholders deeply invested in the dissemination of the project's innovative solutions for ecosystem restoration, conservation, and management. The project enabled a dynamic, multi-purpose delivery mechanism to disseminate evidence-based insights and current findings, ensuring the target audience remained well-informed throughout the project's lifespan.

The dissemination efforts commenced with a press conference at the Botanic Garden of Rome (Italy), marking the project's initiation. This event set the stage for a series of strategically planned activities designed to maximise outreach and impact. Throughout the project's duration, LIFE PRIMED was featured at significant ecological and environmental conferences, such as the 13th European Conference on Ecological Restoration in Alicante, Spain, and the 10th World Conference on Ecological Restoration in Darwin, Australia. These presentations not only reached hundreds of specialists but also facilitated high-level discussions on advanced ecological restoration techniques and strategies. Researchers, competent authorities, policy-makers and restoration practitioners were also convened through three international workshops organised by the Project in Rome (Italy), Tartu (Estonia) and Drama (Greece) to gather experts on transitional freshwater wetlands and coastal forests.

In addition to these large-scale events, LIFE PRIMED emphasised educational outreach, notably through a guest lecture at the University of the Aegean (Greece), where project insights were shared with students specialising in Global Environmental Change. The project also engaged the public and specialists through interactive field trips, providing direct experiences of the project's outcomes and further bridging the gap between theoretical knowledge and practical application, finally engaging more than 2,500 people.

Networking played a crucial role in the dissemination strategy. LIFE PRIMED capitalised on various networking events with other LIFE projects and stakeholders, enhancing the exchange of experiences and visions on nature and biodiversity conservation across Europe. Noteworthy among these were the"LIFE in Forest Marketplace"event and several workshops and networking meetings across Italy, Greece and Spain that fostered future collaborations under the auspices of the EU Nature Restoration Law and within existing funding programmes for nature conservation (e.g. LIFE, Biodiversa +, Cost Action, Horizon).

These diverse activities not only enhanced the visibility and impact of LIFE PRIMED but also ensured the continuity of its innovations in ecological restoration and management across diverse European landscapes. Through a mix of direct engagement, academic collaboration, and participation in high-profile conferences, the project communicated its findings and fostered a sustainable network of knowledge and practice.

Obstacles and challenges

Due to overlapping legal conservation regimes—nature, landscape, archaeology, and hydrogeology—applying in the project sites, but prevalent in many other areas in the Mediterranean basin, executing the hydraulic systems in LIFE PRIMED required permissions from a diverse array of regulatory bodies. For Palo Laziale, this included the Regional Directorate for Environmental Policies, the Archaeological Superintendence of Lazio, the Military Engineering Corps for ordnance clearing, the Metropolitan City Civil

Engineering Department for project verification, and the Regional Forestry Command of Carabinieri, among others.

The authorisation process required navigating complex bureaucratic waters, including a multi-actor service conference, and culminated in a detailed report submitted to all relevant authorities, with the final approvals trickling in throughout a strenuous and time-consuming effort. Project coordinators and procedure managers often had to reach out to each authority individually with separate authorisation requests, leading to disparate response times and procedural complexities. Additionally, arranging necessary field inspections, such as archaeological surveys and military weapon clearances, essential for preparing the hydraulic system's construction, led to unplanned costs and potential halts in restoration efforts. Conversely, in the Nestos area, the authorization process was facilitated by the competent authority (Forest Service of Kavala), which granted full permission for research purposes due to the limited extent of the restoration area under intervention, fully embedded within a public natural protected area. The delays of the Italian site, especially when the least concerning archaeological discoveries occurred, posed significant challenges and introduced significant risks of failure for the project's financial and task management.

Discussion and conclusion

In Europe, the importance of coastal zone ecosystems is a primary driver behind extensive efforts to restore and protect them. Over the past three decades, increasing concerns about aridity, extreme weather events, and direct human pressures have led to growing momentum for conservation initiatives. However, the integration of diverse techniques and methodologies in restoration, conservation, and management practices still lags.

LIFE PRIMED stands out as one of the first ecological restoration projects in the Mediterranean that harmonised ecosystem-based knowledge with the design of restoration practices. The project's operational model—assessing degradation causes, designing targeted nature-based solutions, and monitoring their impact—aims to serve as a holistic, interdisciplinary reference for similar initiatives elsewhere.

The preparatory actions undertaken in the LIFE PRIMED project were crucial for its success. These actions included comprehensive biotic and abiotic data collection and analyses, which informed the design and implementation of restoration interventions. The soil analyses provided insights into salinity levels and soil permeability, directly influencing the selection of plant species and the design of hydraulic systems. Similarly, climatic data helped in understanding the long-term trends of aridity and temperature increases, allowing for the development of adaptive management strategies tailored to anticipate periods of drought and plan for supplemental irrigation through the innovative water harvesting systems. The botanical and forestry surveys confirmed the two sites as biodiversity hotspots, revealing critical information about the past and present composition, structure, health and regeneration potential of native species and plant communities, also providing a baseline for measuring the success of the restoration actions over time (La Montagna et al. 2023, 2024; Lombardi et al. 2023). Future projects could benefit from enhanced data collection methods and more sophisticated modelling tools to deliver data-driven, standardised and transnational restoration practices. Further integration of remote sensing technologies and high-resolution climate and soil models can provide more precise and comprehensive data, improving the accuracy of interventions.

An interdisciplinary team within the project, including foresters, biologists, pedologists, geologists, botanists, zoologists, and environmental engineers, innovatively applied traditional and new practices. These practices were tailored to enhance soil, water and habitat resilience in declined coastal forest ecosystems within Natura 2000 sites in Bosco di Palo Laziale (Italy) and Nestos Delta (Greece). The concrete conservation actions implemented, including the establishment of temporary ponds for recolonisation of aquatic plant and animal species, integrated invasive plant species control, seed viability tests and germination trials for ex-situ conservation of keystone species, free-pathogen plant propagation for woody species, and hydraulic works, can be considered successful based on the project's outcomes and comparisons with similar initiatives.

The temporary ponds established in both project sites provided crucial habitats for species such as the European pond turtle (*Emys orbicularis*), the Italian tree frog (*Hyla intermedia*), and various endemic aquatic plants (Pizzuti Piccoli 2022). These ponds have been shown to support a diverse array of flora and fauna, demonstrating that ecological restoration practices can also be successfully oriented to such particular habitat types. The integrated invasive plant species control measures, including the use of goat grazing and manual removal, effectively reduced the spread of invasive species like *Amorpha fruticosa* and *Acer negundo*. Two years after the removal of these invasive species, the regeneration index (IR) for both decreased significantly in Nestos, creating space for native species to flourish (Avramidou et al. 2023). Seed viability tests and germination trials yielded high germination rates for keystone plant species such as *Quercus cerris*, *Quercus ilex*, *Cyperus fuscus*, *Pycreus flavescens*, and *Pycreus flavidus,* ensuring the availability of healthy seedlings for both present and future habitat reinforcement activities. The use of metagenomic techniques in supporting forest nursery operations has proven invaluable for the relatively easy and quick detection of pathogens in woody species, thereby preventing the cultivation

of seedlings with pathogen presence and the introduction of infected plants (Beccaccioli et al. 2021).

LIFE PRIMED's approach of combining traditional practices with technological innovations, designed and calibrated based on preliminary scientific data collection and analysis, stands out. The project's success is evidenced by the recovery of native plant species and communities, improved habitat conditions and species recolonisation, as well as positive feedback from the local stakeholders involved.

On the other hand, LIFE PRIMED highlighted the fragility of the administrative and bureaucratic processes in the Mediterranean region, a common constraint for restoration practitioners across Europe (Cortina-Segarra et al. 2021). Particularly, the project faced significant hurdles due to overlapping protection regimes in Palo Laziale (naturalist, landscape, archaeological, hydrogeological), necessitating multiple authorisations from a dozen authorities. This regulatory complexity led to severe delays and additional costs, underlining the need for synchronised dialogue among all levels of governance. Aligning national, regional, and municipal legislation and creating harmonised procedures is crucial to balance and prioritise conservation efforts effectively and to prevent conflicts between different protection regimes.

The successful solutions of LIFE PRIMED, as well as the major obstacles and barriers, have been widely disseminated through scientific conferences, workshops, and educational outreach. These activities ensured that the project's findings and methodologies were shared with both the scientific community and the general public. The project's presence at major ecological conferences (e.g. SER, EGU, IBC, SEFS, MPCW, etc.) and its capacity to gather high-level audiences at the international conferences it directly organised (e.g. Natura 2000 Networking Event) facilitated high-level discussions and fostered collaborations that drove to further restoration projects (e.g., NewLIFE4Drylands, LIFE SEEDFORCE, HORIZON-MSCA-2022 TOPIO, Horizon Europe MONALISA) and enabled permanent participation in key sectorial panels (e.g. BiodivRestore Knowledge Hub, GENMEDA, etc.). In situ educational programs and field trips provided hands-on learning and training experiences for early-stage researchers and professionals, further promoting the long-term replicability of the project's innovative approaches.

For future restoration initiatives to succeed without undue delay or resource wastage, such as a streamlined consultation process and multi-actor dialogue must be maintained. A unified approach that supports practitioners and aligns conservation priorities is imperative to reduce bureaucratic friction and enhance the impact of ecological restoration projects across the Mediterranean region and beyond.

LIFE PRIMED underscored the essential role of interdisciplinarity and an ecosystem-based approach in restoration ecology. This project broadens the scope of these practices, advocating for their systematic and regular application to improve biodiversity conservation outcomes. In view of the transposition of the Nature Restoration Law prescriptions in the EU Member States, experiences such as those built within LIFE PRIMED are crucial to promoting a consensus-driven framework for more sustainable and culturally respectful ecological restoration endeavours and environmental stewardship.

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Data availability All data and deliverables are on the official Life Project website "https://www.lifeprimed.eu/en/download"

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