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STUDY PROTOCOL

REVISED Monitoring spatiotemporal changes in global change drivers and their effects on semiarid woodlands and forests - fieldwork protocol

[version 2; peer review: 2 approved]

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Abstract

Training schools play a vital role in COST actions, particularly for young researchers, as they provide opportunities to visit international laboratories and learn new methodologies. In May 2024, CLEANFOREST organized its first training school, *Monitoring Spatiotemporal Changes in Global Change Drivers and Their Effects on Semiarid Woodlands and Forests*, held at the Faculty of Science of the Universidade de Lisboa. The training school included a field trip designed to explore global change drivers and their impacts on semiarid woodlands and forests. Participants engaged in hands-on activities to understand how forest ecosystems interact with key global change factors such as air quality and climate change. They were introduced to various monitoring techniques and parameters for assessing forest health, including ecosystem fluxes, tree physiology, mortality, and regeneration. Additionally, participants examined plant biodiversity and functional ecology, focusing on lichens and their

Open Peer Review

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	1	2
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connection to air quality, and soil physico-chemical properties. Participants applied these methodologies in real-world scenarios, conducting measurements (forest structure assessment, lichen diversity sampling, shrub and herbaceous diversity estimation, deadwood measurement and soil physico-chemical analysis) in different grazing management settings to assess their effects on tree growth, biodiversity, and soil properties. After the practical experience in the field using these experiences, participants were divided into groups to analyze and discuss collected data together with trainers. Key findings were summarized in presentations, together with main take home messages and suggestions on further questions to be explored and related attributes to monitor.

This paper presents the field trip protocol used at Companhia das Lezírias, where simplified versions of established methodologies for sampling various ecosystem components were employed. The protocol provides a valuable reference for replicating similar studies, ensuring consistency in methodologies for future training activities.

Plain Language Summary
Training schools provide young researchers, especially PhD students, with opportunities to learn new skills by working with experts. In May 2024, CLEANFOREST held its first training school, *Monitoring Spatiotemporal Changes in Global Change Drivers and Their Effects on Semiarid Woodlands and Forests*, at the Universidade de Lisboa.

Participants went on a field trip to study how environmental changes, like air quality and climate change, impact forests. They learned methods to monitor forest health, looking at tree growth, soil quality, and biodiversity. The training also included studying organisms like lichens, which reflect air quality, and how different grazing practices affect ecosystems.

Using these experiences, participants analyzed data and suggested ways to manage forests sustainably in response to global environmental challenges.

This paper explains the step-by-step approach from the field trip, offering a guide for future studies.

Keywords
ecosystem health, sampling methods, soil, lichens, vegetation, training school, COST Action, Mediterranean ecosystem

Toronto, Canada

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the COST Actions gateway.

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REVISED **Amendments from Version 1**

In response to the reviewers' comments, we have implemented several key revisions to enhance the clarity, relevance, and impact of our manuscript.

Data Availability & Replicability – To address concerns about replicability, we have added references to multiple published studies that illustrate the application of our methods. This includes datasets related to litter decomposition, humus classification, soil properties, and vegetation cover.

Clarifying Objectives – The introduction was expanded to clearly define the study's aims. We explicitly state that the field trip focused on understanding how grazing, nitrogen deposition, and climatic stressors impact forest ecosystems. The revised text strengthens the link between the methodology and global change drivers.

Interactions Between Global Change Drivers – Although manipulation experiments were not conducted, we included a discussion on how nitrogen deposition and grazing interact with climate change, referencing key studies. This addition provides a broader ecological perspective.

Policy Implications – The manuscript now explicitly links the study to European policies, including the European Green Deal, Nature Restoration Law, and Forest Monitoring Law.

Justification for Methods – We clarified that our protocol compiles well-established methods to offer a cost-effective, practical handbook for training purposes. The selection of methods prioritizes accessibility and feasibility.

Limitations Section – A new section outlines potential biases, including challenges in lichen identification, vegetation sampling, and soil classification, ensuring transparency in methodology.

Any further responses from the reviewers can be found at the end of the article

Introduction

CLEANFOREST COST Action CA21138

The ability of forests to mitigate climate change hinges on their capacity to adapt to global change drivers like frequent climate extremes and changes in atmospheric pollutants (e.g., carbon dioxide, reactive nitrogen, and sulfur compounds). These drivers could play a synergistic, antagonistic or predisposing role in affecting forest ecosystem health and functioning, but they are often studied in isolation. This fragmented approach leads to uncoordinated and scattered information across different research communities. Without a holistic view, the future of Europe's forests and their climate mitigation potential could be misjudged.

CLEANFOREST (https://cleanforest.eu) is a COST Action started in 2022 that aims to establish an inclusive, multidisciplinary pan-European network that leverages existing expertise and infrastructures (e.g., monitoring networks, manipulation experiments) to coordinate research efforts, standardize measurements, and foster collaboration. This network will advance scientific knowledge, identify research gaps, and provide insights for future experiments and monitoring. Additionally, CLEANFOREST will engage key stakeholders, including policymakers, small companies, and citizen science associations, to promote synergies and develop evidence-based solutions to policy, societal, and technological challenges.

Framework and objectives of the training school

Among the networking opportunities available to COST Actions, training schools are particularly important for young researchers, as they allow PhD researchers to visit internationally recognized laboratories and learn new methodologies to apply in their work or consolidate their expertise. Moreover, it also offers an important opportunity for young researchers to build their network of future collaborations. In this context, CLEANFOREST organized its first training school, "Monitoring Spatiotemporal Changes in Global Change Drivers and Their Effects on Semiarid Woodlands and Forests," in May 2024 at the Faculty of Science of the Universidade de Lisboa. The objectives of the field trip of the training school included a comprehensive exploration of various global change drivers and their impacts on natural ecosystems by considering real case studies.

Semiarid woodlands and forested ecosystems are increasingly vulnerable to degradation due to multiple environmental drivers, including climate change, land-use change, fragmentation, and pollution. Understanding how different ecosystem components respond to these pressures is essential for assessing overall ecosystem degradation—both in terms of biodiversity and ecosystem functions/services. With this in mind, our goal was to compile and evaluate different methodologies for assessing these impacts, linking each ecosystem component to the drivers it responds to most strongly. Specifically, the field trip conducted as part of the training school aimed to provide participants with hands-on experience in assessing global change drivers and their real-world impacts on semiarid woodlands and forests. Through practical activities, students gained a deeper understanding of how forest ecosystems interact with key stressors such as air quality and climate change. Participants learned to quantify the effects of varying grazing intensities, atmospheric nitrogen pollution, and climatic stressors on multiple aspects of forest ecosystem health and functioning, including forest structure, tree physiology, carbon and water fluxes, tree regeneration and mortality, biodiversity, and soil health. Additionally, participants delved into the biodiversity and functional ecology of plants, including lichens and their relationship with air quality. The training also covered soil-related aspects, including the characterization of forest soils and their physico-chemical properties. Participants applied their knowledge in real-world scenarios, conducting measurements and assessments (forest structure assessment, lichen diversity sampling, shrub and herbaceous diversity estimation, deadwood measurement and soil physico-chemical analysis) in different grazing management settings to understand the effects on tree growth, biodiversity, soil decomposition and physico-chemical properties. Finally, participants engaged in data processing and analysis, synthesizing their findings to draw conclusions, suggestions on further questions to explore and recommendations for sustainable forest management to promote biodiversity and increase forest resilience under global change. This way, participants had the opportunity to link scientific advances to forest-related strategies and regulations adopted (e.g., Nature Restoration Law) and under discussion (European Forest monitoring Law and soil strategy) within the European Green Deal.

Here, we present the protocol used in the field trip at *Companhia das Lezírias*. In this protocol, the trainers describe simplified versions of well-established methodologies to sample different components of the ecosystem. We focused on presenting these methods in a way that is accessible and practical for users. The methods were selected for their simplicity, cost-effectiveness (requiring no expensive equipment), and comprehensive coverage of the key aspects of a forest study.

This document therefore provides a valuable reference for replicating similar studies and applying consistent methodologies in future training activities.

Methods

Decision Hierarchy

The Decision Hierarchy developed for research in the CLEANFOREST fieldwork protocol outlines the used structured approach to decision-making, through different levels of decisions.

1. Establish Investigation Objectives

- Define the research focus (e.g., climate change, air pollution, grazing, tree decline)
- Identify key parameters to measure
- Formulate hypotheses and expected outcomes

2. Study Area Selection

- Identify representative locations (e.g., farm at Companhia das Lezírias)
- Classify land-use intensity (e.g., high nitrogen, grazing, control areas)
- Select appropriate plots for investigation

3. Data Collection & Sampling Design

A. Climate Data Retrieval

- Obtain meteorological datasets
- Calculate climate variables and indexes (e.g., SPEI, dryspell duration)

B. Air Pollution Assessment

- Identify major pollution sources (e.g., urban, agricultural emissions)
- Measure nitrogen concentration levels and critical load exceedances

C. Grazing Intensity Analysis

- Categorize plots based on grazing pressure (low, medium, high)
- Use stratified sampling to assess historical grazing impacts

D. Tree Decline Investigation

- Identify potential drivers: drought, soil degradation, climate change, pests, air pollution, land use changes
- Record tree mortality rates and natural regeneration patterns

E. Forest Ecosystem Monitoring

- Forest Structure Assessment: Measure tree height, DBH, cork layer width
- Lichen Diversity Sampling: Follow European standard protocols
- Shrub and Herbaceous Diversity: Estimate cover percentage per species/group
- Deadwood Measurement: Assess CWD, snags, stumps, decay classification

F. Soil Physico-Chemical Analysis

- Identify soil horizons (O, A, E, B layers)
- Measure soil properties (texture, pH, organic matter, nutrient levels)
- Assess soil water retention and microbial activity

4. Data Processing & Analysis

- Compile collected data
- Conduct statistical analysis (e.g., multivariate analysis, correlation studies)
- Interpret ecosystem interactions and effects of global change drivers

5. Synthesis & Reporting

- Generate findings and conclusions
- Compare results with existing literature and monitoring data
- Formulate recommendations for sustainable forest management
- Integrate findings into broader environmental and conservation strategies
- Recommend areas for further study

Study area

Companhia das Lezírias (https://cl.pt/en/), located in Portugal close to Lisbon, is one of the country's most prominent agricultural enterprises and land management companies. Situated in the heart of the Tagus River estuary, it encompasses over 18,000 hectares (approximately 44,000 acres) of diverse landscapes, including farmland, forests, wetlands, and natural habitats. The company traces its origins back to the 19th century when it was established to promote agricultural development and land reclamation in the region. Over time, it has evolved into a multifaceted organization involved in various agricultural activities, forestry, nature conservation, and ecotourism initiatives. *Companhia das Lezírias* benefits from its strategic location, enjoying a Mediterranean climate characterized by hot, dry summers and mild, wet winters. The proximity to the Atlantic Ocean moderate temperatures and

influences weather patterns, creating favorable conditions for agriculture and supporting rich biodiversity.

Agriculture is at the core of *Companhia das Lezírias*' activities, with the cultivation of crops such as cereals, vineyards for wine production and olive groves for olive oil. Livestock farming, including cattle and horses, also plays a significant role in the company's operations. In addition to agriculture, *Companhia das Lezírias* is committed to sustainable land management practices and nature conservation. It oversees the preservation of natural habitats and wildlife, including the protection of endangered species and the restoration of wetlands. The company actively promotes ecotourism initiatives, allowing visitors to experience the region's natural beauty while raising awareness about environmental conservation such as the case of EVOA (Espaço de Visitação e Observação de Aves, https://evoa.pt/)

In conclusion, *Companhia das Lezírias* stands as a testament to the intricate interplay between geology, climate, land use, and history. Its story is one of resilience, innovation, and stewardship, reflecting the enduring connection between humanity and the land we inhabit. Its extensive land holdings, diverse ecosystems, and dedication to responsible stewardship make it a key player in shaping the socio-economic and environmental landscape of the region.

Climate

Companhia das Lezírias is located at (38° 47′ 24.01 N; 8° 54′ 11.10 W) 10–20 m above sea level and on a gentle 1.6% slope. Long-term mean annual rainfall in the sampling location is 683 mm with a mean annual temperature of 16.07 °C and 831 mm mean annual potential evapotranspiration, using the Thornthwaite approach (E-Obs ECA&D, 1950–2012). It is representative of the typical Mediterranean climate characterized by hot and dry summers and wet and cold winters as described in Köppen's classification. Rainfall occurs predominantly from October to April, while summer water deficit prevails from June to September. This geographic area of Portugal is considered a semi-arid region (Kurz-Besson *et al.*, 2016). Temperature presents minimum values in January and maximum in August. In this region, the monthly mean maximum temperatures have an intra-annual range of 12 to 18 °C in January and 25 to 36 °C in August; whilst the minimum monthly mean temperature in January fluctuates between 3 to 9 °C and 13 to 19 °C in August. The maximum monthly daily precipitation mean occurs in December with circa 4 mm/day and the minimum occurs during the summer months, with almost no precipitation (Kurz-Besson *et al.*, 2016).

Geology

The geology of *Companhia das Lezírias* is characterized by a variety of sedimentary formations that have been shaped over millions of years. Located in the Tagus River estuary, the region's geological features play a significant role in shaping its landscapes, soils, and agricultural potential. Sedimentary rocks are predominant in the geology of *Companhia das Lezírias*. These rocks are formed by the accumulation and compression of sediments over time. Examples of sedimentary rocks found in the area include sandstone and clay. The proximity to the Tagus River estuary has led to the deposition of alluvial sediments in the region. Over millennia, the river has transported sediments from upstream sources, depositing them in the floodplain and delta areas. These river deposits contribute to the fertility of the soil, making them ideal for agriculture. The entire watershed is located on deep Miocene sands and the main soil types include regosols and podzols. The geological composition of *Companhia das Lezírias* has a direct influence on soil formation processes. The combination of alluvial deposits, coastal sediments, and weathered rock materials gives rise to a diverse range of soil types, each with its own characteristics and agricultural potential. These geological features contribute to the region's diverse landscapes, fertile soils, and agricultural productivity, making it a key factor in shaping the socio-economic and environmental dynamics of the area (Gonçalves *et al.*, 2012; von Essen *et al.*, 2019).

Land-use

For over a century, this area was managed as an agro-silvo-pastoral system. Today, it functions solely as a silvo-pastoral system, with sheep replaced by cattle. Cork is the primary economic product, harvested every nine years, yielding an average of 986 kg per hectare. The cattle stocking rate averages 0.47 animal units per hectare. Cork oak woodlands are the predominant land use and the main focus of management, covering 6,751 hectares (78% of the forested area). Other land uses include pine and eucalyptus plantations (1,900 hectares), agricultural fields, pastures, and reservoirs (Gonçalves *et al.*, 2012; von Essen *et al.*, 2019).

Global change drivers and tree decline

a) Climate change

The drought has been identified as a significant driver of change in ecosystems like the *Companhia das Lezírias*. According to studies, drought exacerbates existing vulnerabilities and stresses the ecosystem, influencing vegetation dynamics, biodiversity, and ecosystem services. In particular, cork oak woodlands, which dominate the area, are highly sensitive to changes in water availability. Extended periods of low precipitation can lead to declines in tree growth, reduced regeneration of cork oaks, and shifts in the understory composition (such as shrub encroachment). Moreover, the added pressure from agricultural activities in the surrounding floodplain, where nitrogen deposition is elevated, can compound the negative impacts of drought. The combination of climatic stress and anthropogenic activities makes the region more vulnerable to long-term ecological changes, affecting both biodiversity and ecosystem functioning (Kurz-Besson *et al.*, 2016).

b) Air pollution

The primary sources of air pollution in the region stem from urban emissions originating in the metropolitan Lisbon area, approximately 45 km to the west, and agricultural activities dispersed across the Tagus floodplain (Figure 1). Locally, fertilized annual crops, including rice fields and irrigated farmland, surround *Companhia das Lezírias* to the west and northeast, contributing to external nitrogen pollution pressures. Volatilized fertilizers from these high-intensity agricultural zones are carried by prevailing winds into the woodlands located downwind.

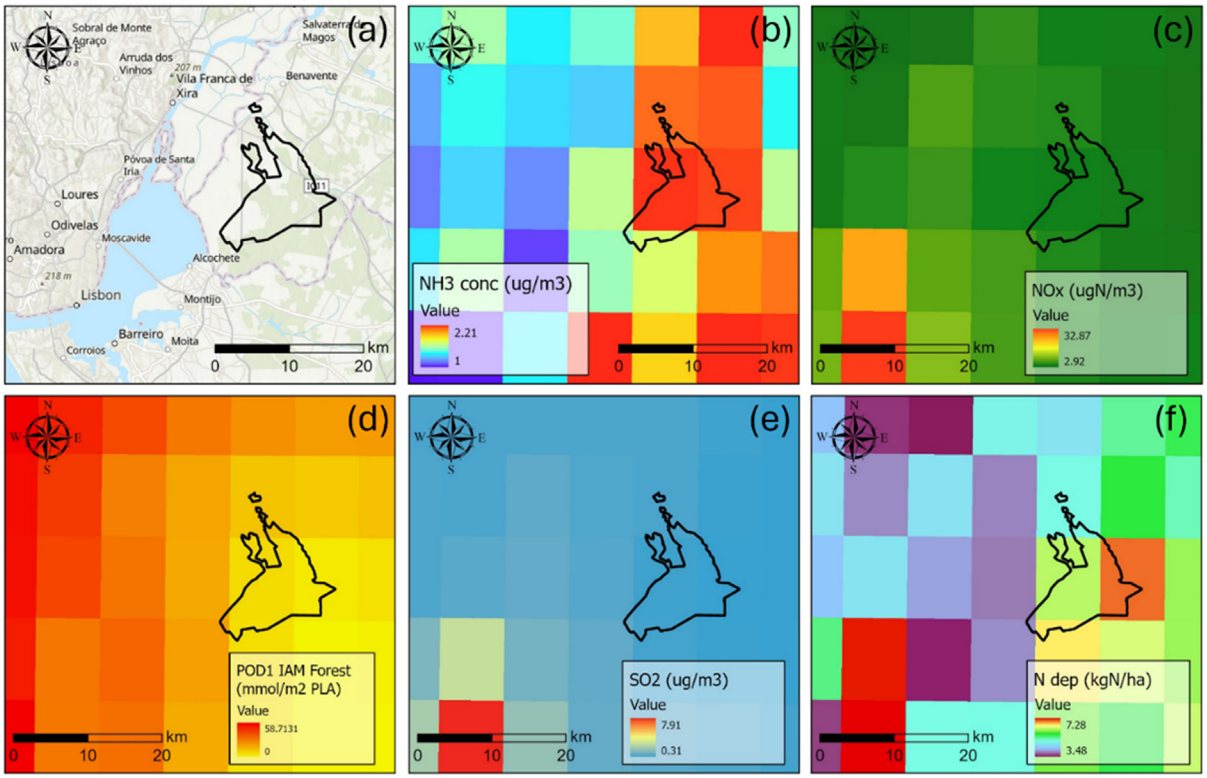

Figure 1. (**a**) Location of the CL estate in the Lisbon area over ArcGISPro world hillshade and topographic map (layer credits: Instituto Geográfico Nacional, Esri, TomTom, Garmin, Foursquare, FAO, METI/NASA, USGS, CGIAR); (**b**) modelled NH_3 concentration (CLe 1 μg/m^3); (**c**) modelled NO_x concentration (CLe 30 μg/m^3); (**d**) modelled POD1IAM for forests (CLe 13.7 mmol/m^2 PLA); (**e**) modelled SO_2 concentration (CLe 10–20 μg/m^3); (**f**) total nitrogen deposition (CLo ~5 kg/ha). Modelled concentration and deposition from the EMEP MSC-W for 2019 (Data from The Norwegian Meteorological Institute, https://www.met.no/en).

Modelled average nitrogen concentrations indicate that critical levels are exceeded only for reduced nitrogen forms (NH_3), with annual concentrations surpassing the general threshold of 1 μg/m^3 for sensitive organisms like lichens and bryophytes. For other pollutants, critical levels (CLe) for vegetation are not exceeded within the study area (Mills *et al.*, 2017; Pinho *et al.*, 2012). Regarding nitrogen deposition, the recorded levels slightly exceed the indicative lower critical load (CLo) of 5 kg/ha for the *montado* ecosystem (Bobbink *et al.*, 2022).

c) Grazing

The study area includes extensive cattle farming, where grazing is managed by dividing the land into fenced patches using barbed wire. Grazing levels vary across patches, ranging from areas completely excluded from grazing since 1998 to those with progressively higher cattle densities, with an average of 9.8 cattle heads per hectare per year, a minimum of 19, and a maximum exceeding 300 cattle heads per hectare per year. Grazing intensity stratification was performed using these density values. A grazing intensity index for each patch was calculated based on the number of cattle heads (C) present per plot during each farming year, defined as October to September, over the period from 2007 to 2016.

$$Index = [\frac{1}{9}\times C_{2007\text{-}2008} + \frac{1}{8}\times C_{2008\text{-}2009} + \frac{1}{7}\times C_{2009\text{-}2010} + \frac{1}{6}\times C_{2010\text{-}2011} + \frac{1}{5}\times C_{2011\text{-}2012} + \frac{1}{5}\times C_{2012\text{-}2013} + \frac{1}{4}\times C_{2013\text{-}2014} + \frac{1}{3}\times C_{2014\text{-}2014} + \frac{1}{2}\times C_{2015\text{-}2016} - (number\ of\ years\ under\ grazing\ exclusion)]$$

(Where C represents the number of cattle heads per hectare in a given year). The increasing importance attributed to the recent years was used to give extra weight to recent years' grazing intensity while subtracting the grazing years intended to differentiate sites with grazing exclusion for many years.

From the state of the art in the subject, we expect that increased nitrogen deposition can initially enhance plant productivity, potentially benefiting grazers. However, grazing can exacerbate the effects of nitrogen deposition by selectively removing certain plant species, leading to a decline in biodiversity and forage quality. Furthermore, climate change,

through increased temperatures and altered precipitation patterns, can further modify these interactions, influencing ecosystem processes and outcomes. These interactions are less known in the Mediterranean climate and need further work despite some works having been already developed (e.g. Nogueira *et al.*, 2018; Ochoa-Hueso *et al.*, 2017)

d) Tree decline

Tree mortality or decline in *Companhia das Lezírias* (Figure 2) could be influenced by various factors, including:

i) Drought and water stress: in regions with Mediterranean climate, like Portugal, droughts can be a significant stressor for trees, leading to reduced water availability and increased susceptibility to diseases and pests.

ii) Soil degradation: poor soil quality, erosion, or soil compaction can affect tree health and growth, leading to mortality or decline over time.

iii) Climate change: climate change can exacerbate existing stressors on trees, such as increasing temperatures, altered precipitation patterns, and more frequent extreme weather events, all of which can negatively impact tree health and increase mortality rates.

iv) Land use management changes: changes in land use practices, such as frequent soil mobilization that might affect trees' lateral roots.

v) Pests and diseases: certain pests and diseases, such as bark beetles or fungal pathogens, can attack trees, causing mortality or decline, especially if the trees are weakened by other stressors like drought or poor soil conditions.

vi) Air pollution: pollution from agricultural activities, industrial emissions, or traffic can contribute to tree stress and decline, particularly if the trees are exposed to high levels of pollutants over an extended period.

Understanding the specific factors contributing to tree mortality or decline in *Companhia das Lezírias* would require detailed assessments and monitoring of tree health, environmental

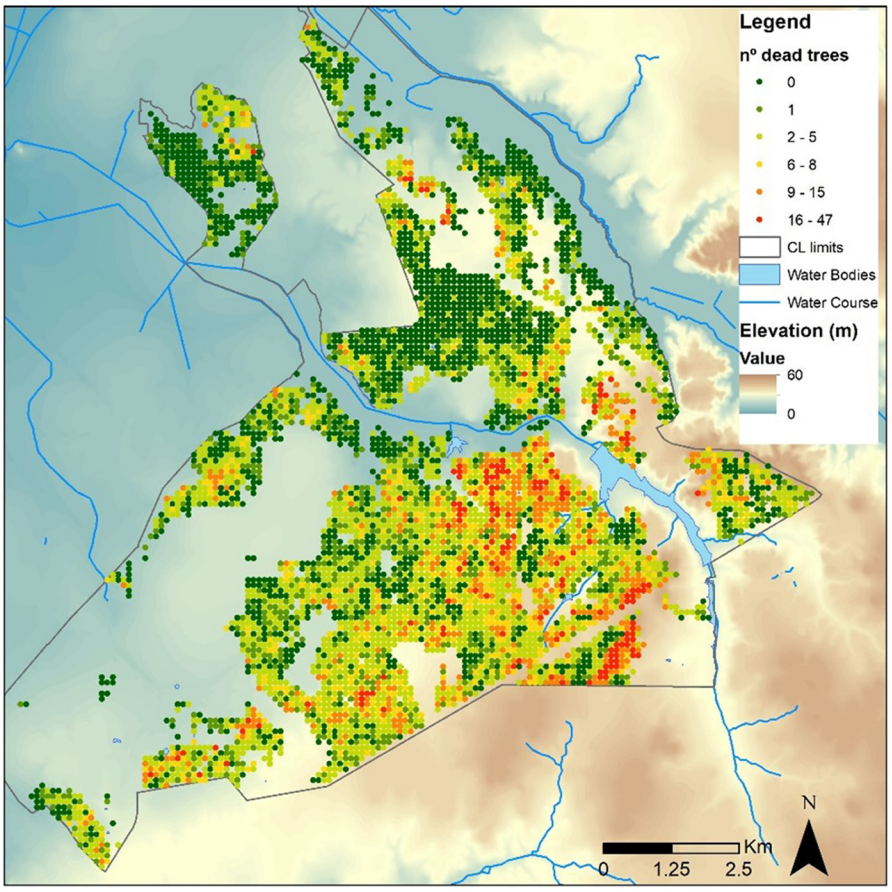

Figure 2. Cork oak tree mortality (nº dead trees/ha) from 2014 to 2019 at *Companhia das Lezírias*. This figure has been reproduced with permission from Príncipe, 2022.

conditions, and potential stressors in the area. Implementing effective management strategies, such as improved water management, pest and disease control measures, soil conservation practices, and sustainable land use planning, can help mitigate tree mortality and promote healthier forest ecosystems.

Tree decline, especially on podzols, has already been observed at *Companhia das Lezírias*. The natural regeneration of the tree stand is not uniform, and it is lacking in some places due to grazing and shrub clearing. No fires have been reported in the last 20 years. Conflicting interests arise from the livestock grazing component that affects the natural regeneration of cork oak trees. Recently, interventions and adjustments to promote natural regeneration and active afforestation (*e.g.*, fencing combined with shrub clearing) have been included in the stakeholder's management plan.

Monitoring ecosystem health

Sampling design

Select areas with different characteristics in terms, for example, of land use, pollution load, anthropogenic activities, etc. In all areas, several metrics should be measured. In our activity, three areas, corresponding to treatments related to land-use intensity, were sampled: high nitrogen (very high cattle pressure), grazing (low-medium cattle pressure) and control (grazing exclusion) (Figure 3). Inside each area, 3 plots of 1 hectare (replicates) with at least 30% tree cover and grasses closest to the patch centroid were sampled (Figure 3). At each plot, the students were divided into groups and assessed: #1 forest structure and lichen diversity; #2 shrub diversity; #3 herbaceous diversity; after assessing all previous indicators all subgroups together sampled #4 soil. Sampling methods for each component (Table 1) are described below.

Assessing forest structure

Inside a plot with 25-meter radius, measure tree height, and diameter at the breast height (DBH) of all the adult trees (> 10 cm DBH) within the limits of the sampling plot. Most of the trees present inside the plot in our study were cork oaks, then the debarking year and the width of the cork layer were registered.

Select the closest trees in four different quadrants to collect 5 leaves of different ages from one apical branch randomly collected in the south-facing aspects at the same canopy location in each tree. Measure the photosynthetically active radiation and infrared leaf reflectance in each leaf, immediately after cutting the branches, using a UniSpec spectroradiometer (PP-Systems, Haver hills, MA, USA). The reflectance spectra must be assessed in the range of visible light (402 nm) to nearinfrared (1148 nm) wavelengths.

Use three linear belts with 2 m width, 1 m along both sides of linear transects 10 m long, to search for saplings and young trees (the same transects will be used to sample Shrub Diversity). For each young tree, estimate the height using a measuring stick of 1 meter. For all the trees higher than 50 cm, register the diameter at the base of each tree.

Sampling lichen's diversity

The European standard protocol for lichen sampling (Asta *et al.*, 2002; Cristofolini *et al.*, 2014) has been applied in numerous studies to assess lichen biodiversity across environmental gradients such as air pollution, climate, land-use intensity, and management practices (Lättman *et al.*, 2014; McCarthy *et al.*, 2009; Munzi *et al.*, 2014). Adopting a standardized approach helps minimize variability and potential biases in the data.

Here, we followed an adaptation of the European standard method, reducing the number of sampled trees and focusing solely on macrolichen species, thus greatly reducing sampling time without compromising the overall lichen diversity assessment (Bergamini *et al.*, 2005). At each sampling location, select four trees nearest to the centroid of the site, ensuring they meet the required conditions outlined in the protocol: trees should be healthy, with a straight trunk (no more than a 20° deviation from vertical), free from injuries or branches up to 1.5 meters, and with a circumference at breast height between 50 and 250 cm. The maximum distance between selected trees should not exceed 50 meters (Figure 4). For replicability purposes, see studies from Rocha *et al.* (2019).

To minimize sampling variability, it is crucial to standardize tree characteristics as much as possible. This can be achieved by selecting trees of the same species to maintain consistency in bark texture, pH, and water retention capacity. Place a 50 x 10 cm sampling grid, divided into 5 squares, on each of the four main tree aspects (N, S, E, W), totaling a sampling area of 2000 cm^2 per tree (Figure 4). Record all visible macrolichens (foliose and fruticose) within the grid, either on-site or for later identification in the laboratory. Estimate species abundance based on the number of squares they occupy (with a maximum of 20 occurrences per tree, 5 per aspect), and then calculate the average abundance per site. Species identification should follow the nomenclature guidelines provided by Nimis and Martellos (2021).

Sampling shrub diversity

The understory of cork oak woodlands consists of a mix of shrub-dominated patches embedded within a grassland matrix (Figure 5). The extent of these shrubland patches, ranging from dense shrub encroachment to sparse or absent coverage, is influenced by various factors. These include grazing intensity, management practices such as shrub clearing, and environmental variables like climate, topography, and soil characteristics (Nunes *et al.*, 2019).

Although dense shrub cover may be considered not suitable for grazing, shrub communities provide significant ecosystem services. They contribute to carbon storage, enhance soil fertility, and increase nitrogen mineralization rates, potentially supporting ecosystem health and promoting tree regeneration.

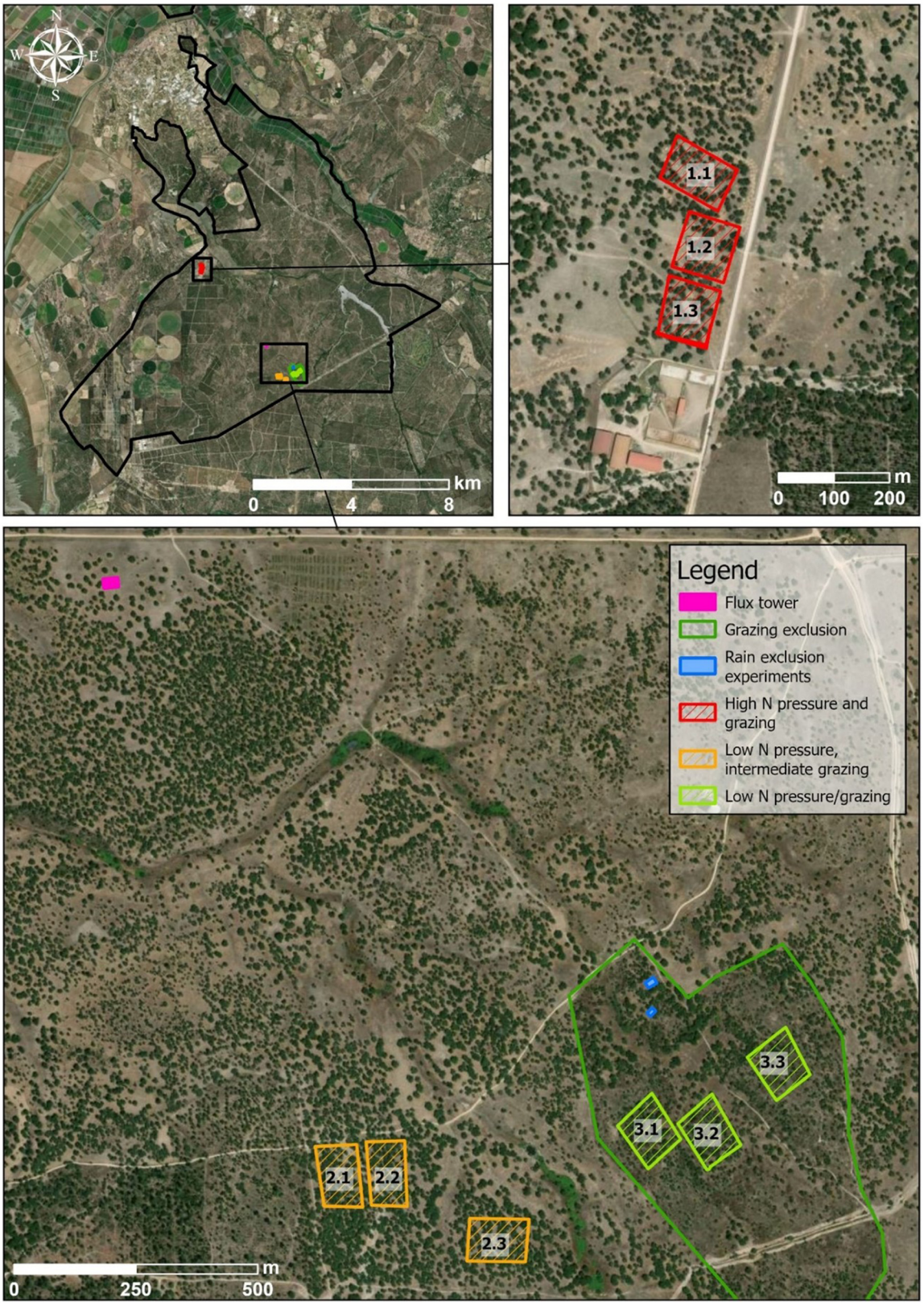

Figure 3. Location of three sampling plots (replicates) within each area (1 - High N pressure and grazing, 2 - low N pressure/grazing and 3 - Grazing exclusion) in *Companhia das Lezírias*. The location of the flux tower and of rain exclusion experiments is also indicated on the map. Ortophoto from ArcGISPro World Imagery layer (Credits: Maxar, Microsoft, Earthstar Geographics).

Table 1. Visual schematic summarizing the data collection process described in the next paragraphs.

Parameter	Area characteristics	Selection/Sampling method	Metrics & measurements
Land use type	High nitrogen (high cattle pressure) Grazing (Low-medium cattle pressure) Control (Grazing exclusion)	Select 3 plots of 1 hectare in each area	Grazing level (proxy for nitrogen availability)
Plot type	30% Tree cover Grasses near centroid	3 replicates of 1 hectare in each area	Size: 1 hectare, Locations with 30% tree cover
Forest structure	25 m radius plot	Measure tree height & diameter at breast height (DBH) of adult trees	Height of tree DBH >10 cm
Young tree diversity	Linear transects, 2m x 10m	Measure saplings in 3 linear transects	Tree height Diameter at base of trees >50 cm tall
Lichen diversity	On Trees (healthy, upright, no injuries)	Use sampling grid (50 x 10 cm) at 4 aspects (N, S, E, W) on 4 trees	Macrolichen species Abundance by squares (20 per tree max)
Shrub diversity	Shrubland patches in oak woodlands (same transects used in 4)	Line intercept method along 3 transects	Shrub species cover (percent) based on transect length intercepted
Herbaceous diversity	Herbaceous communities (semi-natural or sown grasslands)	Quadrats (50 x 50 cm) on 3 transects	% cover by species or functional groups (grasses vs. forbs)
Forest deadwood diversity	Coarse woody debris, stumps, snags, lying deadwood	Measure deadwood components	Diameter (cm) Length (m) Height (m) Decay class (1–5)
Soil composition	All plots	Soil samples from each plot	Soil nutrient levels pH Organic matter Other relevant parameters

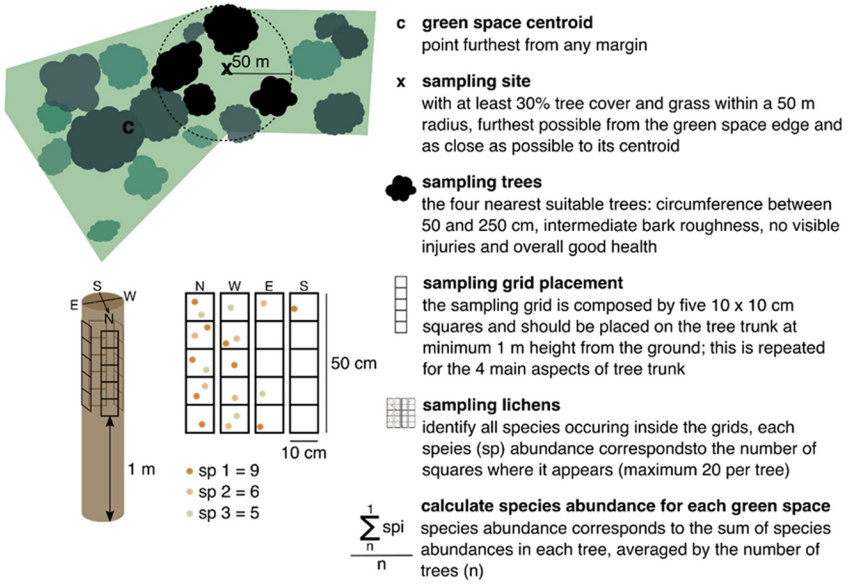

Figure 4. Selection of a single sampling site inside a patch with homogeneous land-cover/use and method to sample lichens diversity, adapted from the European standard method (Asta *et al.*, 2002; Cristofolini *et al.*, 2014). This figure has been reproduced with permission from Rocha *et al.*, 2022.



Figure 5. Shrubland patches in the understory of cork oak woodlands (*montado*).



Figure 6. Semi-natural grasslands dominating the understory of cork oak woodlands.

These benefits depend on the diversity of shrub species and their functional traits (e.g., Eldridge *et al.*, 2024; Köbel *et al.*, 2021).

To sample the shrub community, the line intercept method (Elzinga *et al.*, 2001) can be applied. This involves placing three 10-meter transects in each plot and measuring the proportion of each species intercepting the transects. Shrub cover is expressed as a percentage based on the intercepted length. Each recorded species is subsequently classified into functional groups for further data analysis.

Sampling herbaceous diversity

The dominant matrix in the understory of cork oak woodlands is made of semi-natural or sown grasslands, used as pastures (Figure 6). These communities, dominated by annual herbaceous species, have a remarkably high plant diversity and high resilience to climate interannual fluctuations, characteristic of these ecosystems, showing a faster species turnover than perennials. They play a critical role in ecosystem functioning (*e.g.*, energy flow and nutrient cycling), providing important ecosystem services (e.g. quantity and quality of pasture for livestock, protection against soil erosion), which largely depend on the functional traits of the dominant species.

Sampling of the herbaceous plant community in each plot can be done using quadrats of 50 x 50 cm, estimating the percentage of the area covered by each species or functional group (e.g. grasses vs. forbs) (Figure 7). Place three quadrats on alternate sites along three 10 m transects, totalling nine quadrats in each plot.

The methods used to assess shrub and herbaceous vegetation cover are commonly used in semiarid woodlands and forests, e.g. see Nunes *et al.* (2014), Nogueira *et al.* (2018) and Köbel *et al.* (2021; 2023; 2024) for examples.

Measurements of forest deadwood

Forest deadwood plays a vital role in forest ecosystems, offering habitat, nutrients, and shelter to a wide variety of organisms. It serves as a recognized indicator of forest biodiversity, reflecting habitat quality, ecosystem health, and structural diversity within forests.

Assessing forest deadwood involves measuring several components, including fallen dead trees, coarse woody debris (CWD), snags, and stumps. Coarse woody debris is defined as deadwood with a diameter greater than 10 cm and is typically assessed through full sampling within a known plot area, such as a circular plot with a 10-meter diameter. Fine woody debris, characterized by diameters smaller than 10 cm, can also be measured using a similar approach.

For fine woody debris specifically, pieces with diameters between 5 cm and 10 cm are recorded by measuring both their diameter and length. This ensures a comprehensive understanding of the forest's deadwood composition across different size classes.

Coarse woody debris (CWD) refers to stems, limbs, and branches lying on the ground within the plot. The length of a CWD piece is measured only if its diameter is at least 10 cm. For pieces with irregular diameters along their length, only the sections with a diameter of 10 cm or more are considered, excluding parts below this threshold. Diameter measurements are taken at the midpoint of the segment where the diameter exceeds 10 cm.

Stumps are classified as deadwood with a height (or length, if lying) of less than 130 cm from the base and a diameter of at least 10 cm. The stump diameter is measured at the standard cut height.

A **snag** refers to **standing dead wood** taller than 130 cm with a diameter of at least 10 cm. If the snag retains branches, it is categorized as a standing dead tree, and measurements are taken for diameter at breast height (DBH), at 130 cm from the base. The height of the snag is measured up to the point where its diameter decreases to 10 cm. If the snag is shorter than 130 cm, it is treated as a stump.



Figure 7. Sampling scheme to measure herbaceous community diversity, with three 50 x 50 cm quadrats placed along alternate sides of a 10 m transect.

Decay stages are recorded using a five-class system based on Hunter (1990), to document the degree of decomposition.

The forest deadwood measurements include:

- Diameter (in cm) of coarse woody debris (>10 cm diameter) and length (in m)
- Diameter of stump (cm) less than 130 cm in height with a diameter at normal cut height greater than 10 cm
- Estimated diameter of snag (cm) and snag height (in m)
- Decay state (5 classes) of all deadwoods

Record all measurements in a notebook including **types of deadwoods** (lying, standing, stumps), **length/height (m) and diameter (cm)** and **Declay class** (1 to 5).

Forest soil properties

Soil is a mixture of solids (organic and inorganic), water, air and micro-organisms. The components of the soil interact and affect each other and the study of the physical, chemical, hydrological and biogeochemical properties of soils is called soil science. A desirable composition of soils for plant growth is about 25% air, 25% water and ~50% solid material inclusive of organic matter as source of nutrients for plants and carbon for microbial activities. Soil supports plants and provides water and nutrients to them.

Different physico-chemical processes of soil formation lead to transformation and leaching of the soil materials across the soil's depth, which finally results in the formation of distinctive horizontal soil layers (horizons) (https://en.m.wikipedia.org/wiki/File:Soil_Horizons_Diagram.jpg). Horizons vary in thickness although the boundaries are not always easy to demarcate. Some of the main soil horizons/layers follow:

- **Organic/Humus (O) Layers/Horizons:** (**OL** – Litter layer; **OF** – Fermentation layer; **OH** – Humification layer) The **OL** horizon is characterized by the accumulation of mainly leaves/needles, twigs and woody materials, most of the original plant organs being easily discernible to the naked eye. The **OF** horizon is characterized by the accumulation of partly decomposed litter, mainly from transformed leaves/needles, twigs and woody materials, but without any entire plant organ. The **OH** horizon is characterized by an accumulation of zoogenically transformed material, i.e. black, gray-brown, brown, reddish-brown well-decomposed litter, mainly comprised of aged animal droppings. LFH is also referred to as O-horizon in some classifications and vice versa.
- **A Horizon:** A mineral horizon formed at or near the surface below O/LFH horizon, where mineral material is mixed with organic matter from the O-horizon. This layer shows accumulation of organic matter and loss of minerals (eluviation of Fe, Al and Clay).
- **E Horizon:** A mineral soil horizon formed in some soils where lots of minerals and organic acids are eluviated from it leaving a colorless layer between A and B horizons. Most prominent in podzol soils under coniferous/pine forests.
- **B Layer:** Formed under A-horizon through material accumulation eluted from A horizon and/or by the alteration of the parent material. Accumulation of clay, Fe, Al, Si, humus, $CaCO_3$, $CaSO_4$, or loss of $CaCO_3$; or accumulation of sesquioxides is typical of the B horizon.
- **C Layer:** A soil horizon where evidence of pedogenic [soil forming] activity is not prominent.
- **R Layer:** Consolidated bed rock
- **W Layer:** Water Layer

Dug soil pits in woodland soils under grazing and grazing enclosures to assess the impacts of livestock grazing, nutrient enrichment due to livestock faeces, urine, and atmospheric reactive nitrogen deposition on the physico-chemical properties of soils. In addition to the impact assessment, soil descriptions, decomposition (using the teabag method) and field texture classification by hands should be demonstrated on site. These observations allow discussion of the role of soil in ecosystem health, nutrient supply to trees, carbon sequestration, microbial functions, regulation of the water cycle and biodiversity support and the potential impact of grazing and pollution on soil properties, functions and ecosystem services delivery.

For humus type description we followed the EU humus type classification system (Zanella *et al.*, 2011) and for some of the soil properties the protocol reflects on the EU ICP Forest Soil Manual for soil sampling and analysis (Cools & De Vos, 2020). ICP forest monitoring soil data on soil carbon stocks (De Vos *et al.*, 2015) and C:N ratio (Cools *et al.*, 2014) has been evaluated at European scale.

Humus type classification

Description of humus type follows the European Humus Type Classification only down to the five main terrestrial references (**Mull**, **Moder**, **Amphi**, **Mor** and **Tangel**) which are identified and described by diagnostic features (Zanella *et al.*, 2011).

Soil horizons

Description of soil horizons is critical whenever you undertake site characterisation for various purposes such as soil carbon and GHG, fertility studies, remediation of contaminated soils, restoration of ecosystems and waste recycling. Soil sampling for biogeochemical properties is also influenced by horizons and thus it is critical to consider soils horizons while sampling. For example, organic horizons typically contain much higher organic matter content than underlying mineral soil horizons, making them a key factor in assessing soil organic matter stocks. Additionally, sampling by horizon is fundamental to underpinning soil classification, as it allows a more accurate description of the soil profile and its development. Protocols for soil horizons follow:

- A soil pit (50 cm wide and 100 cm deep) will be dug to observe and record soil horizons (Figure 8).
- Try to minimize impacts on the site.
- Identify soil horizons in the pit and record it in your field notebook.
- Record the organic layers present (LFH) and their depths.
- Record the depth of each horizon in cm starting from 0 cm at the mineral soil surface.
- Record the fragment content in the soil profile.

Soil colour

Soil color is important for soil classification, comparison and identifying influence of water saturation, organic matter accumulation, plant-soil interactions and biogeochemical processes in soil formation, and diagnostic colour development. Soil is a complex matrix whose colour is influenced by soil formation factors including parent material, climate, time, organisms and topography. Therefore, soil color determination is indicative of the soil type and of the prevailing soil management. Determining soil color is thus an important variable in defining and classifying soils. A standard method for soil color determination is based on the Munsell Soil Colour determination (https://munsell.com/color-blog/defining-soil-profiles/; https://www.floridahealth.gov/environmental-health/onsite-sewage/_documents/act-presentation-03-soil-colors-and-their-interpretation-april-2015.pdf). The soil color is characterized by three properties called hue, value and chroma. Using the Munsell soil colour book, determine these properties as below. Determine the hue, value and chroma of each soil horizon using the Munsell soil colour charts (2010).



Figure 8. Soil pit (image source: S. Ullah, University of Birmingham).

- Hue: Dominant colour of soil (yellow, red, blue, etc.)
- Value: Degree of lightness or darkness (values from 2 to 6, where 2 is the darkest and 6 the lightest).
- Chroma: Purity/strength of colour or degree of colour saturation. Soils have 5 levels of chroma where the lower levels are dull (washed) while the higher levels are purer.

Soil texture

Soil texture represents the proportion (percent by weight) of sand, silt, and clay in soil. Soil texture influences soil water retention, permeability, aeration, and retention of nutrients. The texture can be estimated in the field qualitatively by hand. When moist soil is manipulated in the hand, depending on the relative proportion of the particle sizes in it (sand, silt, and clay), the soil feels different. Protocols for texture characterization follow:

- Take a sample of soil after removing litter, organic matter, and stones into the palm of the hand.
- Knead and moisten the soil (where necessary) to make a soil ball in your palm.

- Use the ribboning method supplied to determine texture (printed protocols).
- Report the texture in your report for your presentation.

Rock fragments
The presence of rock fragments influences the nutrient status, permeability, use and management of the soil; the accurate calculation of carbon and nutrient stocks; it also reflects its origin and stage of development. Large fragments (>2 mm) are described according to abundance, size, shape, state of weathering and nature of the fragments. The abundance of classes corresponds with those for surface coarse fragments and mineral nodules. Abundance (in volume %) of rock fragments in each horizon will be estimated using the visual charts (FAO, 2006) and recorded in field notebook.

- 0 None 0 %
- 1 Very few to few 0 – 5 %
- 2 Common 5 – 15 %
- 3 Many 15 – 40 %
- 4 Abundant 40 – 80 %
- 5 Dominant >80 %

Root's abundance
The presence or absence of roots is the most crucial data to record. If there is a relevant change in the quantity or size of the roots, it is important to provide an explanation for this variation. Potential factors that could limit root growth include compaction (which can be assessed through bulk density measurements), cementation, or a discontinuous pore system.

A qualitative description of root size and abundance is also essential. In some cases, noting additional details, such as abrupt changes in root orientation, may be helpful. Root abundance should only be compared within the same size class. For fine and very fine roots, their abundance can be recorded similarly to voids, expressed as the number of roots per decimetre square.

Size (diameter)

- VF - Very fine < 0.5 mm
- F - Fine 0.5 – 2 mm
- M - Medium 2 – 5 mm
- C - Coarse > 5 mm

Abundance (number of roots/dm^3)

<2mm root diameter

- N - None 0
- V - Very few 1 – 20
- F - Few 20 – 50
- C - Common 50 – 200
- M - Many >200

>2mm root diameter

- N - None 0
- V - Very few 1 – 2
- F - Few 2 – 5
- C - Common 5 – 20
- M - Many >20

Rooting depth
Record the depth in the soil profile (in cm) where still visible roots are present. The effective rooting depth may be defined as the depth of the soil at which root growth is strongly inhibited. Rooting depth being plant specific, it is recommended that representative species are used to indicate the effective rooting depth of the soil. The effective rooting depth is governed by such factors as the presence of cemented, toxic or compacted layers, hard rock, or indurated gravel layers. A high permanent water table may also control the rooting depth, but may change after drainage. The effective hydrological depth may be much greater. Apart from obvious situations such as the presence of hard rock, it is realized that the estimation of effective soil depth is subject to individual interpretation.

Biological features
Describe krotovinas, termite burrows, insect nests, worm casts, or burrows of larger animals in terms of abundance and kind. Record specific locations, patterns, size, composition or any other characteristic.

Abundance of biological activity is recorded in the following general descriptive terms:

- N - None
- C - Common
- F - Few
- M - Many

Examples of biological features are the following:

- B - Burrows (unspecified)
- E - Earthworm channels
- BO - Open large burrows
- P - Pedotubules
- BI - Infilled large burrows
- T - Termite or ant channels and nests
- C - Charcoal
- Other insect activity

Soil volumetric water content and temperature
Soil pore spaces are often filled partially or fully with water. Soil water is important for plant growth, root and nutrient uptake, nutrient cycling and transport of organic and inorganic materials including toxic substances. Soil temperature is

important for soil microbial activity, soil biota and soil mineralization and decomposition processes. Volumetric water content (VWC) represents the total volume of soil occupied by water and is often reported in % units. Measure VWC using a portable soil moisture probe to assess if grazing may have changed soil VWC in the topsoil layers. Measure soil temperature by portable temperature probe.

- Using the soil moisture and temperature probes, carefully insert the probes into the soil near the soil pit vertically in the top 10 cm of the soil.
- Remember, if you hit a rock while inserting the probes, then remove the probe and try to insert it in another nearby spot. Don't force it down as this can break down the probe.
- Repeat the VWC and soil temperature measurements at 5 locations with a minimum distance of a couple of meters between locations.
- Record the soil VWC (%) and soil temperature (C°) readings in your notebook.

Soil pH and electrical conductivity

Soil pH is a key variable affecting varied biogeochemical process dynamics and transformations. Soils with high pH (usually >6) are capable of supporting diverse plant species and possess higher potential for nutrient supply than acidic soils. Leaching and absorption of nutrients and heavy metals (As, Cd, Pb etc.) are also influenced by soil pH among other factors and thus pH is an important variable to measure to assess soil health and fertility as a first step.

Electrical conductivity (EC) of soils is a surrogate variable for the assessment of the amount of soluble ions (cations and anions). Excessive EC can be observed in saline and sodic soils (> 5 m Siemens/cm). A very low EC may mean soils may have limited available nutrient ions.

Measurement protocols follow:

- Collect an about 10 g soil sample from the O and A horizons into a plastic beaker and add 20 ml of DI water to it.
- Shake it or mix it by slowly shaking the beaker in your hand to produce a slurry.
- Using the soil pH + EC probe, measure the pH and EC of the slurry (supernatant) and record in your field book.
- Make an effort to compare the pH and EC of different soil horizons under grazing and grazing enclosures in your field notebook.

Soil infiltration capacity

The ability of soil to absorb surface water (rain, irrigation, etc.) is generally reported in mm per hour or per day. Soil infiltration capacity is important for calculating nutrient and pollutant leaching potential into groundwater and surface run-off generation after rainfall, flooding, or an irrigation event. Compaction of soils due to grazing and agricultural machinery results in the reduction of infiltration capacity and an increase in surface run-off, erosion, and flooding. Forests are generally efficient in ameliorating flooding as they have high infiltration capacity.

An instrument like a Geopack's® infiltrometer can be used for calculating the infiltration rates of soils. In this method, a cylinder is inserted into soils (up to 4 cm depth) and is filled with water. The water infiltrates the soil pore spaces and excess water is then transmitted down the soil profiles. Infiltration is initially fast and then becomes constant after the pore spaces are fully saturated with time.

Measurement protocol follows:

- Carefully insert the beveled end of the cylinder into the soil to a depth of 40 mm (4 cm). If you realize that the cylinder is against a rock or hard surface in the soil, then remove it from that spot and try inserting it somewhere else close by. Don't force it as it could be broken.
- Choose a fall unit (a known volume of water within the cylinder). Usually 1 cm to 5 cm. If it is a wet rainy day, a fall unit of 0.5 to 1 cm would be appropriate.

Once you have chosen the fall unit, fill the cylinder up to the 10 cm mark with water and simultaneously start the timer. Repeat the infiltration measurements till you reach an equilibrium as infiltration is relatively faster initially till it reaches an ***equilibrium*** rate.

- Two to five recordings in your notebook need to be taken when doing this experiment. Record the total elapsed time from the start to the point when the infiltration rate becomes constant and record the time taken for a given volume of water to infiltrate the surface (Infiltration rate).
- Record the time taken for the water level to fall by the chosen fall unit (water volume). Record the time it took for the fall unit to infiltrate in the column and next to it record the amount of water (in mm or cm).
- Quickly re-fill the cylinder to the 10 cm mark and record the time again taken for the water level to fall by the fall unit. Record time and fall unit again.
- Repeat the procedure until the time taken for the fall unit becomes constant.
- Finally record the total elapsed time (from the start of the experiment to the end when fall unit infiltration becomes constant).
- Now calculate Infiltration Rates (IR):

  IR = Fall in water level (mm)/Time taken to fall this amount (seconds)

  IR is then in mm/sec or mm/hour or mm/day as you see it appropriate.

Soil ammonium and phosphate concentrations

Key soil macronutrients like nitrogen, phosphorus, calcium, etc. in bioavailable forms are critical for the growth of trees. Whilst forest soils are generally limited by available nitrogen and phosphorus forms, grazing can result in nutrient enrichment with ions like ammonium, nitrate, and phosphate. Thus, we will demonstrate and measure soil ammonium and phosphate concentrations using portable colorimetric devices for ammonium and phosphate ions. In these methods, soil will be extracted with DI water (1:20 ratio of soil to DI water), filtered through 0.45 micrometer filter paper and then a 10 ml filter solution will be poured into cuvettes. The solutions will be treated with compound specific reagents to react with ammonium and phosphate for producing specific stable-coloured compounds. The solution will then be put into portable colorimetric devices for the determination of the concentration of ammonium and phosphate. For details about nutrient colorimetry, refer to the US EPA Methods for nutrient analysis in soil and water as well as Sgouridis *et al.* (2023).

Soil carbonates

The presence of calcium carbonate ($CaCO_3$) can be established in the field by adding some drops of 10% HCl to the soil. Is the matrix calcareous or non-calcareous (the exact quantity of carbonates can be tested in the laboratory) will be determined by if there is a reaction when applying the HCl to the soil. If traces are found in at least one horizon of the profile, the presence/absence should be recorded for all horizons.

Leaf litter decomposition potential – teabag's method

The nutrient cycle in forest soils revolves around the uptake of nutrients for the plant growth, followed by the senescence of leaves back into the soil, their breakdown by physical, chemical and/or by other living organisms (herbivores, macro and micro fauna and flora), in a long process back into their most basic units (mineral nutrients, CO_2) that can be again assimilated by other living organisms.

One method to evaluate the rate of decomposition of leaf litter in soils, and thus, a relative measure of the soil health (presence of biodiversity and potential to perform the ecosystem service of decomposition) is to use standardized leaf litter bags – teabags – incubated in the soil for a period of time (3 months to 3 years). In the last few years a large number of comparisons was possible around the globe, because one of the main factors that determines the rate of decomposition - the composition of the litter - was standardized, using the same type of Green Tea and Rooibos Tea teabags (URLs: teacomposition.org; teatime4science.org). Using leaf litter composition as a fixed factor, we can focus on the factors related to the abundance of soil decomposing microorganisms (namely fungus and bacteria) and to the soil properties (*e.g.*, pH, texture, C:N ratio, organic matter) or climate of the study site.

Lab method I: (1) the teabags are oven dried (≤60 °C) and weighed previously in the lab. (2) each one is labelled with a number ID that will identify it during the duration of the experiment. (3) At least 10 teabags are used as control (not going to the field), dried, weighed, and separated into tea leaves, and teabag with cord and label, and weighted again, to determine the average weight of the packaging to subtract from the original teabags used in the field. (4) calculate a minimum of 2 teabags per site, but preferably more, to account not only for soil niche diversity, but also the probability of losing the teabags during the exposure time (positive correlation, as more time = more losses) due to animal activity or root overgrowth.

Field methods: (5) place the teabags in the organic soil layer (if it exists) or in the mineral soil layer about 5–10 cm below the surface. Unless it is an agricultural soil already disturbed, try not to disturb the soil too much, so natural underground networks are preserved. If that is not possible, try to make the same level of soil disturbance in every plot. (6) They should be spaced ca. 5–10 cm apart, and in a pattern that facilitates the recovery (*e.g.*, in line). (7) the tea tag should be visible and protected, to facilitate the identification of each teabag, GPS coordinates collected, and preferably some form of visual ID or metal detection tags implanted, to easily identify the location later on. (8) after some time (3 months to 3 years) return to the location to (carefully) collect the teabags, making sure the numbers are discernible. Discard any if outside the soil, or with holes. (9) Remove excess soil from the exterior and bring it to the lab to oven dry as soon as possible.

Lab method II: (10) oven dry the teabags (≤60 °C). (11) remove the remaining tea leaves from the bags, making sure all of them are removed and that no soil goes with it (when in doubt if soil entered the bag or contaminated the leaves during the opening of the teabags, burn the leaves to calculate their organic matter content). (12) From the ID of each teabag, identify the initial weight (W_initial) of the leaves and compare with this final weight (W_final), to calculate the amount decomposed.

% Decomposition = (W_initial-W_final)/W_initial *100

For an idea of the results that are possible from this method, we suggest the reading of articles related to the TeaComposition initiative (e.g., Djukic *et al.*, 2018; Kwon *et al.*, 2021), the TBI index (e.g., Keuskamp *et al.*, 2013; Sarneel *et al.*, 2024), or new teabag experimental tests (e.g., Middelanis *et al.*, 2023). As for local data, those articles mentioned for the TeaComposition Initiative include results from some of the study sites where the Training School took place. Results should not be considered absolute, but rather relative and analysed by comparison with appropriate controls.

Organic layer density and soil bulk density determination and soil chemical, physical and biological evaluation

Possible sampling methods:

- Collection of soil samples from the soil profile for chemical, and physical measurements for soil classification purposes.

- Collection of organic layer samples by using known size quadrat and depth measurements to calculate organic layer mass and density for estimation of soil carbon and nutrient stocks.
- Collection of soil samples for estimation of soil bulk density by using 100 cm^3 cylindrical stainless-steel rings horizontally inserted and taken from each horizon from the pit.
- Collection of samples for earthworms count and species evaluation by using quadrats.
- Collection of samples for soil microbial, mesofauna, nematode communities' analysis (e.g. by metabarcoding or other molecular methods).
- Collection of samples for fine roots morphological, biomass, density evaluation using cylindrical cores of known volume.
- Collection of soil sampling by Dutch auger for general chemical analysis.

Different sampling approaches and designs to cover spatial and temporal scale soil variability and change are available.

Potential limitations of the protocol

While the simplicity and low cost of the suggested protocols represent a key strength, we acknowledge that they also have some limitations, particularly for non-experts. Below, we outline the most evident constraints.

For lichens, a key limitation is the expert knowledge required to accurately identify species. Untrained samplers are likely to overlook microlichens, particularly very small species, introducing a potential bias. Additionally, some species groups, such as Physciaceae or Lepraria, are difficult to distinguish, increasing the risk of misidentifications. Another constraint is that the protocol applies exclusively to epiphytic lichens, making it unsuitable for non-forest areas.

The methods used to estimate vegetation cover and diversity are commonly applied in semiarid areas like the one studied. Nevertheless, the number and size of transects or quadrats affect the precision of cover estimates. Their definition relies on a compromise between ensuring that most of the variability in the vegetation is encompassed, which depends also on landscape heterogeneity, and cost-effectiveness in the time and resources needed.

A potential limitation for soils is that the protocol does not provide a fully detailed description of soil characterization and classification. However, it refers to key soil classification systems (e.g., IUSS Working Group WRB, 2022), where comprehensive classification methods are described. The classification of humus types is included at its higher, more practical levels, while more detailed levels are referenced in the EU humus classification guidelines.

Data availability

"No data are associated with this article."

Author contributions

Branquinho C: conceptualization, Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; Rocha B: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Ullah S: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Oliveira MA: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Vanguelova E: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Serrano H: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Nunes A: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Príncipe A: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Pinho P: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Munzi S: Conceptualization, Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Monteiro J: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Alonso R: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Gharun M: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing, Guerrieri R: Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing.

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Version 2

Reviewer Report 07 April 2025

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✔ **Francesco Valerio**
BIOPOLIS, Universidade do Porto Centro de Investigacao em Biodiversidade e Recursos Geneticos, Vairão, Porto, Portugal

The revised manuscript shows a commendable improvement over the earlier version. The introduction now flows much more naturally, offering a clear and explicit description of the study's aims while effectively linking the fieldwork protocol to the broader context of global change drivers. Also, although the study does not involve direct manipulation experiments, the authors have adeptly incorporated relevant literature to provide a nuanced ecological perspective. This approach not only strengthens the theoretical framework of the study but also situates the research within current environmental debates, making the manuscript more engaging for readers.
The connection to European policy initiatives, such as the European Green Deal, Nature Restoration Law, and Forest Monitoring Law, is presented in a clear and compelling manner. This explicit linkage underscores the practical implications of the research and highlights its potential impact on forest management practices across Europe. I find that the way the manuscript bridges the gap between scientific inquiry and policy relevance is particularly appealing. The authors have further emphasized that their protocol is built upon established, cost-effective methods, resulting in a practical handbook that is especially valuable for training purposes. Finally, the inclusion of a new limitations section further enhances the transparency of the study by openly discussing potential challenges, such as biases in lichen identification and issues in vegetation sampling and soil classification.
In light of these substantial improvements, I find the manuscript to be a valuable contribution to the literature on forest ecosystem monitoring under global change drivers, and I recommend its acceptance.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My research areas are Ecology, Landscape Connectivity, Remote Sensing, Conservation Biology, and Natural Resources. This combination reflects an interdisciplinary approach and aligns with studies focusing on environmental monitoring, habitat connectivity, and

resource management.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 28 January 2025

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Javeriya Hasan
[1] Toronto Metropolitan University (formerly Ryerson University), Toronto, Canada
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This protocol adequately covers the different details of parameters that need to be investigated in line with the research objective of measuring spatiotemporal changes on semiarid woodlands.

It only describes the methods which would need to be carried out in an investigation of this sort.

I would have hoped to see a flow chart or a decision hierarchy governing the sequence of steps to be taken in such an investigation. Additionally, it would have been useful to present a table, infographic or a visual schematic showing how data relating to the different parameters is collected. An example of a data collection pertaining to say "biological features" or "soil volumetric water content and temperature" in tabular form would have been helpful for the reader.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Resilient buildings, sustainability, solar energy, solar architecture, data

analytics, machine learning, building science

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Mar 2025

Silvana Munzi

We sincerely thank the reviewer for their constructive time and effort in evaluating our work. We really appreciate the positive evaluation and the valuable suggestions. A decision hierarchy and a visual schematic summarizing the data collection process are now included as suggested by the reviewer.

Competing Interests: No competing interests were disclosed.

Reviewer Report 07 January 2025

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Francesco Valerio
[1] BIOPOLIS, Universidade do Porto Centro de Investigacao em Biodiversidade e Recursos Geneticos, Vairão, Porto, Portugal
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Dear authors, your manuscript presents a valuable protocol for monitoring spatiotemporal changes in global change drivers and their effects on semiarid woodlands and forests. The methods and focus on training activities align well with COST Actions and Horizon Europe priorities. However, several areas require improvement to enhance the scientific rigor, contextual relevance, and practical applicability of your work.

The absence of data associated with this article possibly weakens its replicability. It might be relevant to include illustrative datasets or simulated examples to demonstrate the protocol's effectiveness and provide insights into the expected outcomes. Also, some examples with associated metadata might increase the reachability of your study.

While the introduction discusses the need for holistic approaches to studying global change drivers, it does not specify what the study aims to achieve with this protocol. Even if not mandatory, it is advisable to include defined objectives and research questions to enhance the manuscript's clarity, impact, and alignment with COST's mission. In this direction, this research seems to quantify the impacts of varying grazing intensities, nitrogen deposition, and climatic

stressors on tree regeneration, biodiversity, and soil health. Also, another valuable point is the applicability of standardized methodologies across diverse management scenarios to ensure replicability and scalability. The proposed protocol finally aims to provide actionable insights to inform sustainable forest management and align with EU biodiversity and climate objectives.

Global change drivers (e.g., climate change, nitrogen deposition, grazing) are discussed individually, with no exploration of their interactions. I suggest to introduce a conceptual framework to illustrate the synergies and trade-offs among these drivers. For instance, discuss how nitrogen deposition and grazing jointly influence biodiversity and ecosystem health.

The manuscript mentions sustainable forest management but does not sufficiently link its findings to EU policy frameworks. Please, expand the discussion to highlight how the protocol aligns with European objectives (e.g., Green Deal), such as climate resilience and biodiversity conservation. Provide actionable recommendations for policymakers and stakeholders.

There is a need to clarify how the proposed protocols fill possible gaps in existing methodologies. Basically, even without comparisons with existing frameworks, there is a need to know what is the novelty of your framework more explicitly. In this direction, the methods section is comprehensive, but it lacks prioritization, as some protocols are described without justification for their inclusion. Therefore, provide a clear rationale for their selection and relevance.

Include a section discussing potential limitations of the protocol, such as sampling bias or challenges in adapting methods to other ecosystems.

Minor issues:

"Companhia das Lezírias stands as a testament to the intricate interplay between geology, climate, land use, and history." This is overly poetic and not aligned with the technical focus of the proposed protocol.

"Most of the trees present inside the plot in our study were cork oaks, then the debarking year and the width of the cork layer were registered." The sentence is unclear in construction and lacks specificity about why the debarking year and cork layer width are critical metrics for the protocol.

"Participants applied these methodologies in real-world scenarios, conducting measurements in different grazing management settings to assess their effects on tree growth, biodiversity, and soil properties." This general statement is too vague and does not detail the specific methods used in these assessments.

"Record all visible macrolichens (foliose and fruticose) within the grid, either on-site or for later identification in the laboratory." The option to identify species later in the laboratory could introduce inconsistencies in results if not standardized (e.g., storage or transport conditions).

"Soil sampling for biogeochemical properties is also influenced by horizons and thus it is critical to consider soils horizons while sampling." This sentence is redundant and could be streamlined for clarity. Additionally, it does not specify how horizons influence sampling strategies.

"The teabags are dried (40–60 °C) and weighed previously in the lab." The temperature range is

broad.

"Record the hue, value, and chroma of each soil horizon using the Munsell soil colour charts (2010)." While the Munsell system is standard, this statement assumes familiarity with the charts without providing context or resources for those unfamiliar.

"Detailed protocol follows:" (as an introductory phrase). This phrase appears multiple times without introducing a genuinely detailed protocol. The description often remains high-level and lacks procedural depth.

"Participants analyzed data and suggested ways to manage forests sustainably in response to global environmental challenges." This conclusion is overly general and does not specify how these data analyses translated into actionable forest management strategies.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My research areas are Ecology, Landscape Connectivity, Remote Sensing, Conservation Biology, and Natural Resources. This combination reflects an interdisciplinary approach and aligns with studies focusing on environmental monitoring, habitat connectivity, and resource management.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Mar 2025

Silvana Munzi

We sincerely thank the reviewer for their constructive and insightful feedback. Their comments have helped us refine and clarify our manuscript, strengthening its impact and relevance. Here the detailed **answer (A)** to each **comment (C):**

C: The absence of data associated with this article possibly weakens its replicability. It might be relevant to include illustrative datasets or simulated examples to demonstrate the

protocol's effectiveness and provide insights into the expected outcomes. Also, some examples with associated metadata might increase the reachability of your study.

A: We agree that examples are relevant. Since the methods have been largely employed, there are many published papers that can provide illustrative datasets. We added the following references to some examples present in the literature besides the works already cited in the paper: "For replicability purposes, see studies from Rocha et al,. (2019)." "For the leaf litter decomposition protocol (Teabags) we suggested the reading of many articles published so far related to the TeaComposition initiative (e.g., Djukic et al 2018; Kwon et al. 2021), the TBI index (e.g., Keuskamp et al. 2013; Sarneel et al. 2024), or new experimental tests (e.g., Middelanis et al. 2023). As for local data, those articles mentioned for the TeaComposition Initiative, include results from some of the study sites where the Training School took place." "For humus type description we followed the EU humus type classification system (Zanella et al., 2011) and for some of the soil properties the protocol reflects on the EU ICP Forest Soil Manual for soil sampling and analysis (Cools and De Vos, 2020). ICP forest monitoring soil data on soil carbon stocks (De Vos et al., 2015) and C:N ratio (Cools et al,2014) has been evaluated at European scale." "The methods used to assess shrub and herbaceous vegetation cover are commonly used in semiarid woodlands and forests, e.g. see Nunes et al., 2014; Nogueira et al.(2018) and Köbel et al. (2021, 2023, 2024).

C: While the introduction discusses the need for holistic approaches to studying global change drivers, it does not specify what the study aims to achieve with this protocol. Even if not mandatory, it is advisable to include defined objectives and research questions to enhance the manuscript's clarity, impact, and alignment with COST's mission. In this direction, this research seems to quantify the impacts of varying grazing intensities, nitrogen deposition, and climatic stressors on tree regeneration, biodiversity, and soil health. Also, another valuable point is the applicability of standardized methodologies across diverse management scenarios to ensure replicability and scalability. The proposed protocol finally aims to provide actionable insights to inform sustainable forest management and align with EU biodiversity and climate objectives.

A: We agree with the reviewer. To focus more the introduction on the work developed, we have expanded on the objectives of the field trip adding the following text: "Semiarid woodlands and forested ecosystems are increasingly vulnerable to degradation due to multiple environmental drivers, including climate change, land-use change, fragmentation, and pollution. Understanding how different ecosystem components respond to these pressures is essential for assessing overall ecosystem degradation—both in terms of biodiversity and ecosystem functions/services. With this in mind, our goal is to compile and evaluate different methodologies for assessing these impacts, linking each ecosystem component to the drivers it responds to most strongly. Specifically, the field trip conducted as part of the training school aimed to provide participants with hands-on experience in assessing global change drivers and their real-world impacts on semiarid woodlands and forests. Through practical activities, students gained a deeper understanding of how forest ecosystems interact with key stressors such as air quality and climate change. In particular, participants learned to quantify the effects of varying grazing intensities, atmospheric

nitrogen pollution, and climatic stressors on multiple aspects of forest ecosystems, including forest structure, tree regeneration, biodiversity, and soil health."

C: Global change drivers (e.g., climate change, nitrogen deposition, grazing) are discussed individually, with no exploration of their interactions. I suggest to introduce a conceptual framework to illustrate the synergies and trade-offs among these drivers. For instance, discuss how nitrogen deposition and grazing jointly influence biodiversity and ecosystem health.

A: The complex interactions among global change drivers are the scope of the CLEANFOREST COST Action CA21138, but not of the training school. Moreover, we did not address this from a statistical perspective with the students, as no well-replicated manipulation experiments were available. Instead, our approach to exploring how pollution and climate extremes affect forest structure, soil, and biodiversity was to leverage a gradient of grazing intensity to investigate whether higher nitrogen input from livestock influences ecosystem responses to drought. For clarity, we added: "From the state of the art in the subject, we expect that increased nitrogen deposition can initially enhance plant productivity, potentially benefiting grazers. However, grazing can exacerbate the effects of nitrogen deposition by selectively removing certain plant species, leading to a decline in biodiversity and forage quality. Furthermore, climate change, through increased temperatures and altered precipitation patterns, can further modify these interactions, influencing ecosystem processes and outcomes. These interactions are less known in Mediterranean climate and need further work despite some works having been already developed (e.g. Ochoa-Hueso et al. 2017, Nogueira et al., 2018)."

C: The manuscript mentions sustainable forest management but does not sufficiently link its findings to EU policy frameworks. Please, expand the discussion to highlight how the protocol aligns with European objectives (e.g., Green Deal), such as climate resilience and biodiversity conservation. Provide actionable recommendations for policymakers and stakeholders.

A: Thanks for raising this important point. We linked the goals of the training school and results achieved to European forest strategies and regulations within the European Green Deal. In particular, we added the following text in the summary: 'After the practical experience in the field using these experiences, participants were divided into groups to analyze and discuss collected data together with trainers. Key findings were summarized in presentations, together with main take home messages and suggestions on further questions to be explored and related attributes to monitor. Finally, participants had also the opportunity to link their findings to policy actions, by providing indications on potential sustainable forest management actions to increase resilience of the studied woodland ecosystem, and of mediterranean forests in general, under global change, in line with European strategies and regulations.' Moreover, the following text was added at the end of the introduction: 'Finally, participants engaged in data processing and analysis, synthesizing their findings to draw conclusions, suggestions on further questions to explore and recommendations for sustainable forest management to promote biodiversity and increase forest resilience under global change. This way, participants had the opportunity to link

scientific advances to forest-related strategies and regulations adopted (e.g., Nature Restoration Law) and under discussion (European Forest monitoring Law and soil strategy) within the European Green Deal.'

C: There is a need to clarify how the proposed protocols fill possible gaps in existing methodologies. Basically, even without comparisons with existing frameworks, there is a need to know what is the novelty of your framework more explicitly. In this direction, the methods section is comprehensive, but it lacks prioritization, as some protocols are described without justification for their inclusion. Therefore, provide a clear rationale for their selection and relevance.

A: Our approach does not introduce any novel methodologies but rather utilizes existing and well-established methods that have been successfully applied in similar contexts together to have an holistic perspective on the ecosystem structure and function. The primary aim was to create a handbook that was suitable for participants in our training school and adaptable for other similar initiatives. The methodologies were chosen to maximize the forest aspects considered, avoid the need for costly equipment, and adapt the study to the short duration of the fieldwork. We added the following text to make it clearer: "We focused on presenting these methods in a way that is accessible and practical for users, to have an integrated overview of the ecosystem structure and functioning. The methods were selected for their simplicity, cost-effectiveness (requiring no expensive equipment), and comprehensive coverage of the key aspects of a forest study."

C: Include a section discussing potential limitations of the protocol, such as sampling bias or challenges in adapting methods to other ecosystems.

A: Section added as suggested. "For lichens, a key limitation is the expert knowledge required to accurately identify species. Untrained samplers are likely to overlook microlichens, particularly very small species, introducing a potential bias. Additionally, some species groups, such as Physciaceae or Lepraria, are difficult to distinguish, increasing the risk of misidentifications. Another constraint is that the protocol applies exclusively to epiphytic lichens, making it unsuitable for non-forest areas. The methods used to estimate vegetation cover and diversity are commonly applied in semiarid areas like the one studied. Nevertheless, the number and size of transects or quadrats affect the precision of cover estimates. Their definition relies on a compromise between ensuring that most of the variability in the vegetation is encompassed, which depends also on landscape heterogeneity, and cost-effectiveness in the time and resources needed. A potential limitation for soils is that the protocol does not provide a fully detailed description of soil characterization and classification. However, it refers to key soil classification systems (e.g., WRB, 2020), where comprehensive classification methods are described. The classification of humus types is included at its higher, more practical levels, while more detailed levels are referenced in the EU humus classification guidelines." Minor issues:

C: "Companhia das Lezírias stands as a testament to the intricate interplay between geology, climate, land use, and history." This is overly poetic and not aligned with the

technical focus of the proposed protocol.

A: Removed as suggested.

C: "Most of the trees present inside the plot in our study were cork oaks, then the debarking year and the width of the cork layer were registered." The sentence is unclear in construction and lacks specificity about why the debarking year and cork layer width are critical metrics for the protocol.

A: We removed the sentence and the part of the protocol related to leaf physiology because it was not applied in the training school.

C: "Participants applied these methodologies in real-world scenarios, conducting measurements in different grazing management settings to assess their effects on tree growth, biodiversity, and soil properties." This general statement is too vague and does not detail the specific methods used in these assessments.

A: Methods used are now mentioned both in the abstract and the introduction. Namely, we added the text: “(forest structure assessment, lichen diversity sampling, shrub and herbaceous diversity estimation, deadwood measurement and soil physico-chemical analysis).”

C: "Record all visible macrolichens (foliose and fruticose) within the grid, either on-site or for later identification in the laboratory." The option to identify species later in the laboratory could introduce inconsistencies in results if not standardized (e.g., storage or transport conditions).

A: Many lichen species require microscopic observations or chemical tests and therefore cannot be identified in the field. It is common and safe practice to transport lichens to the laboratory for identification, as they retain their key morphological and anatomical characteristics. For clarity, we added the sentence “Store and transport lichen samples in paper bags, ideally one per sampled tree, to ensure they retain their key morphological and anatomical characteristics needed for identification.”

C: "Soil sampling for biogeochemical properties is also influenced by horizons and thus it is critical to consider soils horizons while sampling." This sentence is redundant and could be streamlined for clarity. Additionally, it does not specify how horizons influence sampling strategies.

A: We replaced the sentence with a clearer text: “Description of soil horizons is crucial when conducting site characterization for purposes such as soil carbon and GHG assessments, fertility studies, remediation of contaminated soils, ecosystem restoration, and waste recycling. Soil properties — including physical, chemical, and biological characteristics — vary significantly between horizons. Therefore, sampling strategies should account for soil horizons to better explain the variability in the targeted physico-chemical properties. For example, organic horizons typically contain much higher organic matter content than

underlying mineral soil horizons, making them a key factor in assessing soil organic matter stocks. Additionally, sampling by horizon is fundamental to underpinning soil classification, as it allows a more accurate description of the soil profile and its development."

C: "The teabags are dried (40–60 °C) and weighed previously in the lab." The temperature range is broad.

A: We use 60 °C in our lab, but for others to use, depending on their lab conditions, the range 40-60 °C is acceptable, what will change is the time needed for drying, adjusted accordingly. In the text, we replaced the range for "oven ≤60°C", as some protocols even mention room temperature as a minimum, although we don't recommend it due to the possible presence of air humidity that will remain on the samples. Temperatures above ~70°C can also be damaging for the samples, potentially accelerating the decomposition.

C: "Record the hue, value, and chroma of each soil horizon using the Munsell soil colour charts (2010)." While the Munsell system is standard, this statement assumes familiarity with the charts without providing context or resources for those unfamiliar.

A: We provided a more detailed description and some online resources in the text: "Soil is a complex matrix whose colour is influenced by the soil formation factors including parent material, climate, time, organisms and topography. Therefore, soil color determination is indicative of the soil type and of the prevailing soil management. Determining soil color is thus an important variable in defining and classifying soils. A standard method for soil color determination is based on the Munsell Soil Colour determination (https://munsell.com/color-blog/defining-soil-profiles/; https://www.floridahealth.gov/environmental-health/onsite-sewage/_documents/act-presentation-03-soil-colors-and-their-interpretation-april-2015.pdf). The soil color is characterized by three properties called hue, value and chroma. Using the Munsell soil colour book, determine these properties as below."

C: "Detailed protocol follows:" (as an introductory phrase). This phrase appears multiple times without introducing a genuinely detailed protocol. The description often remains high-level and lacks procedural depth.

A: We removed the word "detailed".

C: "Participants analyzed data and suggested ways to manage forests sustainably in response to global environmental challenges." This conclusion is overly general and does not specify how these data analyses translated into actionable forest management strategies.

A: The sentence is no longer present due to the rearrangement of the text.

Competing Interests: No competing interests were disclosed.

Author Response 22 Mar 2025

Silvana Munzi

C: The absence of data associated with this article possibly weakens its replicability. It might be relevant to include illustrative datasets or simulated examples to demonstrate the protocol's effectiveness and provide insights into the expected outcomes. Also, some examples with associated metadata might increase the reachability of your study.
A: We agree that examples are relevant. Since the methods have been largely employed, there are many published papers that can provide illustrative datasets. We added the following references to some examples present in the literature besides the works already cited in the paper: "For replicability purposes, see studies from Rocha et al,. (2019)." "For the leaf litter decomposition protocol (Teabags) we suggested the reading of many articles published so far related to the TeaComposition initiative (e.g., Djukic et al 2018; Kwon et al. 2021), the TBI index (e.g., Keuskamp et al. 2013; Sarneel et al. 2024), or new experimental tests (e.g., Middelanis et al. 2023). As for local data, those articles mentioned for the TeaComposition Initiative, include results from some of the study sites where the Training School took place." "For humus type description we followed the EU humus type classification system (Zanella et al., 2011) and for some of the soil properties the protocol reflects on the EU ICP Forest Soil Manual for soil sampling and analysis (Cools and De Vos, 2020). ICP forest monitoring soil data on soil carbon stocks (De Vos et al., 2015) and C:N ratio (Cools et al,2014) has been evaluated at European scale." "The methods used to assess shrub and herbaceous vegetation cover are commonly used in semiarid woodlands and forests, e.g. see Nunes et al., 2014; Nogueira et al.(2018) and Köbel et al. (2021, 2023, 2024).

C: While the introduction discusses the need for holistic approaches to studying global change drivers, it does not specify what the study aims to achieve with this protocol. Even if not mandatory, it is advisable to include defined objectives and research questions to enhance the manuscript's clarity, impact, and alignment with COST's mission. In this direction, this research seems to quantify the impacts of varying grazing intensities, nitrogen deposition, and climatic stressors on tree regeneration, biodiversity, and soil health. Also, another valuable point is the applicability of standardized methodologies across diverse management scenarios to ensure replicability and scalability. The proposed protocol finally aims to provide actionable insights to inform sustainable forest management and align with EU biodiversity and climate objectives.
A: We agree with the reviewer. To focus more the introduction on the work developed, we have expanded on the objectives of the field trip adding the following text: "Semiarid woodlands and forested ecosystems are increasingly vulnerable to degradation due to multiple environmental drivers, including climate change, land-use change, fragmentation, and pollution. Understanding how different ecosystem components respond to these pressures is essential for assessing overall ecosystem degradation—both in terms of biodiversity and ecosystem functions/services. With this in mind, our goal is to compile and evaluate different methodologies for assessing these impacts, linking each ecosystem component to the drivers it responds to most strongly. Specifically, the field trip conducted as part of the training school aimed to provide participants with hands-on experience in assessing global change drivers and their real-world impacts on semiarid woodlands and forests. Through practical activities, students gained a deeper understanding of how forest ecosystems interact with key stressors such as air quality and climate change. In particular,

participants learned to quantify the effects of varying grazing intensities, atmospheric nitrogen pollution, and climatic stressors on multiple aspects of forest ecosystems, including forest structure, tree regeneration, biodiversity, and soil health."

C: Global change drivers (e.g., climate change, nitrogen deposition, grazing) are discussed individually, with no exploration of their interactions. I suggest to introduce a conceptual framework to illustrate the synergies and trade-offs among these drivers. For instance, discuss how nitrogen deposition and grazing jointly influence biodiversity and ecosystem health.
A: The complex interactions among global change drivers are the scope of the CLEANFOREST COST Action CA21138, but not of the training school. Moreover, we did not address this from a statistical perspective with the students, as no well-replicated manipulation experiments were available. Instead, our approach to exploring how pollution and climate extremes affect forest structure, soil, and biodiversity was to leverage a gradient of grazing intensity to investigate whether higher nitrogen input from livestock influences ecosystem responses to drought. For clarity, we added: "From the state of the art in the subject, we expect that increased nitrogen deposition can initially enhance plant productivity, potentially benefiting grazers. However, grazing can exacerbate the effects of nitrogen deposition by selectively removing certain plant species, leading to a decline in biodiversity and forage quality. Furthermore, climate change, through increased temperatures and altered precipitation patterns, can further modify these interactions, influencing ecosystem processes and outcomes. These interactions are less known in Mediterranean climate and need further work despite some works having been already developed (e.g. Ochoa-Hueso et al. 2017, Nogueira et al., 2018)."

C: The manuscript mentions sustainable forest management but does not sufficiently link its findings to EU policy frameworks. Please, expand the discussion to highlight how the protocol aligns with European objectives (e.g., Green Deal), such as climate resilience and biodiversity conservation. Provide actionable recommendations for policymakers and stakeholders.
A: Thanks for raising this important point. We linked the goals of the training school and results achieved to European forest strategies and regulations within the European Green Deal. In particular, we added the following text in the summary: 'After the practical experience in the field using these experiences, participants were divided into groups to analyze and discuss collected data together with trainers. Key findings were summarized in presentations, together with main take home messages and suggestions on further questions to be explored and related attributes to monitor. Finally, participants had also the opportunity to link their findings to policy actions, by providing indications on potential sustainable forest management actions to increase resilience of the studied woodland ecosystem, and of mediterranean forests in general, under global change, in line with European strategies and regulations.' Moreover, the following text was added at the end of the introduction: 'Finally, participants engaged in data processing and analysis, synthesizing their findings to draw conclusions, suggestions on further questions to explore and recommendations for sustainable forest management to promote biodiversity and increase forest resilience under global change. This way, participants had the opportunity to link scientific advances to forest-related strategies and regulations adopted (e.g., Nature Restoration Law) and under discussion (European Forest monitoring Law and soil strategy)

within the European Green Deal.'

C: There is a need to clarify how the proposed protocols fill possible gaps in existing methodologies. Basically, even without comparisons with existing frameworks, there is a need to know what is the novelty of your framework more explicitly. In this direction, the methods section is comprehensive, but it lacks prioritization, as some protocols are described without justification for their inclusion. Therefore, provide a clear rationale for their selection and relevance.
A: Our approach does not introduce any novel methodologies but rather utilizes existing and well-established methods that have been successfully applied in similar contexts together to have an holistic perspective on the ecosystem structure and function. The primary aim was to create a handbook that was suitable for participants in our training school and adaptable for other similar initiatives. The methodologies were chosen to maximize the forest aspects considered, avoid the need for costly equipment, and adapt the study to the short duration of the fieldwork. We added the following text to make it clearer: "We focused on presenting these methods in a way that is accessible and practical for users, to have an integrated overview of the ecosystem structure and functioning. The methods were selected for their simplicity, cost-effectiveness (requiring no expensive equipment), and comprehensive coverage of the key aspects of a forest study."

C: Include a section discussing potential limitations of the protocol, such as sampling bias or challenges in adapting methods to other ecosystems.
A: Section added as suggested. "For lichens, a key limitation is the expert knowledge required to accurately identify species. Untrained samplers are likely to overlook microlichens, particularly very small species, introducing a potential bias. Additionally, some species groups, such as Physciaceae or Lepraria, are difficult to distinguish, increasing the risk of misidentifications. Another constraint is that the protocol applies exclusively to epiphytic lichens, making it unsuitable for non-forest areas. The methods used to estimate vegetation cover and diversity are commonly applied in semiarid areas like the one studied. Nevertheless, the number and size of transects or quadrats affect the precision of cover estimates. Their definition relies on a compromise between ensuring that most of the variability in the vegetation is encompassed, which depends also on landscape heterogeneity, and cost-effectiveness in the time and resources needed. A potential limitation for soils is that the protocol does not provide a fully detailed description of soil characterization and classification. However, it refers to key soil classification systems (e.g., WRB, 2020), where comprehensive classification methods are described. The classification of humus types is included at its higher, more practical levels, while more detailed levels are referenced in the EU humus classification guidelines." Minor issues:

C: "Companhia das Lezírias stands as a testament to the intricate interplay between geology, climate, land use, and history." This is overly poetic and not aligned with the technical focus of the proposed protocol.
A: Removed as suggested.

C: "Most of the trees present inside the plot in our study were cork oaks, then the debarking year and the width of the cork layer were registered." The sentence is unclear in construction and lacks specificity about why the debarking year and cork layer width are

critical metrics for the protocol.
A: We removed the sentence and the part of the protocol related to leaf physiology because it was not applied in the training school.

C: "Participants applied these methodologies in real-world scenarios, conducting measurements in different grazing management settings to assess their effects on tree growth, biodiversity, and soil properties." This general statement is too vague and does not detail the specific methods used in these assessments.
A: Methods used are now mentioned both in the abstract and the introduction. Namely, we added the text: “(forest structure assessment, lichen diversity sampling, shrub and herbaceous diversity estimation, deadwood measurement and soil physico-chemical analysis).”

C: "Record all visible macrolichens (foliose and fruticose) within the grid, either on-site or for later identification in the laboratory." The option to identify species later in the laboratory could introduce inconsistencies in results if not standardized (e.g., storage or transport conditions). **A:** Many lichen species require microscopic observations or chemical tests and therefore cannot be identified in the field. It is common and safe practice to transport lichens to the laboratory for identification, as they retain their key morphological and anatomical characteristics. For clarity, we added the sentence “Store and transport lichen samples in paper bags, ideally one per sampled tree, to ensure they retain their key morphological and anatomical characteristics needed for identification.”

C: "Soil sampling for biogeochemical properties is also influenced by horizons and thus it is critical to consider soils horizons while sampling." This sentence is redundant and could be streamlined for clarity. Additionally, it does not specify how horizons influence sampling strategies.
A: We replaced the sentence with a clearer text: “Description of soil horizons is crucial when conducting site characterization for purposes such as soil carbon and GHG assessments, fertility studies, remediation of contaminated soils, ecosystem restoration, and waste recycling. Soil properties — including physical, chemical, and biological characteristics — vary significantly between horizons. Therefore, sampling strategies should account for soil horizons to better explain the variability in the targeted physico-chemical properties. For example, organic horizons typically contain much higher organic matter content than underlying mineral soil horizons, making them a key factor in assessing soil organic matter stocks. Additionally, sampling by horizon is fundamental to underpinning soil classification, as it allows a more accurate description of the soil profile and its development.“

C: "The teabags are dried (40–60 °C) and weighed previously in the lab." The temperature range is broad.
A: We use 60 °C in our lab, but for others to use, depending on their lab conditions, the range 40-60 °C is acceptable, what will change is the time needed for drying, adjusted accordingly. In the text, we replaced the range for “oven ≤60°C”, as some protocols even mention room temperature as a minimum, although we don’t recommend it due to the possible presence of air humidity that will remain on the samples. Temperatures above ~70°C can also be damaging for the samples, potentially accelerating the decomposition.

C: "Record the hue, value, and chroma of each soil horizon using the Munsell soil colour charts (2010)." While the Munsell system is standard, this statement assumes familiarity with the charts without providing context or resources for those unfamiliar.
A: We provided a more detailed description and some online resources in the text: “Soil is a complex matrix whose colour is influenced by the soil formation factors including parent material, climate, time, organisms and topography. Therefore, soil color determination is indicative of the soil type and of the prevailing soil management. Determining soil color is thus an important variable in defining and classifying soils. A standard method for soil color determination is based on the Munsell Soil Colour determination (https://munsell.com/color-blog/defining-soil-profiles/; https://www.floridahealth.gov/environmental-health/onsite-sewage/_documents/act-presentation-03-soil-colors-and-their-interpretation-april-2015.pdf). The soil color is characterized by three properties called hue, value and chroma. Using the Munsell soil colour book, determine these properties as below.”

C: "Detailed protocol follows:" (as an introductory phrase). This phrase appears multiple times without introducing a genuinely detailed protocol. The description often remains high-level and lacks procedural depth.
A: We removed the word “detailed”.

C: "Participants analyzed data and suggested ways to manage forests sustainably in response to global environmental challenges." This conclusion is overly general and does not specify how these data analyses translated into actionable forest management strategies.
A: The sentence is no longer present due to the rearrangement of the text.

Competing Interests: No competing interests were disclosed.