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ALIVE: A New Protocol for Investigating the Modern Pollen Deposition of Italian Forest Communities and the Correlation with Their Species Composition

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Abstract

Modern pollen deposition studies are essential to forestry and palaeoecological research, as they provide the key to understanding the relationship between the abundance of palynomorphs in natural (moss, litter, top core sediment) or artificial traps and the surrounding vegetation cover. In 1996, the EPMP (*European Pollen Monitoring Programme*) laid the foundations for pollen monitoring research in Europe, involving several countries and dozens of researchers in placing "Tauber-style" artificial traps across a wide range of ecosystems, and legitimising the collection of mosses for comparative studies. Here, we propose a straightforward, fast, and effective procedure—developed within the ALIVE "*TrAcking Long-term declIne of forest biodiVErsity in Italy to support conservation actions*" Project—for the collection of moss polsters and vegetation data, aimed at monitoring modern pollen deposition at the national scale. This protocol addresses a gap in existing literature, as no shared fieldwork guidelines are currently available. We demonstrate how the spatial pattern of modern pollen deposition can be investigated using two of the ALIVE Project's target taxa (*Fagus* and evergreen *Quercus*) to explore the potential of microbotanical data in reflecting the current distribution of forest tree taxa at a national scale. The data collected within the ALIVE Project provide a synoptic picture of pollen deposition across Italy's highly diversified landscapes and allow for preliminary considerations on the relationships between pollen deposition and modern vegetation cover of forest taxa.

Keywords: fieldwork; guidelines; Italy; moss polsters; plant cover; pollen deposition

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1. Introduction

The use of palynomorphs preserved in sediments is well established in palaeoecology as a powerful proxy for reconstructing the structure of past ecosystems, the composition and dynamics of plant populations, and the development of cultural landscapes [1–3], and many more. While palynologists strive to identify fossil pollen grains to the lowest possible taxonomical level (usually groups of species or genera, sometimes families, and more rarely to the species level), the difficulty of "translating" pollen percentages into the actual abundance of each plant in the study area affects the interpretation of pollen records from natural archives [4]. The amount of pollen accumulated at a site is rarely, if ever, in a 1:1 relationship with the species composition of the modern vegetation cover [5–7]. The occurrence and relative abundance of pollen types in a fossil record are influenced by a range of processes affecting primarily the ecosphere but also involving the lithosphere—such as plant productivity and its interannual variations, dispersal mechanisms, climate, runoff, sedimentation [8–11]—as well as taphonomical processes such as reworking [12]. The first step in this chain of processes involves examining modern ecosystems by correlating vegetation composition and structure (e.g., species cover and total forest cover) with the deposition of pollen following its production, dispersal, fallout and rainout. Pollen trapping in moss polsters minimises most sedimentary processes, beginning with surface runoff.

The expressions "*pollen rain*" and "*pollen deposition*" [13,14] are often used as synonyms, although they should not be regarded as identical concepts. "*Pollen rain*" implies an atmospheric origin for particles, neglecting other processes that can transport them into natural archives. Indeed, biological and minerogenic particles can reach their final sink through surface runoff, promoted by rainfall, floods, snowfall and snowmelt, avalanches, and other land surface processes. Several media can be used to investigate pollen rain and pollen deposition [15–18], including: (i) lake sediments, mosses, lichens, and litters: they track both the atmospheric provenance and the surface mobilisation of particles, making them useful records of pollen deposition; (ii) artificial pollen traps: depending on the height of their opening, they can record pollen deposition (if the opening is at ground level) or pollen rain (if elevated above the ground). The use of moss polsters as depositional records of direct fallout and rainout is therefore appropriate to account for the variety and complexity of the processes ultimately responsible for the presence of palynomorphs in natural archives. To produce robust and reliable reconstructions of past environments based on microbotanical proxies, it is essential to have a thorough understanding of the relationship between pollen and vegetation [19] and references therein. In this context, modern pollen deposition studies are valuable, as they allow comparison between the % abundance of pollen types found in natural or artificial traps and the modern cover of each taxon in the landscape. Given the diversity and spatial arrangement of plants across climates and biogeographical regions, a large number of data points are required for a robust understanding and modelling of modern pollen deposition [20]. This highlights the need for a tailored fieldwork protocol aimed at mosses and vegetation data collection, that is both scientifically sound and time efficient.

This paper aims to:

- Recall the origins of modern pollen monitoring projects in Europe and the launch of the EPMP (Section 2);
- Review the available literature and the procedures adopted by other scholars regarding fieldwork activities for modern pollen deposition monitoring and vegetation analysis (Section 2);
- Propose a simple yet effective experimental design integrating vegetation surveys with modern pollen deposition monitoring, developed within the ALIVE "*TrAcking Long-term declIne of forest biodiVErsity in Italy to support conservation actions*" Project, funded by the Italian Ministry of University and Research in 2023 (details in Section 3). This design (Section 4) aims to address the critical gap in modern pollen databases, which often lack corresponding vegetation data, and to generate high-quality paired datasets suitable for refining pollen–vegetation models and improving palaeoecological reconstructions;
- Describe the main results of the ALIVE Project and introduce a new database of modern pollen deposition and data on forest tree species across Italy (Section 5). These

data, collected from a wide range of ecological and biogeographical settings, are intended to support pollen-vegetation calibration studies and palaeoenvironmental reconstructions. They also represent a significant contribution to the existing body of modern palynological data at both national and European scale.

2. Pollen Monitoring in Europe: A Literature Review

Pollen monitoring studies began in Europe in 1996 with the launch of the *EPMP—European Pollen Monitoring Programme*, a Working Group of the Holocene Commission of the International Quaternary Association. The Programme aimed to increase the precision with which pollen analysts can interpret fossil pollen diagrams by studying pollen influx across natural and anthropogenic treelines. According to the Programme's promoters, the goals could be achieved through the study of pollen assemblages characteristic of forest types across Europe and the definition of quantitative relationships between vegetation communities and the pollen they produce. According to the first release of the EPMP guidelines [21] the collection of modern pollen could be achieved using artificial "Tauber-style" traps [22]. Transects were established in several countries (Italy, Switzerland, Bulgaria, Germany, France, Ireland, Greece, Iceland, Finland, Norway, Lithuania, Estonia, Poland, and Great Britain) with artificial traps placed in the field at the end of each flowering season (September–October) for a full year, allowing calculation of the annual pollen influx of the different species recorded by pollen grains. Additional guidelines [23] legitimised the collection of moss polsters as complementary surface samples (mentioned in [21]) to supplement trap results and enable further comparisons. Regarding the collection of vegetation data at the sites, the EPMP protocols recommend that "*both the local vegetation in the immediate vicinity of the trap and that in the wider region surrounding the trap is numerically recorded*". However, instructions for mapping vegetation data in a standardised way are not provided, leaving the method and extent of the surveyed area to national practices.

Over the past 20 years, the collection of moss polsters has become increasingly popular as a tool for monitoring pollen deposition, as it allows rapid sampling of large areas. A review of the available literature (some examples are reported in Table 1) highlights differences in the fieldwork procedures:

(i) The number of mosses collected at each site varies (1, 3, 5, more than 10);
(ii) Vegetation surrounding the traps is not always recorded, and when it is, no common protocol exists.

Table 1. A literature review summarising the various protocols adopted for fieldwork aimed at monitoring modern pollen deposition across Europe, with additional examples from America, Asia, and Africa.

Reference	Study Area	Sampled Ecosystems	Number of Mosses Sampled at Each Site	Vegetation Relevées
[24]	Western Norway	mown and grazed vegetation	Several (not specified), later analysed individually.	Within an area of 10 m^2, five 1 m^2 plot were surveyed. Vascular plants identified to the same taxonomic level as for pollen types.
[25]	Central Pyrenees (Spain)	montane, subalpine and alpine vegetation	2–4 mosses collected in an area of ca. 10 m^2 and then mixed into one sample.	Vegetation survey according to Braun-Blanquet (all plants) at the site and notes on the vegetation around the site.

Table 1. *Cont.*

Reference	Study Area	Sampled Ecosystems	Number of Mosses Sampled at Each Site	Vegetation Relevées
[26]	Western Italian Alps	forest openings above/below the treeline	1	General description of the main vegetation type.
[27]	Western Amazonia	montane forests	Several (not specified), likely mixed in one sample.	Vegetation data from 15 permanent plots of 1 ha.
[28]	Cyprus	coastal/wetlands, orchards, garigue, maquis, forests	15–20, mostly of surface soil, sometimes leaf litter and mosses.	Perennial plant species recorded over an area of about 100 m diameter.
[29]	transect across Finnish Lapland	from tundra-like open communities to boreal conifer forests	1	No site-specific relevées. General description of the vegetation zones encountered along the transect, with plant names (no cover).
[30]	Pechora-Ilych Nature Reserve (Russia)	pristine dark conifer forest	1	Detailed vegetation descriptions in a 1 m radius and at a 400 m^2 scale.
[31]	Namibia	savannas	No mosses, instead surface soils.	Vegetation recorded following Braun-Blanquet (species list and plant cover).
[32]	Tibetan Plateau (China)	alpine meadows and grasslands, sub-alpine shrubs, patchy conifer and deciduous forests	5 mosses later mixed in one sample.	Vegetation survey in each plot (list of vascular species and plant cover).
[33]	Northern China	conifer forest, deciduous forest, deciduous shrub, grass meadow, grass steppe, desert steppe and desert	4–5 subsamples (moss pollsters, litter and topsoil) collected randomly within an area of ca. 50 m^2 and mixed into one sample.	No site-specific relevées. Distinction of 7 vegetation types and list of the most frequent plants within each type.
[34]	Tagus Basin (Spain)	thermo-Mediterranean to oro-Mediterranean vegetation belt	Several moss fragments (usually 5) within a plot of ca. 20 × 20 m^2, later homogenised in the lab.	Vegetation structure and composition recorded, especially for woody taxa. Local tree and (in some cases) shrub cover (%) were recorded.
[35]	Serra da Estrela (Portugal)	meso-Mediterranean cultural landscapes with pine plantations, supra-Mediterranean heathlands, oro-Mediterranean high-elevation grasslands	1	Abundance of vascular plants was surveyed in 1 m^2. Up to 200 m away species were recorded using a 'nearest individual' method of plotless sampling.
[36]	Northern Greece	coastal meso-Mediterranean maquis, temperate and subalpine forests, alpine treeless vegetation	Not specified	Vegetation composition recorded within a 10 m^2 plot, with a focus on woody species. Presence/absence and canopy cover recorded every m along two orthogonal 10 m long transects.

Table 1 highlights the lack of a standardised protocol for fieldwork activities, which arises from a combination of practical, disciplinary, and historical factors:

(a) Interdisciplinary nature of Palynology: Palynology lies at the interface between botany, ecology, geology, and archaeology. Not all palynologists are trained botanists with a strong background in plants identification, taxonomy, and vegetation ecology. Accurate vegetation surveys and plant identification require expertise and time, which not all teams possess, leading to inconsistent or missing vegetation data accompanying modern pollen samples.

(b) Fieldwork constraints: Fieldwork is often limited by tight schedules, restricted funding, and logistical challenges, and it is therefore sometimes skipped or simplified.

(c) Historical evolution of protocols: Modern pollen analysis protocols developed differently across countries. Early studies mostly focused on pollen itself rather than its vegetation context, so vegetation surveys were frequently not integrated.

(d) Methodological challenges in vegetation description: Vegetation descriptions are difficult to standardise across ecosystems, making standard protocols challenging to enforce. In the absence of a central authority or standardised guidelines specific to modern pollen sampling and associated vegetation surveys, researchers often develop local or lab-specific methods.

Finally, the lack of a standard protocol affects data repositories, which often focus solely on storing pollen counts. Metadata such as vegetation descriptions or sampling protocols are frequently missing or incomplete, as they were not consistently collected or prioritised.

3. The ALIVE Project: Establishing an Effective Protocol to Monitor Pollen Deposition in Forest Ecosystems

In 2023, the Italian Ministry of University and Research funded the Project "*TrAcking Long-term declIne of forest biodiVErsity in Italy to support conservation actions*" (acronym ALIVE). The Project aims to identify the spatiotemporal dynamics of twenty key taxa characterising the Italian forests (*Abies*, *Picea*, *Larix*, *Taxus*, *Carpinus betulus*, *Betula*, *Corylus*, *Tilia*, *Ulmus*, *Fagus*, *Ilex*, *Quercus ilex*, *Hedera*, *Vitis*, *Calluna*, *Arbutus*, *Buxus*, *Myrtus*, *Hippophaë*, and *Ephedra*) over the past six millennia. This objective is based on the principle that pollen data provide quantitative insights into past vegetation composition, turnover, and rates of change. To achieve the aims of the project, two tasks were envisaged:

(i) A survey of available pollen records from the last six millennia for the Italian Peninsula, to define the rates, patterns, timing, and modes of range shifts in the target taxa across the country;

(ii) A comparison of past and current distribution of the target taxa using vegetation maps and new evidence on modern pollen deposition collected within the ALIVE project primarily through the analysis of moss polsters.

To accomplish the second task, the ALIVE participants established a new protocol specifically designed for fieldwork activities (Section 4.1). This protocol includes the collection of modern pollen deposition samples, accurate location referencing, and associated vegetation data recorded at various spatial scales. In the ALIVE Project, moss polsters are the preferred medium for monitoring modern pollen deposition. Moss samples have already been the focus of several previous studies (see Section 3). In particular, ref. [14] compared pollen spectra from artificial traps and moss samples and, besides highlighting the lack of a standardised technique for collecting moss samples, the authors raise two additional questions: (i) how many years of pollen deposition are represented by a moss sample, and (ii) how different pollen types are differentially retained in moss. Both factors may vary significantly between artificial traps and moss samples.

4. Material and Methods

4.1. The ALIVE Experimental Design for Field Data Collection

The experimental design developed within the ALIVE Project aims to provide a simple and standardised procedure for field data collection. Between March 2024 and April 2025, 250 sites were selected across Italy for modern pollen deposition studies, and vegetation was recorded. The workflow includes the following steps:

a. Site selection and coding. At each site chosen for moss polsters and vegetation data collection, three subsampling points were identified to account for local variations in vegetation composition and cover, which are likely to be reflected in pollen deposition. Each site was assigned a three-letter code (i.e., the first three letters of the municipality name), followed by a progressive collection number within the project. The three subsampling points were located along a 100 m nature trail, approximately 50 m apart (Figure 1). Urban streets and car-accessible roads were excluded due to potential disturbance. Each subsampling point was additionally labelled with the letter A/B/C.

b. Georeferencing. Each site was identified by the geographical coordinates of its central point. Latitude, longitude and elevation were recorded using GPS devices and common smartphone apps. Under optimal conditions—clear skies and minimal obstruction—the precision of such apps is approximately ±3–5 m. This precision is expected to improve with future advances in GNSS (Global Navigation Satellite System) technology, potentially achieving centimetre-level accuracy in consumer applications.

c. Moss collection. At each subsampling point, one moss polster was collected using a knife. The sample surface should be between 10–20 cm^2, with thickness depending on moss height. The basal section (including roots and sediment/soil beneath the moss) was removed. The moss was placed in a labelled plastic bag (site code + progressive collection number + A/B/C).

d. Vegetation survey (local scale). All species growing within a 4 × 4 m square around each subsampling point were recorded.

e. Repetition at subsampling points. The procedure described above was repeated at the other two subsampling points.

f. Vegetation survey. After moss collection and local vegetation recording, the vegetation growing along the 100 m trail was observed, with special attention to woody species, as Arboreal Pollen (AP) is known to be dispersed over long distance [21]. The percentage cover of these taxa along the entire trail was estimated and recorded.

g. Data recording. All information regarding the site (name, acronym and terrain parameters), the sampled moss polsters and vegetation data were reported on a customised fieldwork form (Figure 2).

To better illustrate the experimental design described above, one site sampled within the ALIVE Project is presented as an example. Using a Google Earth image, Figure 1a shows the location of the Tirli site (Tuscany, central Italy). The sampling area, situated within dense natural woodlands dominated by *Quercus ilex* and *Castanea sativa*, hosts a diverse wild fauna with boar, hare, roe deer and pheasants. The site acronym was TIR, with the progressive collection number 03. Three subsampling points were established, labelled TIR03/A/B/C, respectively (Figure 1b). The site was uniquely identified by the geographical coordinates and elevation of its central point, i.e., TIR03/A. All information collected at the site was recorded in the customised field form (Figure 2).

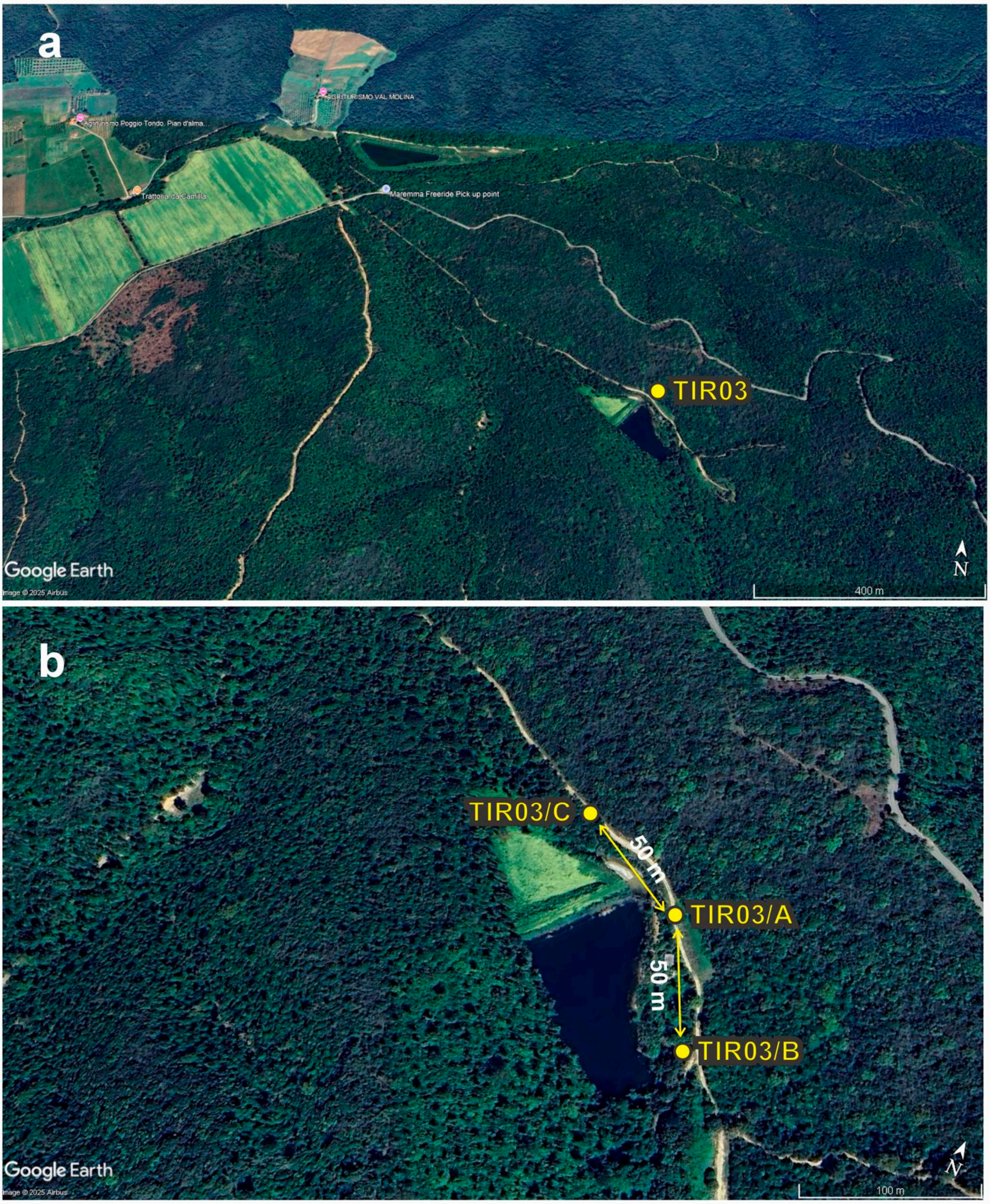

Figure 1. Google Earth image referring to a real case study from the ALIVE Project. (**a**): overview of the Tirli site (acronym TIR03, Central Italy); (**b**): identification of the 3 subsampling points, labelled A/B/C, used for the collection of moss polsters and vegetation data. At each subsite, the list of species occurring within a 4 × 4 m square was recorded, together with the cover of each woody species identified along the 100 m trail.

Fieldwork form

part 1 - general data

Site name: **TIRLI** Site acronym and number: **TIR03**
Date: **02/03/2024**

Operators: F. Di Rita, L. Caucci, E. De Luca, F. Michelangeli, R. Pini

Coordinates (WGS 84): **42.849°N** **10.866°E**

Elevation: **117 m asl** Aspect and slope: **to be calculated in lab**

Notes: ______________________________

part 2 - subsampling sites

site TIR03/A
vegetation type: **seminatural evergreen shrubs on the shore of the lake**
tree cover (%): **10%** shrub cover (%): **60%** herb cover (%): **40%**
list of species within a 4x4 m square:
Arbutus unedo, Asparagus acutifolius, Carduus sp., *Cistus salviifolius, Coleostephus myconis, Crepis* sp., *Geranium* sp., *Lapsana* sp., *Myrtus communis, Phillyrea angustifolia, Phillyrea latifolia, Plantago lanceolata,* Poaceae, *Sanguisorba minor, Quercus ilex, Sonchus* sp., *Stellaria media, Viburnum tinus*

site TIR03/B
vegetation type: **seminatural evergreen shrubs**
tree cover (%): **10%** shrub cover (%): **40%** herb cover (%): **10%**
list of species within a 4x4 m square:
Cistus salviifolius, Erica arborea, Hieracium sp., *Inula viscosa, Phillyrea latifolia,* Poaceae, *Quercus ilex, Spartium junceum, Viburnum tinus*

site TIR03/C
vegetation type: **seminatural evergreen shrubs**
tree cover (%): **20%** shrub cover (%): **30%** herb cover (%): **30%**
list of species within a 4x4 m square:
Arbutus unedo, Cistus monspeliensis, Cistus salviifolius, Coleostephus myconis, Crepis sp., *Erica arborea, Euphorbia helioscopia, Geranium* sp., *Inula viscosa, Lavandula stoechas, Plantago lanceolata,* Poaceae, *Prunella* sp., *Urospermum dalechampii*

part 3 - woody species identified along the 100 m trail

vegetation type: **Mediterranean maquis**
list of species and cover (values according to the Braun Blanquet system)
Quercus ilex (4), *Myrtus communis* (1), *Arbutus unedo* (2), *Erica arborea* (+), *Quercus suber* (+), *Phillyrea latifolia* (+), *Phillyrea angustifolia* (+), *Rhamnus alaternus* (+), *Cistus salviifolius* (+), *Cistus monspeliensis* (+), *Lavandula stoechas* (+), *Fraxinus ornus* (+), *Pinus pinaster* (+), *Viburnum tinus* (+)

Figure 2. Example of a completed fieldwork form developed within the ALIVE Project. Part 1 of the form records general site information, including the site name, acronym, acquisition number, elevation, and geographical coordinates. Part 2 provides a list of plant species growing within a 4 × 4 m area around each of the three subsampling points, together with the vegetation type and the percentage cover of trees, shrubs, and herbs. Part 3 lists the woody species occurring along the 100 m trail and their cover, expressed according to Braun-Blanquet cover abundance classes (r, +, 1, 2, 3, 4, 5).

4.2. Moss Samples Lab Processing and Pollen Analysis

The collected material was transferred to the palynological laboratories for the physico-chemical treatment aimed at extracting palynomorphs. The three moss polsters collected at each site were subsampled and combined into a single composite sample. The dry weight and volume of the composite sample were then estimated. *Lycopodium* tablets with a known number of spores were added to estimate pollen concentration [37]. The chemical protocol used to extract and enrich pollen from mosses was based on standard procedures, including treatment with HCl 37%, HF 40%, hot NaOH or KOH 10%, and acetolysis [38–42]. Each sample was sieved through a 250 μm mesh and rinsed with distiller water to remove moss tissues, followed by sieving through a 7 μm mesh to remove the fine organic fraction that might otherwise hinder microscope observation. Pollen samples were stored in glycerol.

All samples proved suitable for microscope analysis, confirming the effectiveness of the lab treatments. Pollen analysis was performed under light microscopes at 400–1000× magnification, with reference to pollen morphology atlases and identification keys [40,43,44], online databases (Non-Pollen Palynomorph Database—https://non-pollen-palynomorphs.uni-goettingen.de/; The Global Pollen Project—https://globalpollenproject.org/; Paldat-Palynological Database—https://www.paldat.org/, accessed on 1 February 2025) as well as reference collections. Pollen percentages from moss polsters were calculated based on the sum of terrestrial plant taxa, excluding aquatics, ferns spores, and non-Pollen Palynomorphs.

4.3. Pollen Distribution Mapping, Data Visualisation and Explorative Statistical Analysis

Pollen percentages from the ALIVE project were integrated with modern pollen spectra available in the European Modern Pollen Database (EMPD2—https://empd2.github.io/, accessed on 1 February 2025) [45]. From this dataset, only records with reliable georeferencing (EMPD location reliability code "A" and "B") were included. Soil samples were excluded due to potential differences in pollen deposition and preservation arising from taphonomical problems. When multiple pollen spectra referred to the same coordinates, pollen counts were summed, and percentages were recalculated from the combined total pollen count. To enhance the spatial resolution of the pollen dataset, additional modern surface samples were retrieved from the Neotoma Paleoecology Database & Community (https://www.neotomadb.org, accessed on 1 February 2025) [46]. When possible, pollen surface samples from well-dated sedimentary sequencies (with at least one chronological control between 6–3 ka cal BP and another between 3–0 ka cal BP) were digitised from the literature.

To visualise relationships between pollen percentages in moss polsters and the modern vegetation cover of two target forest taxa of the ALIVE Project, XY scatterplots were produced.

Major ecological gradients within the ALIVE dataset were explored using Detrended Correspondence Analysis (DCA), applied to the square-root-transformed (sqrt) pollen percentages of the target taxa. Data standardisation and ordination were performed using the vegan package [47] in the R environment [48].

4.4. Spatial Modelling

The spatial distribution of pollen percentages was interpolated using a Bayesian thin plate regression spline [49,50], implemented in the open access averageR application of the Data Search and Spatiotemporal Modelling tool (DSSM ver. 25.06.1; https://isomemoapp.com/app/iso-memo-app, accessed on 1 June 2025), developed within the Pandora & IsoMemo initiatives [51]. This approach enables continuous surface mapping of pollen data, facilitating the exploration of spatial variability and the visualisation of regional/local patterns in pollen representation of forest taxa. To highlight areas of potential local presence, we applied isolines of pollen percentages to the results of Bayesian spatial interpolation. In the following section, we provide an example using two of the most widespread forest taxa in Italy: evergreen *Quercus* (*Q. ilex* and *Q. coccifera* type) and *Fagus*, selected among those targeted by the ALIVE Project. We applied the thresholds proposed by [52] (2%–5.6% for *Fagus* and 2%–5% for evergreen *Quercus*).

5. Results

5.1. The New Dataset of Modern Pollen Deposition Sites for Italy

Using the protocol presented in this study, we substantially increased the number of modern pollen deposition sites available for Italy, focusing on areas that were poorly or not represented in the EMPD2, namely the Po Plain of Northern Italy, the easternmost northern region of Friuli Venezia Giulia, the central Apennines, and Sardinia. The ALIVE project contributed 250 new modern pollen records, while the literature survey and diagram digitization yielded 65 pollen spectra from core top sediments. Selection of sites and merging of duplicates from the EMPD2 database resulted in 353 records. In total, 668 modern pollen records were compiled for analysis (see Supplementary Table S1). The spatial distribution of these sites is shown in Figure 3.

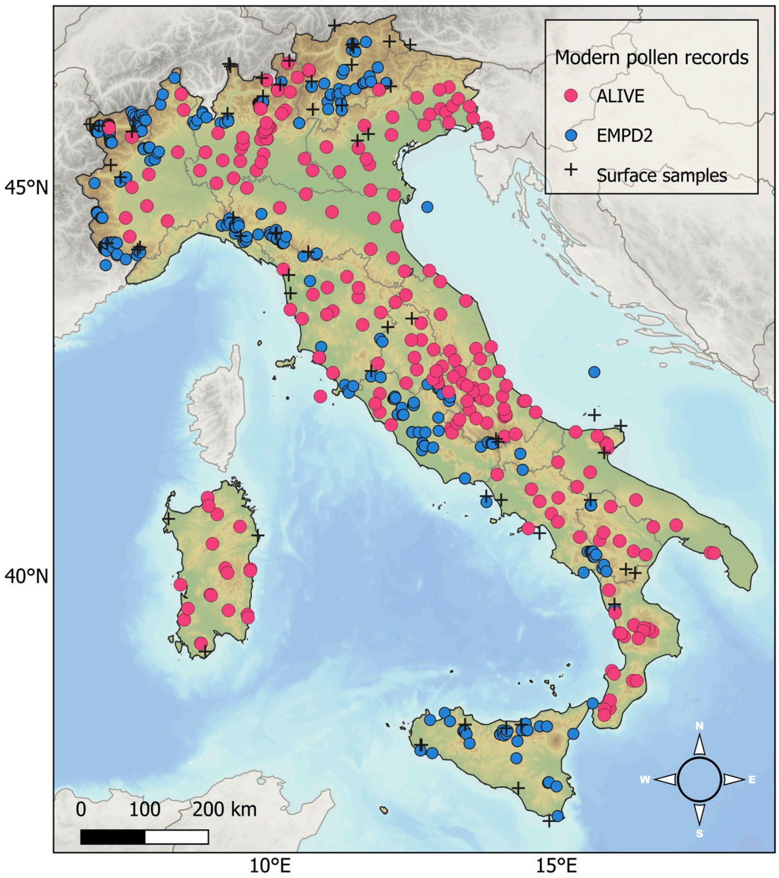

Figure 3. Distribution of the available modern pollen data: New sites contributed by the ALIVE Project are shown as red dots, while sites stored in the EMPD2 are indicated with blue dots. Black crosses represent core top sediments from well-dated sedimentary sequences.

5.2. Overview of the ALIVE Dataset: Relationships Between Variables and Detrended Correspondence Analysis on the 20 Target Taxa

The XY diagram shown in Figure 4 focuses on *Fagus* and evergreen species of the genus *Quercus*, two important components of Italian forest ecosystems. For both taxa, we observe a statistically significant correlation between pollen percentage and forest cover (*p*-value < 2.2×10^{-16}), with increasing pollen abundance corresponding to higher plant cover. The correlation is generally moderate, with a higher R^2 value for evergreen *Quercus* (R^2 = 0.69). In the case of *Fagus*, its absence or scarcity along the 100 m trail (0% plant cover, r, + or 1 in the Braun-Blanquet system) is accompanied by up to 6% pollen abundance, likely reflecting the presence of scattered plants or small populations within a maximum 2 km distance from the sites. A similar pattern is observed for evergreen *Quercus*, whose absence or scarcity in the vegetation corresponds to up to 12% of pollen abundance. This likely reflects the higher productivity and more efficient dispersal mechanisms of oaks compared to beech, which is evident across all the abundance classes. In addition, the moderate correlation between plant and pollen data may also result from uncertainties inherent in Braun-Blanquet classes, which encompass wide ranges of percentage cover values.

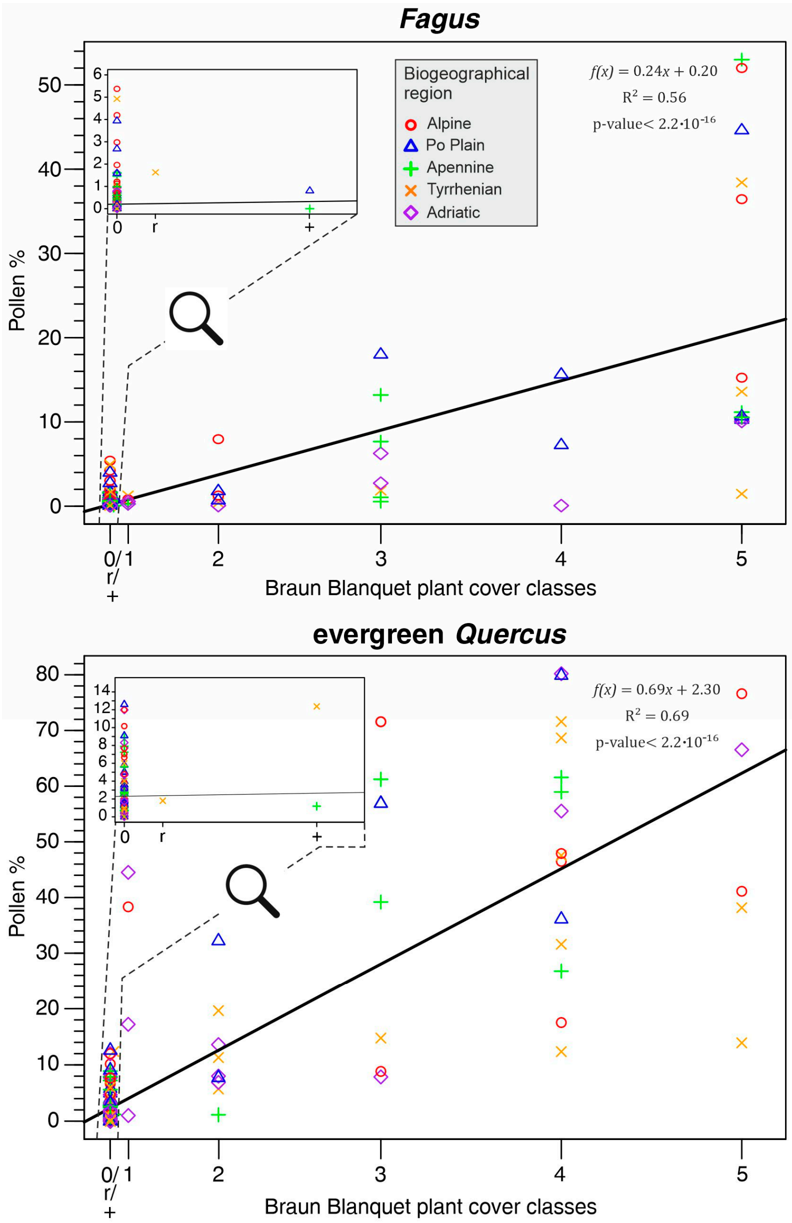

Figure 4. Braun-Blanquet plant cover classes plotted against pollen percentages of *Fagus* and evergreen *Quercus* recorded in the moss polsters collected by the ALIVE Project. Sites are represented with symbols (see upper panel) corresponding to their biogeographical region.

5.3. Overview of the ALIVE Dataset: Detrended Correspondence Analysis on the 20 Target Taxa

The ordination analysis was performed using modern pollen deposition for the 20 target taxa considered within the ALIVE Project. To facilitate interpretation of the biplot (Figure 5), sites are represented with symbols corresponding to their associated biogeographical region (Index of/Natura2000/Lista Rossa degli Ecosistemi Italiani/_prodotto_D). In the DCA biplot, sites with similar species composition cluster near each other, reflecting their biogeographical distribution in the main groupings. The first axis represents a latitudinal gradient, separating Alpine sites on the left from the Adriatic-Tyrrhenian on the right. *Corylus*, *Hedera* and *Carpinus betulus* are the most common taxa occurring across all biogeographical regions. In contrast, *Ilex*, *Taxus*, *Myrtus*, *Arbutus*, *Ephedra*, *Buxus*, *Ulmus*, *Tilia* and *Larix* are underrepresented in the dataset, with only a few sites showing high percentages. *Picea* and *Betula* characterise the Alpine sites, together with *Larix*, which is underrepresented (only one site in Trentino-Alto Adige reaches 10%). *Fagus* occurs along the entire latitudinal gradient but is most abundant in Apennine sites; *Abies* shows a similar pattern, but it is less represented in the dataset. Evergreen *Quercus* species are emblematic of Tyrrhenian sites (mainly Sardinia), where their pollen percentage exceeds 50%. Sites in the Po Plain are represented by taxa common to the whole dataset and are therefore clustered near the centre of the plot.

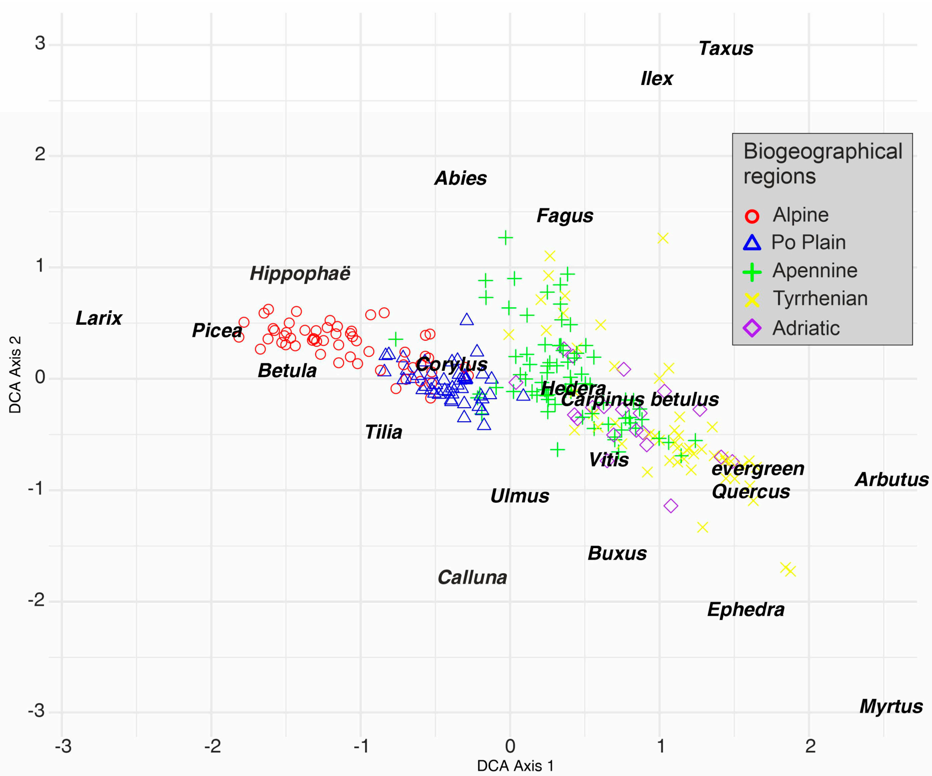

Figure 5. DCA biplot of the ALIVE dataset. Sites are represented with symbols corresponding to their biogeographical region.

5.4. Occurrence Maps and Bayesian Modelling on Modern Pollen Deposition Data: An Example for Two Forest Tree Taxa (Fagus and Evergreen Quercus)

Current occurrence record maps were compiled using multiple data sources, including the Italian National Biodiversity Network (https://www.nnb.isprambiente.it/, accessed on 1 June 2025), the national vegetation plot database [53], and expert-validated citizen science observations (https://www.inaturalist.org/, accessed on 1 June 2025). The resulting occurrence records were assigned to a 10 × 10 km grid and displayed as presence-only cells (Figure 6a,d). These data were compared with Bayesian interpolation maps based on modern pollen samples (Figure 6c,f) to evaluate the reliability of the modelled distributions against current occurrences of the target species. The analysis of pollen deposition data for *Fagus* and evergreen *Quercus* (Figure 6b,e) highlights a good correspondence with their occurrence record maps.

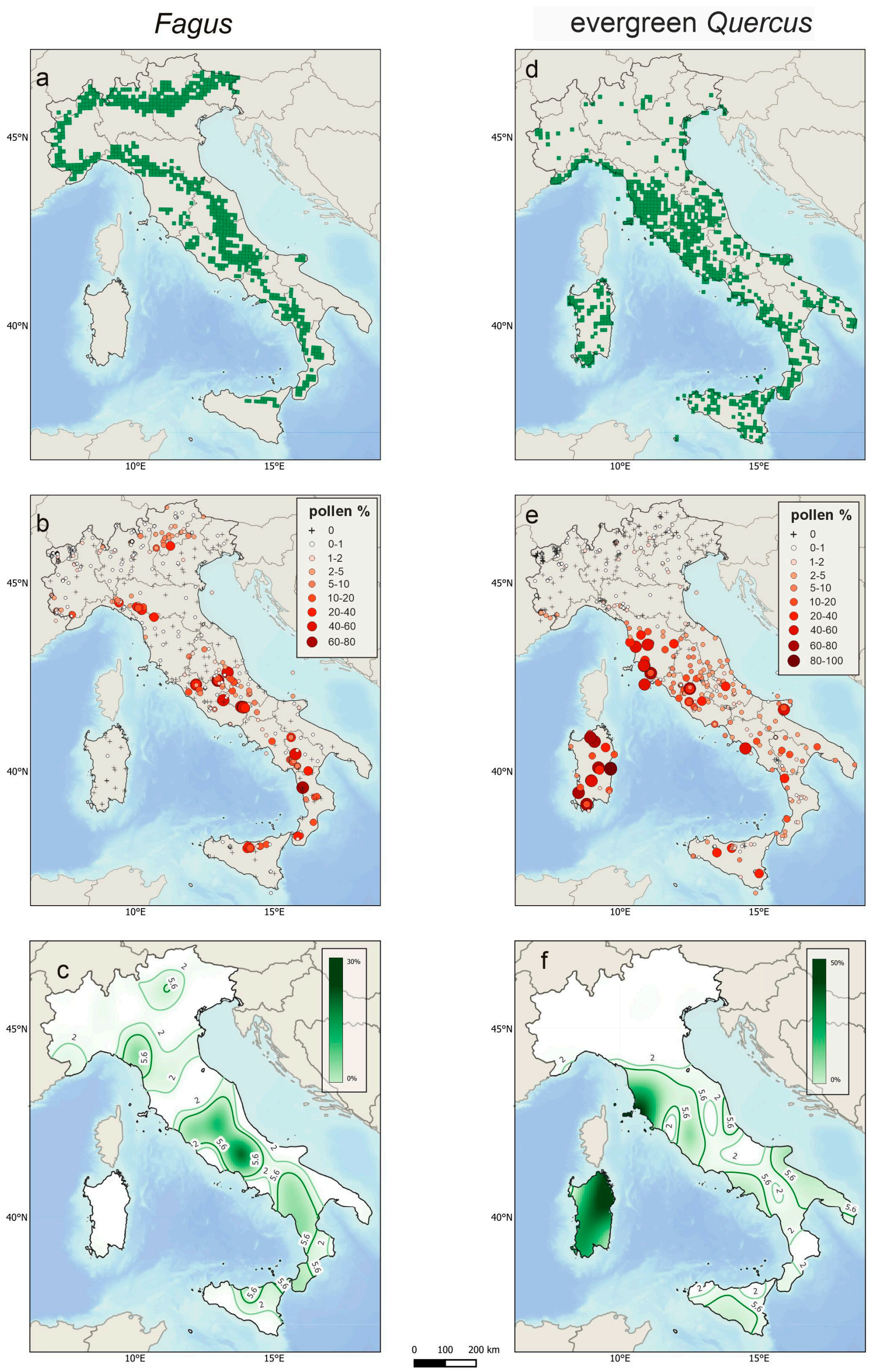

Figure 6. Distribution maps (**a**,**d**), pollen percentages (**b**,**e**) and Bayesian spatial interpolation (**c**,**f**) for *Fagus sylvatica* and evergreen *Quercus* (*Q. ilex* and *Q. coccifera*).

For *Fagus*, pollen is absent in 189 out of 668 pollen records (28.3%) and recorded under the threshold of 2% in 325 sites (48.6%). In 69 sites (10.3%) pollen percentages fall between 2%–5.6%, while only 94 sites (14%) exceed 5.6%. The geographic distribution of *Fagus* pollen exhibits clear spatial patterns, with clusters reflecting the local presence of beech trees (>2%). In northern Italy, high values are observed in the Venetian Prealps and

Dolomites, the Maritime Alps, and the north-western Apennines. In central Italy, high percentages occur in the central Apennines of Latium, Abruzzi and Molise, with a small cluster in the volcanic caldera of Vico lake and Cimini Hills. In southern Italy, a main cluster is detected in the Lucanian Apennines and Pollino national parks, with localised high values along the Catena Costiera and Aspromonte ranges. In Sicily, *Fagus* is primarily recorded in the Madonie and Nebrodi Mountains. This spatial pattern is also evident in the modelled distribution of modern pollen deposition, supporting evidence for local presence and potential areas of higher density of *Fagus* (Figure 6c).

For evergreen *Quercus* (*Quercus ilex* and *Quercus coccifera*), pollen is absent in 280 out of 668 pollen records (41.9%) and it is recorded under the 2% threshold in 216 sites (32.3%). In 71 sites (10.6%) pollen ranges from 2%–5%, while in 110 sites (16.5%) it exceeds 5%. The map of pollen deposition records shows high spatial coherence with current occurrence records map, broadly reflecting the distribution of mediterranean evergreen forests and maquis dominated by *Q. ilex*. Pollen is often absent or present in low amounts (<1%) in moss polsters from Northern Italy (Figure 6e), reflecting the scattered and limited occurrence of these plants in modern vegetation. Higher pollen percentages are detected along the Tyrrhenian coast, extending inland across parts of Tuscany and Latium (Figure 6e). Lower percentages punctuate the Adriatic coast except for higher values in Apulia (Gargano and Salento sub-regions) where *Q. coccifera* contribute to the pollen signal of evergreen *Quercus*. Similarly, *Q. coccifera* also contributes to the pollen percentages in Sicily and Sardinia. In Sardinia *Q. coccifera* occurs mainly in the south-western sector of the island, while the highest values in the eastern and northern sectors are attributed to *Q. ilex*. The spatial model highlights broad and continuous areas with potential local presence of evergreen *Quercus* (>2%), with the highest modelled estimates corresponding to the northern Tyrrhenian sector and Sardinia where dense *Q. ilex* forests and maquis currently occur (Figure 6f).

5.5. The ALIVE Database

Pollen percentages and plant cover data from Italian forest communities, collected during the project, will be made available at the end of the research (early 2026) as a stand-alone database. These data will also contribute to a future version of the Eurasian Modern Pollen Database. For each site, pollen and vegetation cover data will be accompanied by geographic coordinates, elevation, and mean climate variables retrieved from international databases such as WorldClim or CHELSA.

Given ongoing climate change, we emphasise the importance of including precise georeferencing of sampling points and detailed vegetation surveys in modern pollen deposition databases. This approach will facilitate long-term monitoring and allow for future resampling at the same locations.

6. Discussion

6.1. Progresses in Pollen Deposition Studies and Open Questions

The protocol developed within the ALIVE Project provides a simple and effective approach for field data collection. This method refines sampling practices and improved monitoring of pollen deposition through a fast collection procedure with precise georeferencing (±3–5 m: see Section 4.1), enabling the assessment of the relationship between pollen deposition and modern vegetation in small forest parcels. The ability to monitor vegetation changes and the resulting pollen deposition—while returning to the same sites over time with high precision—represents a significant advancement for palynological research and studies on vegetation dynamics. Furthermore, selecting 3 subsampling points within the same area allows for averaging differences among individual samples both at the macroscopic level (vegetation data), and the microscopic level (pollen composition). This approach provides a more robust picture of species occurrence and their ecological associations.

That said, we are aware that pollen grains retained in a trap represent input from both very local sources and broader surrounding areas (see examples in [54–57]), implying that data on species occurrence and distribution are needed well beyond the 100 m trail used in our protocol. The issue of determining the appropriate spatial extent of vegetation to associate with modern pollen data is often discussed within the palynological community; however, no universally applicable or practical solution has yet been proposed. Despite concerted efforts to define pollen percentages thresholds indicating the local or regional presence of forest taxa, relatively few studies have explored empirical thresholds derived from modern pollen data [52] and references therein. Conducting tens or hundreds of phytosociological relevées using traditional methods across large areas is nearly impossible due to the time intensive nature of such work. Aerial imagery has been used for several decades [58,59] but the limitations of this approach are well known: while effective for delineating physiographic units, vegetation and land-use patterns, it cannot produce accurate maps of species distribution (particularly for the understorey and the forest floor) unless complemented by field-based phytosociological relevées. Vegetation maps, produced by botanists, phytosociologists and Forest Services at various scales, are available for many European regions. These can provide a broader perspective on species occurrence, distribution and abundance, and should be integrated with small scale relevées carried out in conjunction with moss sampling.

When comparing pollen and vegetation data, the different taxonomic systems used by palynologists and botanists can be a concern. Considering that several plant families cannot be identified to the genus or species (or species group) level by palynologists, an important question emerges: to what level of identification should plants be recorded in the field? There are of course several possible approaches, i.e.:

(i) Identify plants to the lowest possible taxonomical level. Once the species list is compiled, each plant name can be matched to its corresponding pollen morphological type harmonising the two taxonomies by downscaling the botanical one;
(ii) Identify plants in the field according to pollen taxonomy (e.g., Poaceae, instead of individual grass species). This approach speeds up fieldwork and simplifies the comparison between pollen and vegetation, but inevitably results in the loss of detailed botanical information that could be valuable for future research.

Another issue that arises when comparing pollen and plant data concerns their differing representativeness. Some taxa are overrepresented in pollen samples relative to vegetation relevées because of their high pollen productivity and dispersal capacity (e.g., conifers and anemophilous plants), while others show the opposite pattern, with higher plant cover but lower pollen percentages—as is typical of zoophilous taxa. To achieve robust reconstructions of plant cover, particularly in palaeoecological research, it is essential to estimate the RPP (Relative Pollen Productivity) of each taxon across different biomes and climatic gradients.

The data collected within the ALIVE Project provide a synoptic overview of pollen deposition across the highly diversified Italian landscapes and allows for some preliminary considerations regarding the relationships between pollen deposition and the modern vegetation cover of forest taxa. The case studies presented in Section 5 demonstrate that increasing the spatial resolution of modern pollen data greatly refine the relationship between modern pollen deposition and the current distribution of forest taxa at a national scale. Monitoring modern pollen deposition represents a powerful tool for identifying areas of high pollen productivity in tree taxa, which likely correspond to areas where these taxa occur at higher density within forest communities (Figure 6a,c). In the case of evergreen

Quercus, the area showing high modern pollen percentages in Sardinia and in the northern Tyrrhenian sector closely correspond to the estimates of "very high probability" (from 70 to >90%) of *Q. ilex* presence [60].

One final issue warranting further attention is the biochronology of mosses, a topic also of interest to scholars involved in biomonitoring heavy metals [61]. Moss growth rates vary considerably by species and environment, ranging from extremely low in xerophytic mosses (steppes, semideserts and rock cliffs) to several cm/months in aquatic species. Optimal conditions include adequate edaphic and climatic moisture combined with low light availability. In boreal forests, under favourable conditions, common mosses exhibit linear growth increments of approximately 10–30 mm per year [62]. In contrast, in Mediterranean or drought-prone ecosystems, these values can be significantly lower due to moisture stress [63]. It may be hypothesised that very fast-growing mosses could introduce seasonal biases in their ability to retain particles (through particle dilution or concentration), which in turn affects their reliability as analogues in palaeoenvironmental studies. Before using mosses, it is advisable to obtain precise identification by an expert in order to accurately constrain their growth rates.

6.2. Different Ways to Express the Pollen Concentration of Moss Samples

Pollen concentration provides an estimate of the abundance of pollen in a sample, expressed as the number of grains per gram or per cubic centimetre (cm^3). Traditionally, pollen concentration is referred to the weight or volume of the entire sample, which includes various components (organic matter, mineral particles, and voids). To provide a standardised and comparable metric for quantifying and interpreting pollen deposition across different media and sites, it may be reasonable to express pollen concentration relative to the total organic content of the sample.

To test the feasibility of this approach, we used a LECO TGA601 Thermogravimetric Analyzer to estimate the organic content of a moss sample that had already been analysed for its pollen content. Table 2 reports the sample details (number of grains counted, *Lycopodium* spores added and counted, initial sample weight and volume, and the weight and volume of the organic component calculated after thermogravimetric analysis), along with the different ways of expressing pollen concentration. The concentration values based on the weight or volume of the whole (wet moss) sample and those based solely on the organic content differ by as much as one to two orders of magnitude.

Table 2. Different ways to express the pollen concentration of a moss sample. Column 1 reports the name and provenance of the moss sample. Column 2 indicates the pollen sum (the total number of identified pollen grains) obtained for the sample, the number of *Lycopodium* spores added to calculate concentrations and the number of *Lycopodium* spores counted. Column 3 reports the initial sample weight (g) and volume (cm^3), as well as the total organic content (expressed in g and cm^3) estimated through thermogravimetric analysis. Column 4 provides an estimate of pollen concentration relative to the initial moss weight and volume, as well as to its total organic content.

Sample Name and Provenance	Pollen Grains Counted, *Lycopodium* Spores Added/Counted	Sample Weight, Volume, TOC vs. Weight and Volume	Pollen Concentration
Larix M3_2m Mount Spundascia (Central Italian Alps)	Pollen sum: 1001 grains	moss weight: 2.6 g	102,797 grains/g
	Lycopodium spores added: 13,761	moss volume: 12 cm^3	17,133 grains/cm^3
	Lycopodium spores counted: 67	moss total organic content: 0.1872 g	1,177,129 grains/g of total organic matter
		moss total organic content: 0.0678 cm^3	3,030,084 grains/cm^3 of total organic matter

7. Conclusions and Perspectives

Understanding the relationships between modern pollen deposition and vegetation across different biomes and climates is essential for accurate interpretations of fossil pollen records. Furthermore, pollen monitoring can make a substantial contribution to forestry by providing insights into the relationship between areas of high pollen deposition and the relative abundance of tree taxa within forest communities. The adoption of a shared protocol for the collection of moss polsters and associated vegetation data should be a prerequisite for meaningful data comparison among different research groups. The experimental fieldwork design presented in this paper aims to address a key gap in current pollen monitoring research, where data collection often lacks standardisation.

The data gathered through the ALIVE Project significantly enhances current knowledge on modern pollen deposition across Italy by improving the spatial resolution of existing datasets. The combined analysis of modern pollen data and contemporary vegetation distribution paves the way for further applications, including:

- Estimating tree species density in forest ecosystems;
- Exploring pollen dispersion patterns and identifying percentage thresholds for the local presence of forest taxa;
- Developing transfer functions to model the relationship among modern pollen data, present-day vegetation, and climate variables, thereby supporting palaeoecological reconstructions;
- Improving the statistical comparison between pollen and vegetation data through a more precise recording of plant cover, since Braun-Blanquet classes involve a degree of uncertainty, whereas pollen data are expressed as exact percentage values.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16111722/s1, Table S1: List of the sites of modern pollen deposition compiled for analysis and associated metadata.

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Data Availability Statement: The original data produced during the ALIVE Project will be made available in 2026 as a stand-alone database. These data will also contribute to a future version of the Eurasian Modern Pollen Database. Inquiries regarding access to the stand-alone database can be directed to the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

ALIVE	acronym of the project "*TrAcking Long-term declIne of forest biodiVErsity in Italy to support conservation actions*"
EPMP	European Pollen Monitoring Programme
EMPD	Eurasian Modern Pollen Database

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