Towards an effective in-situ biodiversity assessment in European forests

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Highlights:

- The current information on forest multi-taxon biodiversity is deficient for most taxa
- Existing data may help define an effective monitoring network for European forest
- Sampling effort to assess species diversity varies across taxa and forest types
- Sampling effort varies with the level of species diversity
- Taxa may require different sampling efforts across species richness and composition

Towards an effective in-situ biodiversity assessment in European forests

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Abstract

Assessing multi-taxon biodiversity is crucial to understand forests' response to environmental changes and to inform management strategies. In Europe, forest biodiversity monitoring is still scattered and heterogeneous, although a long-term monitoring network has long been advocated. Given the monitoring aims reported in various EU policies, this network should be accurately designed also through the estimation of its sampling effort, here limited to the number of sampling plots and sites.

We used a novel database of forest multi-taxon biodiversity for a pilot study to: estimate the minimum sampling effort needed to assess variation in species richness and composition; compare these estimates with the efforts invested in the pilot database; discuss estimates' differences across taxonomic groups and forest categories.

We focused on six taxonomic groups (vascular plants, birds, epiphytic lichens and bryophytes, wood-inhabiting fungi and saproxylic beetles) across six forest categories. Based on 6,165 plots at 2,084 different locations across Europe, we benchmarked the effort to achieve: a complete species richness estimate through interpolation/extrapolation curves, and a precise evaluation of species composition variation through multivariate standard error.

Our estimates differed widely, especially among taxonomic groups. For species richness, estimates range from 3 to 147 plots per site across 3 to 29 sites per forest category, with birds and epiphytic bryophytes requiring the least effort. For species composition, estimates range from 5 to over 25 plots per site across 5 to 20 sites per forest category, with saproxylic beetles, vascular plants, and fungi displaying the highest estimates.

The taxonomic groups requiring an effort comparable to existing data were the least diverse, all the others need greater efforts, either for species richness (e.g., saproxylic beetles), or species composition (e.g., vascular plants), or both (e.g., wood-inhabiting fungi). An effective monitoring network of European forests' biodiversity should thoroughly account for these benchmarks and for their taxon-dependency.

Keywords: birds, epiphytic lichens, epiphytic bryophytes, forest biodiversity, monitoring network, multivariate standard error, rarefaction curves, saproxylic beetles, vascular plants, wood-inhabiting fungi.

Introduction

Forests host about three-quarters of the global terrestrial plant, fungi and animal species (FAO 2020), and have a great economical value, as well as a prominent role in climate regulation and human well-being (IPBES 2019).

Implementing forest monitoring at different spatial scales is crucial to comprehend forest ecosystems' response to environmental changes (Senf et al. 2020), and to define pathways towards preserving biodiversity while profiting from forest resources in the framework of sustainable forest management (Aubin et al. 2013; Flensted et al. 2016). In this view, the

European Union (EU) recently proposed a Forest Monitoring Law as a framework for the collection and reporting of forest data that integrates Earth observation and in-situ monitoring (EC 2023). The EU Forest Strategy for 2030 (EC 2021) defines the information on forest ecosystems as patchy, and advocates for an integrated forest monitoring framework with a special focus on biodiversity and forest management. In this view, the European Environmental Agency implemented the Forest Information System for Europe (FISE) (https://forest.eea.europa.eu/) to stimulate the integration of forest data, but still, biodiversity inventories are insufficient, even for forest habitats within Natura2000 sites (Alberdi et al. 2019).

Based on the policies mentioned, long-term monitoring (sensu Lindermayer and Likens 2010) should be initiated in the EU. According to policy requirements, such monitoring could be categorized as mandated monitoring and should be focused on the identification of trends in forest biodiversity (Lindermayer & Likens 2010). Although multi-taxon biodiversity assessments are recommended as a direct approach to comprehensively study and monitor forest biodiversity (Flensted et al. 2016; Schall et al. 2018), they are often replaced by indirect proxies, ranging from the extent of forest or of protected forests at the broadest spatial scales, to the sampling of tree-related microhabitats at the finest scales. These indirect approaches are justified by the cost of the multiple hard skills needed for direct biodiversity data collection and management (Larrieu et al. 2019). For this reason, national forest inventories mostly do not include direct biodiversity sampling, but are rather focused on the measurement of structural variables, whose relation to biological diversity is still not broadly demonstrated (Gao et al. 2015; Penone et al. 2019; Zeller et al. 2022). Similarly, international forest monitoring networks (e.g., ICP - https://www.icp-forests.org) focus on forest health, i.e., crown condition, tree growth, and do not include comprehensive biodiversity assessments.

Species-based biodiversity surrogates (*sensu* Lindenmayer & Likens 2011) give direct insights on biodiversity but cover only partially the wide range of forest organisms. These surrogates refer to individual species or groups of species that are either characteristic of certain habitat features, of conservation value, charismatic, or well known, e.g., indicator, umbrella, focal, and keystone species. Such partial assessments contribute only limitedly to defining the relationships between forest biodiversity and environmental conditions, including management regimes (Oettel & Lapin 2021).

Multi-taxon surveys, even though challenging, allow for a comprehensive representation of the forest conservation status by giving insights on a wide range of ecological interactions at different scales (Burrascano et al. 2018; Pereira et al. 2013). Such surveys are still the exception rather than the rule in forest inventories, but they have been increasingly applied from local to national scales, especially to address research questions on the effect of management on forest biodiversity (Elek et al. 2018; Flensted et al. 2016). Such question-driven monitoring efforts (Lindermayer & Likens 2010) have been providing support to local management issues, but cannot provide answers to questions deriving from the ongoing environmental crises at the continental scale (Pereira et al. 2013; Stadt et al. 2006), for which a purposedly designed long-term mandated monitoring program is needed to provide guidance to the EU forest and environmental legislations (Lindermayer & Likens 2010).

A comprehensive multi-taxon forest monitoring network at the European scale would allow detecting substantial changes in species richness and composition that may derive from changes in environmental conditions, e.g., climate change, nitrogen depositions, and/or in disturbance regimes, e.g., pathogens outbreaks, changes in harvesting intensity. The implementation of such a network is challenging primarily because each taxonomic group reacts to environmental conditions at different spatial scales, and likely requires different sampling methods to allow for broad-scale assessments (Brunbjerg et al. 2019). For these reasons, taxon-specific sampling strategies should be adopted to optimize resources in the context of a broad-scale forest biodiversity monitoring. Secondly, lack of coordination in the definition of sampling designs and protocols still hamper such initiatives (Burrascano et al. 2021; Feld et al. 2009). Estimating beforehand the effort needed, here intended as the number of sites and plots, for an efficient inventory would play a key role to implement multi-taxon monitoring and demonstrate the effect of external variables (e.g., climate, forest management) on forest biodiversity (Hoffmann et al. 2019; Montes et al. 2021). An insufficient sampling effort could lead to a biased estimation of forest biodiversity due to low statistical power (Alessi et al. 2023; Underwood & Chapman 2003). Conversely, an oversized inventory would waste time and resources, which are often limited for monitoring activities (Nuñez-Penichet et al. 2022). Thus, finding the right balance between obtaining a representative and cost-effective sample size is crucial in monitoring projects (Bruel & White 2021; Gardner 2010).

The increasing availability of biodiversity data from heterogeneous sources across large extents allows for ecological predictions at large scales; however, the upscaling issues of such heterogeneous datasets are often neglected or only partly handled in modeling approaches (Ovaskainen & Abrego 2020). For this reason, these datasets should not lead to abandoning the idea of targeted, soundly designed large-scale monitoring programmes, but rather underpin their development by using the acquired experience and data for designing new and more effective schemes. In this view, investigating the effectiveness of local sampling intensities at broader spatial scales would represent a relevant asset for the development of a coordinated network for multi-taxon biodiversity assessment across European forests.

We used multi-taxon biodiversity data spread across 12 European countries (Burrascano et al. 2023) to link sampling effort to sampling completeness and precision for respectively the species richness and composition of six taxonomic groups. Our aims are to: i) estimate the minimum sampling effort needed to capture variation in species richness and composition; ii) compare these estimates with the sampling effort of the pilot data; iii) discuss the estimates' differences across forest categories and taxonomic groups. Our ultimate goal is to inform the realization of a cooperative European forest biodiversity monitoring network in support of forest conservation and sustainable management policies.

Materials and methods

Biodiversity data

The data used in this study derives from the merging of 30 datasets (see Appendix A: Table 1) across 12 European countries (Burrascano et al. 2023). Each dataset includes forest multi-taxon biodiversity data for multiple plots, i.e., a concretely delimited sampling unit of known geographical coordinates, within one to 23 sites, i.e., an environmentally homogeneous forest area (Fig. 2). Here, we limited our analyses to the most commonly sampled taxonomic groups (vascular plants, birds, epiphytic lichens and bryophytes, wood-inhabiting fungi and saproxylic beetles) rising to a total of 112,323 observations of 3,229 species in 6,165 plots (Fig. 3). By selecting this subset, we obtained a dataset in which not all but most plots have information on two or more taxonomic groups, resulting in a total of 2,084 spatially distinct sampling locations across 87 sites (Fig. 1), with each site having an average size of 106 km^2 containing at least six plots.

Although extensive, the database is centered on central Europe, for this reason we limited our analyses to temperate forest categories: (3) 'Alpine': alpine coniferous forests; (4) 'Acidophilous oak': acidophilous oak and oak-birch forests; (5) 'Mesophytic deciduous': mesophytic deciduous forests; (6) 'Lowland beech': beech forests; (7) 'Mountainous beech': mountainous beech forests., and including also (2) 'Hemiboreal': hemiboreal forest and nemoral coniferous and mixed broadleaved-coniferous forest, although the latter are underrepresented as compared to the other categories. Overall, these six forest categories are estimated to cover about 42% of European forest area (Barbati et al. 2014). These catogories refer to the classification into 14 categories of ecologically distinct forest communities in Europe dominated by specific assemblages of tree species (EEA 2007), which were designed to facilitate the interpretation and communication of indicators on the status and trends of forests in Europe (Barbati et al. 2014).

Sampling protocols vary across datasets but use similar sampling approaches locally adjusted to capture the species diversity of each site (see Burrascano et al. 2021 and Appendix A: Table 2): vascular plants and wood-inhabiting fungi were sampled in plots or blocks of plots rising to an overall sampling area mostly ranging from 78 to 1000 m^2; epiphytic lichens (called lichens henceforth) and epiphytic bryophytes (called bryophytes henceforth) were sampled respectively on two to 12, and five to 18 standing trees per plot, with complete censuses performed in very few studies; birds were sampled by point counts during time frames mostly ranging from 5 to 20 minutes, see e.g., Bouvet et al. (2016);

saproxylic beetles were sampled with window-flight interception traps (1 to 6 in each plot), in some cases coupled with emergence traps and Winkler extractors (see e.g., Janssen et al. 2016).

Species nomenclature was checked using the *gnr_resolve*() function in the 'taxize' package (Chamberlain & Szocs 2013) in R version 4.1.1 (R Core Team 2021), with species names with scores lower than 0.9 corroborated by experts or checked against the GBIF.org (2023) database.

The selection of the most commonly sampled taxonomic groups and forest categories ensured the broadest possible spatial overlap across taxonomic groups so that potential differences in sampling effort estimates among them would not depend on substantial differences in their sampling distribution.

Data visualization and statistical analysis

Data was analyzed at two different spatial scales: plot-grain/site-extent, henceforth plot scale, and site-grain/continental extent, henceforth site scale. Note that the heterogeneity in sampling methods affected neither the plot scale, since the same sampling methods were used within each site; nor the site scale for which plot data was pooled at the site level, thus blurring plot-scale differences potentially induced by varying sampling efforts. However, in order to exclude a substantial effect of the variables related to the sampling methods we quantified the effort invested in terms of sampling methods (e.g., area sampled, number of sampling elements) and showed that this is not related to the estimated species richness at the site level (see Appendix A: Fig. 1).

Species diversity patterns across taxonomic groups and forest categories

Since we based our estimates of sampling effort on accurately capturing species diversity, which is deemed more informative than the mere extent of the area sampled (Chao & Jost 2012), we visualized alpha, beta and gamma diversity of the pilot database at plot and site scale in two separate heatmaps using the forest category on the x-axis and coloring the tiles based on the taxonomic group. At the plot scale, alpha was calculated as the average species richness per plot in each site, gamma as the overall richness per site, and beta as a ratio between gamma and alpha in each site following a multiplicative approach (Whittaker 1972). For the site scale we calculated the average site alpha richness, the overall gamma richness per category, and the among-sites beta-diversity in each category through a multiplicative approach. Diversity values are divided into five separate classes (low; low-mid; mid; mid-high; high) based on the quantiles defined by the distribution of overall plot and site values.

The pilot database shows different patterns of species diversity, especially across taxonomic groups, with slight differences across spatial scales (site or plot), and differences between forest categories emerging at the site scale (Fig. 4). At the plot scale, the only taxonomic groups reaching the mid-high and high diversity ranks are fungi, saproxylic beetles, vascular plants, and lichens, with the latter never reaching the high diversity rank. Bryophyte diversity values were almost exclusively within the low rank, with few values in the mid class especially for beta and gamma diversity. Values for birds mostly occurred at the low-mid and mid levels.

At the site scale, these patterns are more dramatic. Bryophytes are totally included in the low diversity class, and the mid-high and high classes exclusively occupied by fungi, saproxylic beetles and vascular plants. At this scale, differences across forest categories emerge. Vascular plants reached only mid values for hemiboreal, acidophilous oak and lowland beech forests, and high values in the remaining forest categories; while saproxylic beetles have a completely opposite pattern. Fungi had mid-values for the alpine coniferous and acidophilous oak forests, mid-high values for the remaining categories, with high values only for the hemiboreal category.

Estimates for species richness

Rarefaction is traditionally used to compare species richness values by down-sampling larger samples to the sampling effort of the smallest sample. Similarly, extrapolation estimates the species richness associated with a larger sample (Chao & Jost 2012). In this framework, sampling completeness for species richness is the ratio of the observed species richness to the true richness, i.e., observed plus undetected (Chao et al. 2020). We used rarefaction or extrapolation of our pilot dataset (R package iNEXT, Hsieh et al. 2016) to calculate the number of sampling units needed for each combination of taxonomic group and forest category to reach a desired sample completeness, which we set to 90% as in several studies (Grey et al. 2018; Monleon-Getino & Frias-Lopez 2020; Nikkeshi et al. 2021). The rarefaction/extrapolation approach is widely used to assess the magnitude of the differences in richness among communities across space and time (Gordó-Vilaseca et al. 2023) and comparing samples showing the same degree of sampling completeness was deemed more efficient than traditional approaches applied to equal sample sizes (Hsieh et al. 2016). We tested the differences between the median number of plots per site calculated on the iNext estimates and observed in the pilot database through the Mann-

Whitney test. Through this approach we estimated the sampling effort needed to detect 90% of the species richness or more.

Estimates for species composition

Sampling designs often rely on the precision of the arithmetic mean of a response variable. This may be estimated as the standard error of the mean of a previous pilot study from which the number of replicates that are needed to improve that precision can be derived. Similarly, a multivariate pseudo standard error (MultSE) can be used as a proxy for the precision in the assessment of the variation in species composition (Anderson & Santana-Garcon 2015). This approach has been improved through simulations that allow to extrapolate the MultSE beyond the sampling effort of the pilot study (Guerra-Castro et al. 2021).

We ran the simulations and MultSE calculations (R package SSP, Guerra-Castro et al. 2021) at both plot and site scale. For each forest compositional category, 10 data matrices were simulated based on the pilot dataset using the “sim.data” function, each matrix containing 25 virtual sites and 75 virtual plots per site.

A two-stage random sampling was performed using sites from 2 to 25 and plots from 2 to 25 with each combination repeated 10 times followed by the MultSE computation for each repeated combination. This approach is usually aimed at defining optimal and suboptimal sampling efforts by analysing the pattern of MultSE as the sampling effort increases. Here we focused on the minimum necessary sampling effort to assess variation in species composition in European forests since currently no pilot database may be assumed to encompass the total biodiversity of each forest category to assess an optimal sampling effort. In this perspective, we retrieved the sampling effort corresponding to a 0.1 multivariate standard error. Since the values of MultSE may vary across the dissimilarity measures used to compare sampling units, we used the Jaccard index for all taxonomic groups and forest categories. Similarly, MultSE may vary depending on the number of variables, i.e., species (Anderson & Santana-Garcon 2015), therefore, it should be discussed in relation to patterns of species diversity (Fig. 4). Through this approach we estimated the sampling effort needed to detect a change in species composition exceeding 0.1 multivariate standard error.

Results

Estimates of the minimum sampling effort

The minimum number of plots needed to assess species richness and variation in species composition for forest biodiversity varied widely (Table 1) primarily across taxonomic groups and secondarily across forest categories, with somewhat different results for species richness and species composition. For species richness, the taxonomic group requiring the highest number of plots was wood-inhabiting fungi, whose estimates for a 90% sampling completeness were highly variable across sites, ranging from 7 to 147 plots (see Appendix A: Fig. 2), with very high values occurring in sites across different forest categories and regions. In contrast, birds required the lowest number of plots for the recovery of 90% of species richness, from 2 to 17 depending on the site and forest category. Overall, all taxonomic groups gave similar results for species richness and composition, with a comparable number of plots and sites resulting from the two analyses, with some exceptions. The estimates of vascular plant species composition were much higher than for species richness (Table 1).

Also for assessing species composition, fungi were the taxonomic group that required the largest sampling effort in terms of plots per site (over 25 which was the highest threshold for our modeling approach). Overall, the number of plots per site to achieve a precise (multivariate standard error lower than 0.1) assessment of variation in species composition (Table 1) was always higher than 12, with the lowest values for birds in Alpine coniferous forests (12) and in mountainous beech forests (13), and for lichens in alpine coniferous forests (14) (Appendix A: Fig. 3).

The estimates for the site scale were more homogeneous than those at the plot scale both across forest categories and taxonomic groups, but with differences between species richness and species composition values (Table 1). Interestingly, for species composition, besides birds and bryophytes, also fungi resulted in low estimates for the number of sites. Indeed, within the estimated number of sites to be sampled for fungi for each forest category ranged from 5 to 9, with values substantially lower than those for vascular plants, ranging from 8 to 19 (Table 1).

Comparison with current knowledge

Depending on the taxonomic group and forest category, the estimates for the number of plots per site to achieve a 90% sampling completeness for species richness were either higher or lower than the values in our pilot database (see Appendix A: Fig. 2). For fungi the estimates were always higher than in the pilot database, while birds were sampled over the estimated minimum effort in terms of number of plots per site in the pilot dataset (Fig. 5). Vascular plants, bryophytes and lichens were sampled beyond the estimated minimum effort, although these differences have a lower degree of significance for most forest categories, except for vascular plants in mountainous beech forests.

When pooling biodiversity information at the site scale, we found that the number of sites sampled in the pilot database was mostly not sufficient to reach a fair (90%) sample completeness for species richness within each forest category. Thus, the estimate for the minimum number of sites was mostly higher than the number of currently sampled sites (upper left part of the diagram in Fig. 6). The highest gaps were found for vascular plants, saproxylic beetles, wood inhabiting fungi, and only in beech dominated forest categories for lichens. These results differ substantially from those obtained at the plot scale. Conversely, we obtained lower estimates of the number of sites for birds as compared to the pilot database across all forest categories.

We found different results for species composition (Fig. 7). The number of plots per site are generally high, always higher than 20 for fungi, vascular plants and beetles. At the site scale, except for saproxylic beetles that resulted as undersampled in all forest categories, only few combinations of forest categories and taxonomic groups required a substantially higher effort than the pilot database: vascular plants in hemiboreal and acidophilous oak forests, lichens in hemiboreal forests and bryophytes in mesophytic deciduous forests.

Discussion

Setting the minimum threshold for forest biodiversity assessment

Using a large pilot database with information deriving from multi-taxon sampling across Europe is a novel and unique opportunity to estimate the sampling effort needed to cover European forest biodiversity. Setting the minimum effort based on the existing knowledge means putting the basis for a coordinated European forest biodiversity assessment and long-term monitoring network, as advocated in the proposal for a EU Forest Monitoring Law (EC 2023).

Once this minimum sampling effort is applied through data collection campaigns, it could be progressively refined through periodic assessments of sampling completeness and multivariate precision until an optimal sampling effort is achieved that could support evidence-based forest and conservation policies in the EU (Oettel & Lapin 2021).

Initiating this process is highly relevant in the current political and environmental context. Biodiversity is listed among the priorities for sustainable forest management in the EU Taxonomy Regulation (2020/852), and for the enhancement of forest resilience to environmental changes and disturbance events in the European Forest Strategy for 2030 (EC 2021); finally, biodiversity is in the focus of the proposal for a Forest Monitoring Law (EC 2023). Nevertheless, none of these political documents, not even the latter, refers to an operational framework for a sound in-situ assessment of forest biodiversity, which is at the very base of developing forest policies and management strategies that comply with the definition of Sustainable Forest Management, and of detecting changes in forest biodiversity that derive from the increasingly impactful stresses (Senf et al. 2020) and disturbance events (Patacca et al. 2023) related to ongoing climate change.

Are we there yet?

As we may have expected, the short answer to this question is “no”.

This primarily derives from the relevant knowledge gaps for the biodiversity of several European forest categories (Burrascano et al. 2023). Based on data availability, we were forced to limit our analyses to only six out of the 14 forest categories identified by the EEA (2007). This is particularly unfortunate since, among the forest categories that we could not include in our analyses, are those centered in the Mediterranean biogeographical region that likely display the highest forest biodiversity values for the European continent, e.g., thermophilous deciduous and broadleaved evergreen forests (EEA 2007). The high tree species richness of this region (Médail et al. 2019; Rivers et al. 2019) drives the occurrence of both several different forest types at the regional scale and species-rich overstoreys at the local scale, with cascading positive effects on the diversity of forest-dwelling organisms (Groote et al. 2017; O'Brien et al. 2017; Vockenhuber et al. 2011). Southern Europe hosts the highest number of tree species threatened with extinction, mainly in direct or indirect relation to climate change (Rivers et al. 2019), which will impact especially this region through progressive subtropicalisation and desertification (Pörtner et al. 2022).

Also, for the relatively well-studied categories, much work still have to be done. Across the forest categories we analysed, we estimated a total need of 109 sites for reaching 90% sampling completeness in species richness, and of 71 sites to achieve a 0.1 multivariate standard error in species composition. The estimated number of plots per site goes beyond 20 to assess species composition variation in several taxonomic group/forest category combinations but is often lower than for species richness. As compared to the largest ongoing forest monitoring program, i.e., ICP forests comprising 6000 first-level plots across Europe, even if it does not comprehensively account for forest biodiversity, the overall number of sampling units needed for forest multi-taxon monitoring would be in the same order of magnitude, but different in its spatial structure. Although this effort may seem insufficient to detect changes at the local or regional scales, at the continental scale it would contribute to detect broad-scale changes and to provide a data-driven dynamic benchmarks for species richness and composition to be used also by national or local monitoring programs operating at finer scales.

Our results point to the relevance of an accurate sampling stratification across forest categories, since we found different estimates for both plots and sites across forest categories, e.g., estimates for hemiboreal forests are often much lower than those for other categories. This stratification may be useful both for representative monitoring, as ICP Forest level I (Ferretti et al. 2020), and for a purposive sampling, i.e., following different criteria among which environmental conditions and dominant species may often occur. The lack of a reliable European map of forest categories hampers the inclusion of this key information in the design of future biodiversity monitoring schemes. Unlike National Forest Inventories, which have been used since their origin mainly to estimate and manage timber resources, the development of a biodiversity monitoring network would strongly benefit from spatially-explicit information on forest categories based on dominant tree species, since the latter substantially incorporate a complex set of environmental drivers of biodiversity patterns. However, even if a probabilistic sampling would be deemed as a more robust strategy since it does not depend on a priori stratification, being able to link the outcomes of biodiversity monitoring to different forest categories would allow for the definition of tailored, thus more effective, strategies (Barbati et al. 2014). We found very high estimates for the number of plots within a site, e.g., for fungi, suggesting that a nested sampling design (i.e., several plots per site) is advisable when aiming at the analysis of forest biodiversity, whose patterns are also expressed at fine spatial scales (Burrascano et al. 2018).

Currently, the most relevant forest biodiversity coordinated monitoring in the European Union is the one put in place by Member States for the Habitats Directive obligations (Article 17). Although aimed at providing similar information, the monitoring programs to comply with Article 17 follow very different approaches among the Member States, e.g., some Member States established a special standardized monitoring program, while others use already existing data, e.g., habitat maps, forest inventories. Where *in-situ* monitoring is applied, data is collected through different protocols, which in many cases include lists of typical species, but seldom record complete plant species lists. Sampling of animal species is even rarer, and usually limited to few habitat types and taxonomic groups, i.e., birds and butterflies (Ellwanger et al. 2018).

Overall, the existing coordinated efforts for biodiversity assessment are strongly limited and need substantial investments at the European scale for improvement. A mandated monitoring of forest biodiversity could use these existing networks to have an estimate of the baseline forest heterogeneity but would need to develop independently based on the current distribution of forest categories, and on the changes and trends that are foreseen in European forest ecosystems.

On which basis should we calibrate our sampling efforts?

A wide body of scientific literature studied the extent and pathways through which sampling effort influences biodiversity assessments (Gotelli & Colwell 2001; Grey et al. 2018; Nuñez-Penichet et al. 2022). Within a certain habitat, an increasing sampling effort results in an initial increase in the observed species richness and in species composition precision, which then levels off following, respectively, the general species-area relationship (Turner & Tjørve

2005) and the decrease of the multivariate standard error (Guerra-Castro et al. 2021). As the share of species that are rare or hard to detect varies greatly across taxonomic groups and environmental contexts, so does the relationship between sampling effort and observed diversity (Anderson & Santana-Garcon 2015; Chao & Jost 2012; Coddington et al. 2009).

The other side of this evidence is that the more a taxonomic group in a certain context is rich with rare and hard to detect species, the higher sampling effort is needed to achieve a comprehensive assessment. This influences not only alpha-diversity, but also beta-diversity since rare and hard to detect species are more likely to vary across plots and sites either because they are linked to specific habitat features or because they were undetected in some sampling units (Pärtel et al. 2011). The variation of environmental conditions at different spatial scales, adds to the drivers of variation in species composition across plots and sites, and in turn to the patterns of beta-diversity (Graco-Roza et al. 2022).

Accordingly, our estimates reflect the diversity patterns of the different taxonomic groups in the first place, and, secondarily, of the different forest categories. It is important to underline that EU member states defined the sampling effort for habitat monitoring in the framework of the Habitats Directive mostly based on the habitat area or occurrences, but very rarely, the monitoring effort accounted for the habitat variability (Ellwanger et al. 2018). Based on our data, birds and bryophytes showed very low diversity levels across all forest categories and spatial scales. This is not surprising since the total number of species censused for these groups in Europe are relatively low, 912 for birds (Clements et al. 2023) and 1392 for bryophytes (Hodgetts et al. 2020). In European forests, these taxonomic groups would therefore achieve a relatively complete assessment of species richness and composition by applying a feasible sampling effort, which is similar to the one applied in the pilot database. Notwithstanding their similar diversity patterns, the amount of available data and conservation efforts for these two taxonomic groups is extremely different. Birds are among the most studied organisms globally, with species checklists updated annually (Clements et al. 2023), also through vast programs of citizen science (Sullivan et al. 2009; Jiguet et al. 2012) and have been protected in Europe since 1979 through an exclusive Directive (79/409/EEC) that was recently amended (2009/147/EC). Bryophytes are far less studied, especially in some European regions (Sabovljević et al. 2001) and often marginal in conservation efforts (Vellak et al. 2010), not to mention their lower appeal for citizen science activities.

Given the relatively low effort and the large amount of available data, it is not surprising that birds, limited to 34 common bird species, were the only organisms (other than trees) included among the indicators for the maintenance, conservation and enhancement of biodiversity by Forest Europe (2020). However, we should question the informative value of this indicator, which is by definition focused on one of the least species-rich taxonomic groups in European forests, and particularly on very common species whose abundance cannot say much about the temporal and spatial patterns of forest biodiversity, also in relation to their low congruence with the diversity of other taxonomic groups (Burrascano et al. 2018).

Alternative options exist but require greater effort. We found very high diversity levels for wood-inhabiting fungi, with high levels of diversity at the plot scale. This means that very high numbers of fungi species may be found in one sampling unit, and that their species composition varies greatly among the plots within the same site, i.e., they may have very high levels of alpha and beta-diversity at the plot scale. This general pattern may be related to the amount and diversity of senescing and dead wood within a forest site and makes wood-inhabiting fungi particularly interesting as fine-scale indicators of sustainable forest management. In general, fungi pose several challenges for sampling, since their surveys usually rely on reproductive structures that for most species are ephemeral and somewhat unpredictable (Lodge et al. 2004). This issue, however, is larger for soil fungi producing agaricoid reproductive structures than for saproxylic species (Runnel et al. 2015) often producing perennial reproductive structures, as in polypores (Halme & Kotiaho 2012). These challenges are being progressively addressed by environmental DNA techniques (Burrascano et al. 2021; Leclerc et al. 2023), and are counterbalanced by the possibility of identifying complex spatial and temporal patterns of biodiversity as nuanced by the presence/absence of a wealth of species associated with different habitat features and ecological functions, e.g., deadwood decay.

At the site scale, fungi may be sampled through a relatively low effort as compared to other taxonomic groups, i.e., saproxylic beetles and vascular plants, both of which will have a particular relevance for forest monitoring.

The exact number of saproxylic beetle species in Europe is still unknown but has been estimated to about 4,000 (Bouget et al. 2008). Of these species, about 650 underwent a red list assessment: 20% of these resulted as threatened with extinction, and about a quarter as data-deficient (Calix et al. 2018). Monitoring saproxylic beetles would provide crucial information on the distribution and abundance of a group that includes several species of conservation concern but for which a profound knowledge is missing. Furthermore, timber harvesting with deadwood release limited in quantity and diversity is by far the primary threat to the species within this group that could therefore give relevant and nuanced indications on the sustainability of forest management for biodiversity, as required by the aforementioned European policies and regulations.

Finally, vascular plants are also a well-studied group, whose species lists were used to gather information on the ecological patterns and community features since the early 1900s. Our estimates for this group are intermediate as compared to other groups but reflect high levels of diversity across forest categories and spatial scales. Plants are the major structural and functional component of forest ecosystems and therefore influence forest structure and are the source of a diverse producer-based food chain. They are renown as good surrogates, especially of spatial composition patterns in temperate forests (Blasi et al. 2010; Sætersdal et al. 2004), and are used by several conservation initiatives, including the Habitats Directive within the European Union and Natura2000 implementation, as a base for habitat interpretation and conservation status assessment (Burrascano et al. 2018).

Opportunities and limitations

We used the best available data to demonstrate that the efforts we are investing in monitoring biodiversity is not focused on the most diverse taxonomic groups and forest types. The current focus on birds (e.g., by Forest Europe) and on vascular plants (e.g., by Habitat Directive) should be coordinated and further implemented and should be complemented with assessment for saproxylic fungi and beetles to test the sustainability of forest management in Europe. Other functional and taxonomic groups, especially of arthropods, may also be suitable but were not analysed here due to scarce and scattered available information. Further sampling effort should be directed towards Mediterranean and thermophilous forests, which are currently underrepresented in multi-taxon forest assessments and thus not included in our study.

Our approach did not allow us to account for the sampling design, which may influence sampling effort estimates (Montes et al. 2021), thus further studies using spatially explicit models are needed in this regard. However, our results point to a nested sampling scheme to investigate the within and across sites variation of species diversity for some taxonomic groups that may display very different diversity levels across spatial scales, i.e., wood-inhabiting fungi. In this regard, it is important to underscore that, while the pilot database may generally be considered exhaustive at the individual site extent, it is surely defective at the continental extent, since no forest category may be deemed as fully represented. Notwithstanding this limitation, we presented results at both scales to point out how some taxonomic groups may need a limited within-site effort while being more demanding in terms of number of sites or viceversa, in relation to their specific patterns of diversity.

It is also important to note that our data derive from sampling performed through different protocols and that further estimates based on standardized sampling strategies (Burrascano et al. 2021) could give sounder insights into the implementation of a European forest biodiversity monitoring network. It is also noteworthy that we did not account for the criteria through which the sites and plots are selected and distributed across space and environmental gradients. In the datasets included in the pilot database, these criteria derived from a trade-off between the project aims and local constraints (e.g., ownership, stakeholder availability). When setting a European monitoring network, the target spatial and environmental gradients should be defined at a broader scale, though the actual network implementation would have to account anyway for local constraints. We assumed the relevance of compositional categories, which are those designed to tune forest indicators (EEA 2007). However, the levels of species diversity may vary substantially across areas under different management regimes, ranging from clearcut to no intervention. Further efforts accounting for this additional classification could pay special attention to those forest areas in which management strategies allow for high levels of forest diversity and that could serve as a reference for forest biodiversity monitoring.

Author contribution

SB and LC designed the study, all authors contributed and/or harmonized the data, LB, LC, SB, FC and EH performed the data visualization and analysis, SB led the manuscript writing, to which all co-authors contributed.

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Data availability statement

The data used for this study are available upon request using the Data Explorer within the Cost Action website (https://www.bottoms-up.eu/en/results/data-explorer.html).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XXXXX.

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Table 1.

Table 1. Estimates of the number of plots per site and the number of sites across Europe for each forest category and taxonomic group to assess species richness with a 90% sampling completeness, and the variation in species composition with a 0.1 multivariate standard error.

		Species richness		Species composition	
	Forest category	n° plots	n° sites	n° plots	n° sites
Beetles	2. Hemiboreal	8-14	7	24	11
	3. Alpine coniferous	8-29	11	23	12
	4. Acidophilous oak	8-14	7	22	5
	5. Mesophytic deciduous	7-16	13	23	14
	6. Lowland beech	6-34	19	>25	11
	7. Mountainous beech	7-32	27	21	19
Birds	2. Hemiboreal	3-12	4	23	5
	3. Alpine coniferous	2-8	3	12	4
	4. Acidophilous oak	3-5	3	15	2
	5. Mesophytic deciduous	4-7	4	19	4
	6. Lowland beech	3-8	5	18	2
	7. Mountainous beech	3-17	6	13	5
Bryophytes	2. Hemiboreal	-	-	-	-
	3. Alpine coniferous	-	-	-	-
	4. Acidophilous oak	3-18	8	19	4
	5. Mesophytic deciduous	3-21	7	25	7
	6. Lowland beech	2-28	13	22	8
	7. Mountainous beech	4-50	9	19	8
Lichens	2. Hemiboreal	3-17	5	16	9
	3. Alpine coniferous	2-11	9	14	11
	4. Acidophilous oak	-	-	-	-
	5. Mesophytic deciduous	-	-	-	-
	6. Lowland beech	5-39	13	21	9
	7. Mountainous beech	5-26	21	24	10
Fungi	2. Hemiboreal	10-16	6	>25	7
	3. Alpine coniferous	10-147	15	>25	8
	4. Acidophilous oak	23-58	11	>25	5
	5. Mesophytic deciduous	14-96	16	>25	9
	6. Lowland beech	7-42	17	>25	8
	7. Mountainous beech	9-56	12	>25	8
Vascular plants	2. Hemiboreal	6-18	11	21	19
	3. Alpine coniferous	4-12	12	22	8
	4. Acidophilous oak	8-20	12	>25	9
	5. Mesophytic deciduous	3-19	25	>25	11
	6. Lowland beech	3-46	21	>25	12
	7. Mountainous beech	6-34	17	>25	9

Figure captions

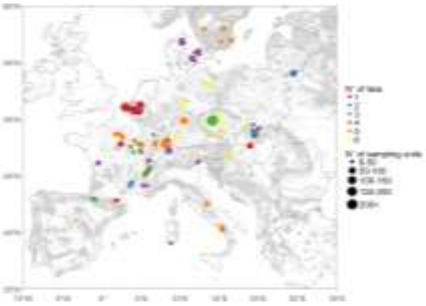

Fig. 1. Distribution of sites in Europe. Dot color and size indicate, respectively, the number of sampled taxonomic groups and of sampling units in each site. Gray areas are covered by forests with a tree cover greater than 40% according to Kempeneers et al. (2011).

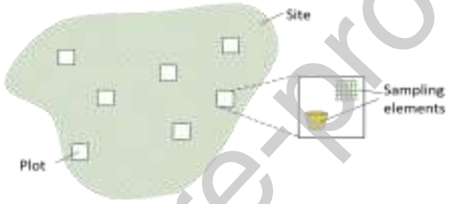

Fig. 2. Scheme representing the spatial structure of the database. Site is intended as an environmentally homogeneous forest area, plot is intended as a concretely delimited sampling unit of known geographical coordinates. Within each plot different sampling elements were used for different taxonomic groups.

Fig. 3. Heatmaps showing the distribution of plots, observations, and species records across taxonomic groups and forest categories according to EEA (2007).

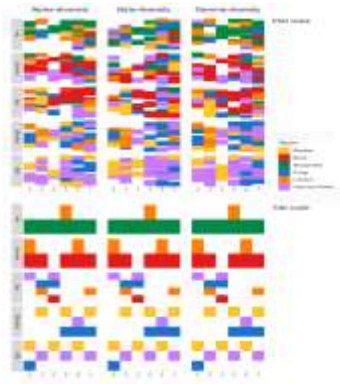

Fig. 4. Distribution of alpha, beta and gamma diversity at the plot and site scale. Diversity values were calculated for each taxonomic group in each plot and site and sorted in ascending order towards the bottom of the graphs. For the plot level, gamma diversity is the site species richness at 90% sample coverage, alfa is the plot richness standardized by gamma and beta is derived through a multiplicative approach. For the site scale, gamma is the overall species richness of a taxon in a forest category, alfa is the average species richness of a site and beta was calculated through a multiplicative approach. Diversity values were classified into five classes (low; low-mid; mid; mid-high; high) based on the quantiles of the overall plot and site values' distribution. The x axis distinguish the six forest categories: 2 = 'Hemiboreal'; 3 = 'Alpine'; 4 = 'Acidophilous oak'; 5 = 'Mesophytic deciduous'; 6= 'Lowland beech'; 7 = 'Mountainous beech'.

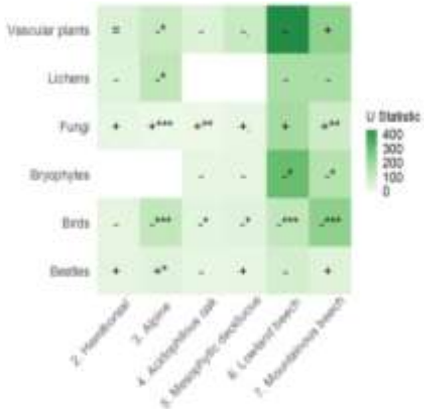

Fig. 5. Differences between the median of the number of plots per site in the pilot database and in the estimate for a sampling completeness of 90% (+: estimate>pilot; -: estimate < pilot; =: estimate equals pilot) and significance of the Mann-Whitney test between the pilot and estimated number of

plots per site distributions (. p<0.1; * p<0.05; ** p< 0.001; *** p< 0.0005). The color intensity represents the U statistic from the Mann-Whitney U test, with darker shades indicating larger U statistics and thus greater differences between the pilot and estimated distributions.

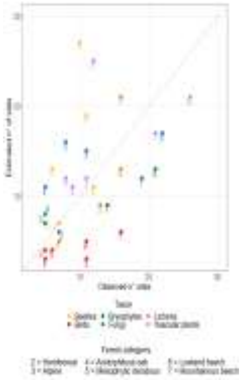

Fig. 6. Scatterplot of the number of sites across forest categories in the pilot database and estimated to achieve a 90% sampling completeness for species richness. The dotted diagonal line separates the taxon/category combinations that would and would not need further sampling effort based on the pilot database.

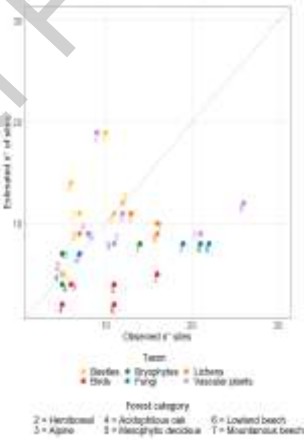

Fig. 7. Scatterplot of the number of sites across forest categories in the pilot database and estimated to achieve a 0.1 value of multivariate standard error when assessing variation in species composition. The dotted diagonal line separates the taxon/category combinations that would and would not need further sampling effort based on the pilot database.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for Basic and Applied Ecology and was not involved in the editorial review or the decision to publish this article.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: