Handbook of field sampling for multi-taxon biodiversity studies in European forests

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ABSTRACT

Forests host most terrestrial biodiversity and their sustainable management is crucial to halt biodiversity loss. Although scientific evidence indicates that sustainable forest management (SFM) should be assessed by monitoring multi-taxon biodiversity, most current SFM criteria and indicators account only for trees or consider indirect biodiversity proxies. Several projects performed multi-taxon sampling to investigate the effects of forest management on biodiversity, but the large variability of their sampling approaches hampers the identification of general trends, and limits broad-scale inference for designing SFM. Here we address the need of common sampling protocols for forest structure and multi-taxon biodiversity to be used at broad spatial scales. We established a network of researchers involved in 41 projects on forest multi-taxon biodiversity across 13 European countries. The network data structure comprised the assessment of at least three taxa, and the measurement of forest stand structure in the same plots or stands. We mapped the sampling approaches to multi-taxon biodiversity, standing trees and deadwood, and used this overview to provide operational answers to two simple, yet crucial, questions: what to sample? How to sample? The most commonly sampled taxonomic groups are vascular plants (83% of datasets), beetles (80%), lichens (66%), birds (66%), fungi (61%), bryophytes (49%). They cover different forest structures and habitats, with a limited focus on soil, litter and forest canopy. Notwithstanding the common goal of assessing forest management effects on biodiversity, sampling approaches differed widely within and among taxonomic groups. Differences derive from sampling units (plots size, use of stand vs. plot scale), and from the focus on different substrates or functional groups of organisms. Sampling methods for standing trees and lying deadwood were relatively homogeneous and focused on volume calculations, but with a great variability in sampling units and diameter thresholds. We developed a handbook of...
1. Introduction

Three-quarters of known terrestrial plant, fungi and animal species need forests as a part of their habitat (FAO, 2020). Sustainable forest management (SFM) is globally recognized as a crucial tool for halting biodiversity loss, and to promote sustainable development (UN, 2015), whose biodiversity maintenance principle (MCPFE, 1993) was particularly stressed in the recent European Union Taxonomy Regulation (2020/852).

In line with this, biodiversity is the focus of one of the six sustainability criteria in the Pan-European region (FOREST EUROPE, 2020). However, existing indicators for this criterion either account only for stand structure and tree species (e.g. species composition, regeneration), or are indirect biodiversity proxies, some of which are not tested or remain vaguely defined (e.g., naturalness, fragmentation, protection status). Only recently, the criterion has included common forest bird species as a direct biodiversity indicator (FOREST EUROPE, 2020), but those taxonomic groups that are strictly related to forest ecosystems and that contribute most to their biodiversity are still neglected (e.g., deadwood dependent groups or soil organisms). This crucial gap stems from the lack of broad scale forest biodiversity studies (Gao et al., 2015), and is only partially addressed by literature reviews (Oettel and Lapin, 2021) and meta-analyses (Westgate et al., 2017).

Forest stand structure has been traditionally measured to inform silviculture but is now commonly used as a proxy for other forest functions, including biodiversity conservation (Franklin et al., 2002; Heym et al., 2021). However, forest inventories can be used as reliable indicators of biodiversity only if they measure specific structural attributes with evident causal importance for specific groups of organisms (Barton et al., 2020). Some useful approaches based on deadwood amount, type and decay class (e.g., Lassauce et al., 2011) or, recently, on tree related microhabitats (Larrieu et al., 2018) have been suggested. However, these structural variables only partially inform about the diversity and composition of different taxonomic groups since their responses to environmental conditions are variable and complex (Larrieu et al., 2019; Paillet et al., 2018). Also analyses on cross-taxon congruence point to the need to directly sample multiple taxonomic groups to soundly assess the status of forest biodiversity and guide sustainable management (Burrascano et al., 2018).

International observation networks, either specifically focused on forest ecosystems functioning (i.e., ICP Forests, FunDivEurope) or on the long-term change of a wide range of aquatic and terrestrial ecosystems (i.e., LTER), collect biodiversity data. However, given the geographical and the conceptual scope of these networks, their biodiversity data are mostly unevenly distributed across space (e.g., different LTER sites focus on different samplings, Frenzel et al., 2012), time (e.g., ICP Forests sampled vascular plants and lichens only in some years, Ferretti & Fischer, 2013), and organisms (e.g., FunDivEurope collects information on trees only, Baeten et al., 2013).

On the other hand, several research programs are primarily focused on forest multi-taxon biodiversity and on its response to forest management (e.g., Elek et al., 2018; Lelli et al., 2019; Paillet et al., 2018; Remm et al., 2013; Sitziu et al., 2017). These studies range from local to regional and national spatial scales and are mostly based on the sampling of multiple plots or stands across single or multiple sites. Although limited in scale, these projects invested considerable resources in collecting data for a number of biodiversity, structural, environmental and management characteristics, as well as in developing protocols for sampling these data. Overall, the protocols used in these multi-disciplinary projects have a focus on cost-effectiveness but are highly heterogeneous. Whereas this variability partly stems from sound scientific reasons (i.e., differences in research questions or forest types, EEA, 2006), in most cases it merely derives from different traditions and local experiences.

The heterogeneity in sampling approaches limits studies comparability and hampers broad multi-taxon analyses on forest biodiversity responses to management. The first comparability issue derives from a heterogeneous sampling coverage at the plot and stand scales, with substantial effects on alpha (Chao & Jost, 2012) and beta (Engel et al., 2020) diversity estimates. The second problem is the heterogeneous use of spatial scale: since the multi-taxon studies address organisms that use forest resources across different ranges, various trade-offs have been used between sampling grain and extent (Burrascano et al., 2018). The reviews and meta-analyses that combined the results of published multi-taxon studies (Westgate et al., 2014; Wolters et al., 2006) or multiple single-taxon studies (Chaudhary et al., 2016; Paillet et al., 2010) have acknowledged these problems, and have recognized that they hamper the understanding of forest biodiversity mechanistic response to management at multiple spatial scales.

Ecological data incomparability is increasingly being solved by establishing common data platforms (Brueelheide et al., 2019; Kattge et al., 2011), through guidelines on data management (e.g., the INSPIRE infrastructure in Europe) and open science practices (e.g., Cooper and Hsing, 2017; Nosek et al., 2015). However, in the field of forest biodiversity, building a common database represent a partial solution (Burrascano et al., 2018; Sabatini et al., 2018), since data collected through unstandardised protocols will always need a long and complex (and not always feasible) process of harmonization that inevitably results in information loss and blurry estimate of effect sizes. In the long-term, these issues should be addressed by using sampling protocols that ensure the comparability across studies, with a key stimulating role played by handbooks. Previous experiences represent excellent examples, and demonstrate the long-term effectiveness of handbooks in ecology (Cornelissen et al., 2003; Moretti et al., 2017; Pérez-Harguindeguy et al., 2013; Säck et al., 2010).

We first present a synthesis of a wide range of field protocols used up to now in Europe for forest multi-taxon biodiversity studies including stand structure measurement and discuss their similarities and differences. Then, based on this overview, we propose a handbook of field sampling protocols (see SI 3) for the study of forest multi-taxon biodiversity in relation to management. The wide application of these protocols will allow for broad scale comparative studies. We address two key questions that researchers may face while designing these studies: what to sample? and how to sample?

The first question is addressed by analysing the most commonly sampled taxa and structural variables in forest multi-taxon studies, as well as by motivating the choice of specific taxonomic groups. The second question is answered by reviewing the most common approaches used in previous multi-taxon studies at the plot scale. This review was the base for developing two standards for sampling protocols provided in the form of a handbook (SI 3).

This multi-disciplinary operational handbook promotes standardised sampling for the assessment of forest biodiversity responses to management at large spatial scales. It would enable a wider applicability of forest biodiversity data to face the current challenges of management sustainability and environmental changes.
2. Methods

2.1. Data collection

This work was carried out through the collaboration network established by the COST Action BOTTOMS-UP (CA18207: https://www.bottoms-up.eu/en/). We collected and harmonized the vast majority of the available multi-taxon datasets in Europe (41 datasets), each dataset being a homogeneous range of data sampled through the same protocols by a given research group. All datasets include data on multiple taxonomic groups, forest structure and forest management, and together they encompass 13 European countries. To qualify as multi-taxon, a dataset should include a minimum of three taxonomic groups representing the Animalia kingdom and at least one of the kingdoms of Plantae and Fungi. The sampled groups represented heterogeneous taxonomic ranks, from kingdom to orders, so when merged some taxonomic groups display partial overlap. For instance, Coleoptera and Carabidae are reported separately since some studies focused on all Coleoptera, while others only sampled Carabidae. In some cases, the sampled taxonomic groups corresponded to morphological or functional groups, e.g., lichenized fungi, for which we used common names, i.e., lichens. The nomenclature we used for high rank taxonomic groups follows Roskov et al. (2019).

The bryophytes included in this work belong to two separate phyla, i.e., mosses (Bryophyta), liverworts (Marchantiophyta) that are usually considered together in ecological studies due to their similar life history, photosynthetic and poikilohydric ecophysiological structure (Goffinet and Shaw, 2009). Lichens constitute a highly paraphyletic group of fungi species (mainly Ascomycota) that form stable symbiotic relationships with cyanobacteria and/or algae. For fungi, most datasets considered only macrofungi, i.e., those fungi that can be detected by naked eye, which constitute a pragmatically defined group of Ascomycota and Basidiomycota forming macroscopically recognizable fungi with ascomata or basidiomata larger than 1 mm.

Management practices affect forest stand structure and, in turn, forest biodiversity (Farská et al., 2014) both directly, e.g., providing habitat structures, and indirectly by altering forest environmental conditions, e.g., pH, light radiation, soil humidity. Forest stand structure is therefore highly informative when linking biodiversity to forest management since it has direct links to both management practices and to the environmental conditions to which forest-dwelling organisms are subjected. For these reasons the combination of multi-taxon biodiversity data and structural information is common to most forest biodiversity datasets and was maintained here, thus complying with the framework of essential biodiversity variables (see Pereira et al., 2013). For structural data we focused on those measurements that are used to assess the main features of stand horizontal and vertical structure (Hui et al., 2019) and of deadwood, such as tree/fragment diameter and height/length. Deadwood was included in the handbook due to its high relevance for forest biodiversity, even if it was not available for some datasets (5 out of 41). Other environmental variables, e.g., microclimate or soil variables, are not discussed in this handbook.

2.2. Data harmonization

Sampling methods for biodiversity followed heterogeneous approaches and used different levels of effort and detail. For these reasons, a first step was necessary to agree on some common terms needed to describe the sampling designs (Table 1).

Initially, we collected qualitative descriptions of each sampling protocol to identify the main commonalities and sources of variation across datasets. This allowed to constrain the heterogeneity of sampling approaches into a limited number of quantitative and categorical variables that we divided across three main ecosystem components: multi-taxon biodiversity (SI 1), standing trees SI 2 and lying deadwood (SI 2). With standing trees, we refer both to living and dead trees or part of trees (snags and stumps) that have not fallen on the ground, whereas lying deadwood refers to deadwood fallen on the ground.

The inclusion of all relevant information on a single table summarising the protocols used for 35 taxonomic groups across 41 datasets needed several iterative phases of refinement. We also estimated the time and number of persons needed to sample individual units and of the equipment costs to provide a benchmark of the effort needed for each protocol.

2.3. Data analysis and visualization

To create a background for answering our first question: “What to sample?”, we calculated (from table SI 1) the number of plots (column: numb_plot) for which cross-taxon information between all the possible pairs of taxa (column: taxon) is available. To visualise the cross-taxon information most commonly available, we created a chord diagram using the package “circize” (Gu et al., 2014) in R (R Core Team, 2020).

To create a background for answering the second question: “How to sample?”, we analysed the share of plots across the variables describing the sampling methodologies based on the table synthesizing protocols for all the taxonomic groups (SI 1) and for standing trees and lying deadwood (SI 2) and visualised this information through alluvial plots. These plots represent a map of the approaches used in previous studies and were critically evaluated and discussed to develop the handbook (SI 3). In the alluvial plots, vertical blocks represent clusters of plots for which the same sampling parameter (e.g., square plot shape) was used, regardless of distribution across taxonomic groups. The higher the block the higher the number of plots for which that parameter was used. Flows between the blocks show the combination of sampling parameters for each taxonomic group (e.g., number of vascular plant square plots with a size comprised between 100 and 500 m²). By following the flow of a specific taxonomic group, it is possible to identify the most common sampling approaches for that group. Alluvial plots were constructed using the R-package “ggalluvial” (Brunson, 2020) in R.

The tables summarizing the sampling protocols (SI 1 and SI 2) and the graphs were made available to a network of experts that are representative of almost all forest multi-taxon studies performed in Europe. Within this network, subgroups of experts were defined for each of the most commonly sampled taxonomic groups and for forest structure sampling. Each subgroup drafted the protocol for each taxonomic group and for stand structure. These drafts were then commented and edited by all the other network participants to check for the feasibility of the proposed protocols by different research groups with experiences across very different forest types. This phase served to add a multi-taxonomic perspective to the handbook of field protocols (SI 3), since during this process all the approaches potentially overlapping or conflicting have been harmonized.

Eventually, the handbook includes detailed descriptions of the sampling methodology of different variables according to two sampling
standards that allow for cross-comparisons.

3. Results

3.1. Common standards on “What to sample?”

3.1.1. Forest multi-taxon biodiversity

The taxonomic groups that were most commonly sampled in multi-taxon forest biodiversity datasets (sorted by decreasing number of sampled plots) are: vascular plants (Tracheophyta), beetles (either sampled across the whole Coleoptera order or limitedly to Carabidae), lichens (mainly Ascomycota), bryophytes (Bryophyta, Marchantiophyta), fungi (Basidiomycota and Ascomycota pro parte), birds (Aves), bats (Chiroptera), spiders (Araneae) and harvestmen (Opiliones). The most widely sampled groups include organisms with preferences for different habitat elements of forest ecosystems, from soil and litter (fungi), ground (vascular plants and bryophytes, carabids), to epiphytic, epixylic, and saproxylic organisms (lichens, bryophytes, fungi and beetles), to airborne arthropods occurring in the subcanopy (beetles), and canopy-dwelling organisms, represented by some bird and bat species. The underrepresented habitat elements were soil and litter, and the canopy layer.

Also in a trophic network perspective, the groups sampled to a wide extent cover primary producers and decomposers, as well as consumers of these two groups, and secondary consumers. Fungivores and large herbivores instead were mostly neglected.

Several invertebrate groups of different ranks, from phyla to families, were sampled in relatively few studies (Fig. 1) leading to hardly comparable data among studies. This heterogeneity derives from the great effort needed to sample entire orders or classes of invertebrates, and to the high degree of specialization required for taxonomic identification.

3.1.2. Forest structure

Sampling methods for standing trees and lying deadwood were relatively homogeneous and mostly focused on assessing the living and deadwood volumes through measures of tree diameters and height (length of the fragment for lying deadwood). Only a fraction of datasets includes tree vitality and decay stages of deadwood, about 20 and 60% respectively. Regeneration and the shrub layer were mostly sampled in the context of the vascular plant survey.

Sampling differences occurred mostly in the shape, size and nest-edness of the sampling units (see section 3.2.2) and in the completeness of the sample with regards to the smallest trees/deadwood pieces, i.e., diameter thresholds.

Lying deadwood was mostly sampled in the same sampling units used for standing trees, but in some cases different methods were used, e.g., line intercept sampling (Van Wagner, 1968; Warren and Olsen, 1964).

![Fig. 1. Chord diagram representing the extent of simultaneous and overlapped sampling for each possible pairs of taxonomic groups across the plots/stands included in the 41 analysed datasets (see SI 1). Sector and links width show the cumulative number of available plots (column “numb_plot”) with cross-taxon information for each taxonomic group and pair of groups, respectively. Taxonomic groups encompass various taxonomic ranks that may partly overlap (column “taxon”). Taxonomic groups sampled in less than 60 plots are not shown.](https://example.com/image.png)
3.2. Common standards on “How to sample?”

3.2.1. Forest multi-taxon biodiversity

The sampling approaches used in existing multi-taxon datasets differed substantially across taxonomic groups and ecosystem components, with additional variation among datasets for the same taxonomic group. As expected, the main differences occurred between sessile (i.e., plantae and fungi) and vagile organisms (i.e., animals), and within the latter between vertebrates and invertebrates.

Sessile organisms were sampled visually, and their abundance was mostly estimated as cover or frequency across nested elements (pseudo-abundance), rather than by counting individuals (Fig. 2). Within sessile organisms, substantial methodological differences occurred between ground-dwelling groups and taxa occurring on specific substrates (trunks, logs, rocks). Ground-dwelling organisms were recorded mainly within a fixed circular or square area (plot), with a surface ranging from 100 to 1000 m². Organisms dwelling on other substrates were often sampled through designs where substrate elements (e.g., trees, logs, rocks) were nested within a plot, mostly by assigning presence/absence values to each species on each element. The sampling of vascular plants was generally associated with intermediate size, ranging from 75 to 1256 m² in about 60% of the datasets, only 4 datasets used smaller plots. Larger sampling units (2500–20,000 m²) were used in 10 datasets but mostly to identify nested subplots (7 datasets) ranging between 100 and 400 m².

Differences across protocols for taxa did not show any geographical pattern, indicating that there are no common approaches related to a country or a region. Results very similar to those of vascular plants were found for fungi, bryophytes and lichens, though with a greater share of nested designs accounting for specific nested elements, whose species occurrences were mostly aggregated at the plot or stand level.

The sampling unit (intended as a plot) is not substantially relevant for animals, since the sampling is mostly performed either in nested elements, for invertebrates, or across large areas for vertebrates.

Invertebrates show the greatest heterogeneity in sampling approaches (Figs. 3 and 6). They are included in studies aggregating cross-taxon information at the plot level by using nested elements, mostly traps or soil samples, depending on their preferred substrates and behaviors. The two types of most commonly used traps are pitfall traps and window traps, mostly two or three of each of them were used in each plot.

More than one visit within the same year is common due to the complex life-cycles that characterize some groups of invertebrates that may even require different sampling methods at different life-cycle stages. Plots were revisited mostly two to five times per year, when a higher number of visits were performed, they ranged from six to nine and only in one case reached up to 16 revisions.

Among vertebrates, birds were by far those sampled in the highest number of plots mostly through point counts (Fig. 4), but also bats were often surveyed, mostly based on echolocation signal recording. Other mammals were sampled through different strategies depending on their size, baited traps were used for small mammals, while camera traps were used for larger ones. Apart from camera traps, most sampling strategies relied on one element (trap or sampling point) per plot, since these approaches are based on a punctual information that is meant to express the species diversity of a relatively wide surrounding area.

3.2.2. Forest structure

Forest structure sampling (SI 2) was based on sampling standing trees (living and dead trees, snags and stumps), and lying dead wood (dead downed trees, coarse woody debris). Even if standing trees were sampled through nested schemes in 64% of the total number of plots,
Fig. 3. Alluvial plot synthesizing the methods for the sampling of the most commonly sampled invertebrates across the total number of plots (3153) in 41 studies. Columns from left to right report on: level for cross-taxon aggregation (field “aggr_level” in SI 1), sampling method (field “samp_meth” in SI 1), number of visits within one year (field “n_repl” in SI 1); type of abundance estimation (field “abun_score” in SI 1); sampling unit size (in hectares) and shape as derived from fields “plot_size” and “plot_shap” in SI 1 respectively; type and number of nested elements (fields “n_elem” and “type_elem” in SI 1). Only the upper limits of ranges are reported in the columns. Labels referring to less than 50 plots are not shown.

Fig. 4. Alluvial plot synthesizing the sampling methods used for vertebrates across the total number of plots (2245) in 41 studies. Columns from left to right report on: level for cross-taxon aggregation (field “aggr_level” in SI 1), sampling method (field “samp_meth” in SI 1), number of visits within one year (field “n_repl” in SI 1); type of abundance estimation (field “abun_score” in SI 1, P/A is for presence/absence); sampling unit size (in hectares) and shape as derived from fields “plot_size” and “plot_shap” in SI 1 respectively; type and number of nested elements (fields “n_elem” and “type_elem” in SI 1). Point counts are separated into those made by automatic recording (‘r’) and by human sampling (‘h’), where automatic recording includes camera traps. Labels referring to less than 50 plots are not shown.
most studies (25 out of 41) used a non-nested sampling scheme (Fig. 5). Nested schemes were primarily adopted in broad scale studies, in some cases related to National Forest Inventories.

Circular shape with intermediate size (from 1000 to 3000 square meters) was the most frequent sampling unit for forest structure (SI 2). Plots larger than 1 ha were seldom used (about 9% of the plots) mostly in northern and eastern Europe. Diameter thresholds have a wide range for the largest sampling units (up to 40 cm); lowest diameter thresholds (from 1 to 10 cm) are mostly associated with the smallest plot sizes (lower than 1000 m$^2$), with 5–7 cm thresholds being those used most commonly. As expected, nested units usually have the same shape but lower diameter thresholds (from less than 1 cm to 11 cm) than the largest unit.

To calculate standing tree volume, the direct measurement of tree diameter and height is the most common adopted methodology. However, in 32% of the sampling units tree height was not sampled and tree volume was hence calculated through diameter-based tables (single entry production tables). Tree height is sampled through either a fixed number of trees per plot (i.e., 1–50 trees) in 25% of the plots, or a constant proportion of trees in each plot (10–100% of the trees). Although both methods are biased (Zeide and Zakrzewski, 1993), given the great variability in plot size and tree densities, the constant proportion ensures a greater degree of comparability than the fixed number approach. In two datasets only, height values were obtained through LiDAR data.

When recorded, tree vitality mostly followed Kraft (1884) or IUFRO standard classification (Nieuwenhuis, 2000) with respectively five and three classes. Some studies adapted these classifications based on the needs of the survey.

Most protocols used a plot-based method for sampling lying deadwood, mostly with diameter thresholds, plot sizes and shape consistent to the ones used for standing trees SI 2. In eight protocols (58% of the overall plots), lying deadwood was sampled through line intersect method with a threshold diameter lower than 10 cm (5 cm and 10 cm being the most common thresholds). Half of these protocols used a nested scheme for smaller deadwood elements (i.e. lower diameter thresholds). Only three protocols sampled lying deadwood using a combination of the line intersect method and the plot-based method. In these cases, the line method is used for sampling logs with lower diameter thresholds. For stumps (h < 1.30 m) protocols include the measurement of both base and top diameters. Overall, fine woody debris was not inventoried in most studies, though this compartment can represent a greater proportion of the total volume of deadwood (du Cros and Lopez, 2009), and play an important role for some taxa (e.g., fungi, soil biota).

When recorded, deadwood decay stages were mainly sampled through five point classifications based on well-established methodologies (e.g. Maser et al., 1979; Waddell, 2002), or on national and international manuals (Hunter, 1990; Keller, 2011). Few protocols used original classifications based on local studies, but always including five classes (e.g., those regarding boreal forests of Soderstrom, 1988; Renvall, 1995).

3.2.3 Less commonly sampled taxonomic groups

Many groups of invertebrates were sampled in a low share of datasets. Although currently overlooked in biodiversity studies and monitoring, these taxonomic groups may still have a great potential for future monitoring and assessment. Furthermore, many of them may be sampled without adding sampling effort to the sampling of other invertebrates, although their identification will certainly require additional work.

Fig. 5. Alluvial plot synthesizing the methods for sampling forest structure across the total number of plots (7608) in 41 studies. Columns from left to right report on: approach to length/height measurement; shape, size, and associated diameter threshold of the largest and in the nested sampling unit. In the length/height column length refers to lying deadwood, “no” means no height was measured, “fixed” and “proportion” mean that a fixed number or a constant proportion of tree heights were measured respectively. Information derives from the field “tree_height” in SI 2 for height methods: proportion, no, fixed; length was associated to all lying deadwood sampling units. The other columns derive from the fields “first_shape”, “first_size”, “first_min_dia”, and “second_shape”, “second_size”, “second_min_dia” in SI 2. Only the upper limits of ranges are reported in the columns. Labels referring to less than 150 plots are not shown.
additional time and economic resources.

For instance, pitfall traps used for carabids, spiders and opiliones can be considered also for the sampling of Diplopoda, Isopoda (Oniscidea), Heteroptera and Coleoptera (Staphilinidae). The latter however have an extremely complex taxonomy and identification time is definitely higher than for other insect families.

Also, the window traps used for beetles, if provided with an additional funnel above the transparent panels with a container at its end, may serve the sampling of Diptera and Hymenoptera in addition to Coleoptera with no additional equipment cost.

Although not frequent among existing forest multi-taxon studies, soil samples can provide valuable information on several phylogenetically different taxa, such as Fungi through eDNA analysis, Gastropoda, Annelidae, and small-sized but highly abundant taxa like Acari and Collembola that are valuable biodiversity indicators in relation to forest management (Boros et al., 2019; Oettel and Lapin, 2021).

4. Discussion

4.1. Gaps in knowledge and emerging opportunities

By focusing primarily on biodiversity and collecting information on a wide range of taxonomic groups through highly diverse methodologies, the studies on forest multi-taxon biodiversity provide a different perspective for forest observations as compared to existing broad scale observation networks (Frenzel et al., 2012; Baeten et al., 2013; Ferretti and Fischer, 2013). The high degree of heterogeneity that can be found in the sampling protocols used in these studies is counterbalanced by consistent goals and similar sampling approaches. One of the commonalities is the sampling of taxonomic groups that were often pointed out as potential biodiversity indicators for European forests (Oettel and Lapin, 2021). This may derive from the indication value of these groups, which give information on the condition of forest ecosystems (e.g., including species that act as tree pathogens such as fungi and beetles), or on biogeochemical cycles (e.g., vascular plants). However, this overlap may also point to a certain degree of circularity that may lead to neglecting less studied taxonomic groups and ecosystem components. Except for fungi, soil and litter dwelling organisms were included in very few multi-taxonomic studies mostly accounting for soil macro-fauna such as Annelida, Gastropoda, Isopoda (Oniscidea) and Myriapoda, likely due to a limited tradition of using these taxa in forest biodiversity assessments. Soil meso- and micro-fauna, such as Collembola, Acari and Nematoda, were hardly sampled in any of the assessed multi-taxon studies despite their high abundances, and their key roles in ecosystem functioning. By contributing to biogeochemical cycles (Hättenschwiler et al., 2005), these taxa influence plant diversity and abundance, succession and productivity (Bardgett and Van der Putten, 2014; Kardol et al., 2006). One of the reasons why soil-dwelling organisms are often not included in multi-taxon studies but rather studied separately is that their sampling coverage is generally lower as compared with other groups, e.g., vascular plants. This gap can be filled through the analysis of environmental DNA (Taberlet et al., 2018) as an important complement to traditional field data collection. Environmental DNA techniques are rapidly developing, but still have limitations. The reference databases are often incomplete, and include confusing species annotations, complicating the translation from sequence to species data (Frøslev et al., 2019). Furthermore, commonly used marker genes may poorly distinguish between intraspecific and
species level diversity (Estensmo et al., 2021), similarly to what happens when relying on morphological species concepts, e.g., in fungi (Nilsson et al., 2003). Environmental DNA techniques also have limitations in quantifying plot level species abundances, and have a coarse temporal resolution (Turner et al., 2014) especially for those species with a distinct bank of propagules or other biological legacies (Foslev et al., 2019).

Also the sampling of canopy dwelling organisms should be noted as limited, mainly due to practical constraints, i.e., sampling is generally performed from the ground even if this is not appropriate for some taxa different than birds and bats. Sampling methods for canopy arthropods (Floren and Schmidl, 2008) include some destructive techniques, such as fogging with insecticide, as well as other approaches, i.e., cranes, that are more sustainable environmentally but not economically. By neglecting tree canopy during sampling some rare species of lichens and Bryophytes (Fritz, 2009), and spiders (Hietz and Lienennair, 2011) may be undetected, especially where large senescent trees occur. Also for saproxylic beetles the overall species detection probability is higher in the understory (1.5–2 m) when compared to the canopy (15–20 m) (Bouget, Brin and Brustel, 2011).

Based on the current knowledge, the risk of a limited knowledge for some ecosystem components may be addressed through the adoption of novel sampling techniques in the case of soil biodiversity, whereas the additional costs of approaches focused on the canopy layer may not be compensated by the share of species that this would add to traditional sampling. For these reasons, in the handbook we developed (SI 3), we introduced soil environmental DNA techniques, but suggested canopy sampling only for studies with a focus on this ecosystem component and a compatible budget.

### 4.2. Plot vs. stand aggregation level

Two main spatial approaches were used to aggregate data for different taxa and stand structure: in most cases (70% of studies), all the taxonomic groups and stand structure were sampled in the same plots, i.e., a sampling unit with a unique identification in which different sampling were performed in an overlapping area (Kiraly et al., 2013; Lohmus and Runnel, 2018; Sitzia et al., 2012). This approach, i.e. plot aggregation level, allows for cross-taxa analyses and for the use of structural attributes as explanatory variables for biodiversity at the plot scale. In the other cases (30% of studies), different taxonomic groups and structural attributes were sampled either across a whole stand, without specific sampling units, or in plots that differed not only for size and shape, but also for their locations across the stand (see for instance Lelli et al., 2019; Vandekerkhove et al., 2011). This approach allows for full cross-taxa analysis only at the stand level.

The main advantage of plot-level aggregation is that it results in a larger number of sampling units that can be used in ecological models, if pseudoreplication issues are adequately handled (Spake and Doncaster, 2017). Furthermore, plot level data can be easily aggregated at the stand level (Burrascano et al., 2018), or used to investigate patterns and drivers of within-stand multi-taxa diversity (Jones et al., 2008; Sabatini et al., 2014). The number of plots that is representative for a stand depends on plot and stand size, stand heterogeneity, and on time and economic constraints. The sampling coverage should be, but is rarely, estimated based on rarefaction techniques (Huck et al., 1975).

Plot-based sampling is generally very efficient in capturing typical species and habitat features, but is prone to overlook rare species, unique microhabitats or other unusual habitat features, unless the number or size of sample plots is very high. This shortcoming is the main reason why some studies have combined different sampling protocols at stand level, to allow for customized, cost-effective sampling of specific taxonomic groups and structures that are less efficiently sampled using joint plots, even if nested. For instance, some studies (Balestriere et al., 2015; Lelli et al., 2019) mapped the full population of breeding birds at stand level, as a more comprehensive alternative to point-counts. Some studies using the stand aggregation level performed several reiterations, thus approximating a complete census that is substantially independent of a specific sampling design (Hofmeister et al., 2017). As a kind of compromise between plot level and stand-level aggregation, Lohmus et al. (2018) suggested an opportunistic sampling of biodiversity within 2 ha macroplots, using fixed time bounds to secure adequate sampling depth. Although this approach proved effective in terms of sampling completeness, most studies of forest biodiversity response to management used smaller sampling units, likely due to their focus on fine resolution heterogeneity in forest structure and ecological conditions (Sabatini et al., 2014).

Based on the above considerations, we suggest that plot-level sampling should be preferred in forest multi-taxon biodiversity studies. The spatial overlap of the sampling area for taxonomic groups with large home ranges should be addressed in each individual study. Solutions may include large distances between sampling units, or an uneven density of sampling units across taxonomic groups.

### 4.3. Limitations of the study

The first limitation of our study is geographical: our data collection has a strict European focus. However, it is also true that the majority of non-tropical forest multi-taxon studies were performed in Europe. Based on the search on ISI-WOS of “forest AND multi-taxon AND biodiversity” performed in August 2021 81% of the 59 studies performed in non-tropical regions were located in Europe. Hence, Europe is the only continent with a fair tradition of multi-taxon studies, and a substantial need for shared standards. The handbook (SI 3), however, can be applied in other temperate, boreal or Mediterranean forests, whenever researchers deem the proposed protocols appropriate for the forest type under study. If this will not be possible, the handbook will promote the creation of alternative standards in other continents with the highest degree of comparability to the ones here presented.

The second limitation of our study relates to the fact that it takes a synthetic, rather than analytic approach. Answering the question on “what to sample”, we primarily emphasized the most commonly used variables and species groups to describe forest ecosystems, enabling comparisons with a broad range of previous studies. In our recommendation of specific sampling methods, we take the same synthetic approach. This builds on a traditional, common understanding of forest ecosystems, rather than on an explicit assessment of cost-effectiveness for the sampling of multi-taxon biodiversity and its drivers in a management context.

The focus on well-studied organism groups and structural variables has pros and cons. It takes advantage of previous assessments of feasibility and addresses well-studied taxonomic groups (e.g., vascular plants, beetles, birds) and structures that are well known conservation targets, or have a wider applicability as indicators (Oettel and Lapin, 2021).

On the other hand, the historical bias derived by an uneven availability of taxonomic experts or effort needed for a comprehensive sampling of different groups is maintained. Similarly, the focus on a common set of structural variables most likely promotes variables used in traditional timber production-oriented surveys (except for standing dead trees and lying deadwood) but lacking a final proof as relevant to biodiversity. However, it should be recognized that these well-tested variables are easy to measure and effective in relating forest structure to management, and biodiversity (Storch, Dormann and Bautois, 2018).

When coming to “how to sample”, this handbook does not explicitly address the sampling efficiency and coverage of different methods since presently this would not be feasible given the tight association between forest types, management regimes, site conditions and sampling protocols.

Sampling protocols normally face a trade-off between allocating resources to attain sufficient sample quantity (i.e., extending coverage) and quality (to ensure reliability of individual estimates and detection of
species that are difficult to monitor) (Gardner, 2010). Here we cover the quality of plot-level sampling but not how the quantity of sites and plots relates to sampling completeness. This will represent the next effort and will be based on a wide database that was purposely merged and harmonized.

We did go slightly beyond the synthetic approach, and identified underrepresented taxonomic groups that may have a specific indicator value and should be progressively incorporated into monitoring schemes, such as Collembola (Oettel and Lapin, 2021) or enchytraeid worms (Boros et al., 2019). Similarly, we do advocate for use of novel schemes, such as Collembola (Oettel and Lapin, 2021) or enchytraeid potential for many groups of soil- and litter dwelling organisms.

Notwithstanding the limitations outlined above, this handbook represents a pragmatic synthesis and an important step forward to direct monitoring of forest biodiversity, in Europe and elsewhere. It gives the state of the art to build on in the future: it derives from an effort of networking and synthesis aimed at defining standard approaches for forest monitoring, with the goal to ensure sampling robustness and comparability. We are certain it can contribute to more efficient monitoring of biodiversity response to the numerous pressures and threats related to management to which forest ecosystems are currently subjected (EEA, 2020).

Declaration of Competing Interest

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.

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This review was funded by the EU Framework Programme Horizon 2020 through the COST Association: COST Action CA18207: BOTTOMS—Biodiversity Of Temperate forest Taxa Orienting Management underrepresented taxonomic groups that may have a specific indicator relates to sampling completeness. This will represent the next effort and resumed.

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Authors’ contributions

SB and GT developed the idea of the manuscript and collected and harmonized the data. RA, GB, AC, ED, ID, JH, JaH, PJ, SKR, NK, DK, TL, AL, RL, AM, MM, SM, BN, MP, JoP, MR, PS, KR, MS, FT, MU, KV, KV contributed the data. SB, FC and GT realized the graphs. SIB, ABO, TC, Brunson, J., ggaluvial: Layered Grammar for Alluvial Plots. Journal of Open Source Software 5 (49), 2017. https://doi.org/10.21105/joss.02017.

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