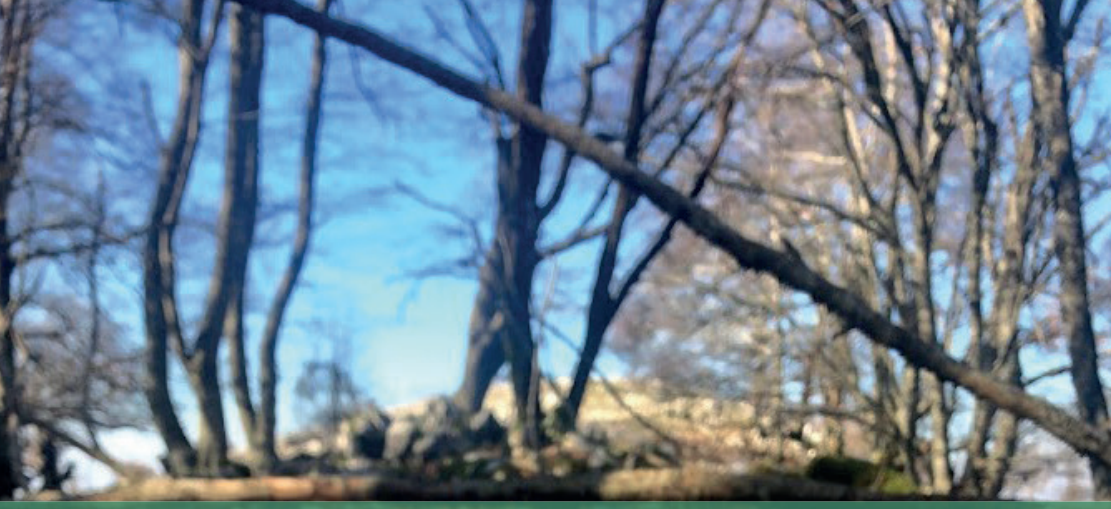

HANDBOOK OF FIELD SAMPLING FOR MULTI-TAXON BIODIVERSITY STUDIES IN EUROPEAN FORESTS

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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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BOTTOMS-UP

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EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Funded by
the European Union

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EDIZIONI

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ISBN 978-88-31222-50-1
First edition: May 2022

Printed in Italy by Press Up Srl – Stabilimento di Nepi (VT)

Suggested citation: Burrascano S., Trentanovi G., et al., 2022. *Handbook of sampling for multi-taxon biodiversity studies in European forests*. PM edizioni, Varazze (Savona). ISBN 978-88-31222-50-1. DOI https://doi.org/10.48250/1051.
Retrieved from: https://www. cost.eu/publications/.

This publication is based upon work from COST Action BOTTOMS-UP CA18207, supported by COST.

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What is COST?
COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

What is the COST Action BOTTOMS-UP?
The COST Action BOTTOMS-UP (CA18207: https://www.bottoms-up.eu/en/) took up the challenge to increase the degree of sustainability of European temperate forest management for biodiversity. It adopts a bottom-up approach by establishing a synergy of local research efforts to collect information on multi-taxon biodiversity, structure and management that already resulted in the most comprehensive knowledge of European multi-taxonomic forest biodiversity. Outcomes will include shared research and monitoring tools for forest biodiversity, innovative indicators for sustainable forest management (SFM) and management guidelines at the stand and landscape scale. These outcomes will improve forest management sustainability, ecosystem functioning and provisioning of services. The Action involves about 200 researchers and stakeholders from more than 30 countries and presents an outstanding opportunity to develop a strong network of collaboration for standardized broad-scale multi-taxon studies in Europe.

The text and tables included in this handbook mostly derive from the article:
Burrascano, S., Trentanovi, G., Paillet, Y., Heilmann-Clausen, J., Giordani, P., Bagella, S., Bravo-Oviedo, A., Campagnaro, T., Campanaro, A., Chianucci, F., De Smedt, P., García-Mijangos, I., Matošević, D., Sitzia, T., Aszalós, R., Brazaitis, G., Cutini, A., D'Andrea, E., Doerfler, I., Hofmeister, J., Hošek, J., Janssen, P., Kepfer Rojas, S., Korboulewsky, N., Kozák, D., Lachat, T., Lõhmus, A., Lopez, R., Mårell, A., Matula, R., Mikoláš, M., Munzi, S., Nordén, B., Pärtel, M., Penner, J., Runnel, K., Schall, P., Svoboda, M., Tinya, F., Ujházyová, M., Vandekerkhove, K., Verheyen, K., Xystrakis, F., Ódor, P., 2021. Handbook of field sampling for multi-taxon biodiversity studies in European forests. Ecological Indicators 132, 108266. https://doi.org/10.1016/j.ecolind.2021.108266

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FOREWORD

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 the diversity of multiple taxonomic groups, 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 their heterogeneous sampling approaches hamper broad-scale inference for designing SFM. The COST Action BOTTOMS-UP (CA18207) addressed the need of common sampling protocols for European forest structure and multi-taxon biodiversity. We established a network of researchers involved in 41 projects on European forest multi-taxon biodiversity across 13 European countries. These projects comprised the assessment of at least three taxonomic groups, 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? Here we comprehensively address these questions for nine different taxonomic groups and for the sampling of standing trees and lying deadwood. For each of these forest components, we provide two standards that differ in spatial scale and effort. Both standards were specifically designed towards the greatest possible comparability across taxonomic groups and studies. 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 to ensure sampling robustness and comparability. We are certain it can contribute to more efficient monitoring of biodiversity response to forest management.

Birch forest in southern Finland.
Photo by: Tommaso Sitzia

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Beech forest in northern Italy.
Photo by: Giovanni Trentanovi

1. BACKGROUND AND GENERAL APPROACH

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 both sustainable development (UN, 2015) and biodiversity maintenance (MCPFE, 1993) as promoted in the most recent European Union regulations (EU 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, the biodiversity indicators for this criterion either account only for stand structure and tree species (e.g., tree 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 biodiversity 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 & 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 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).

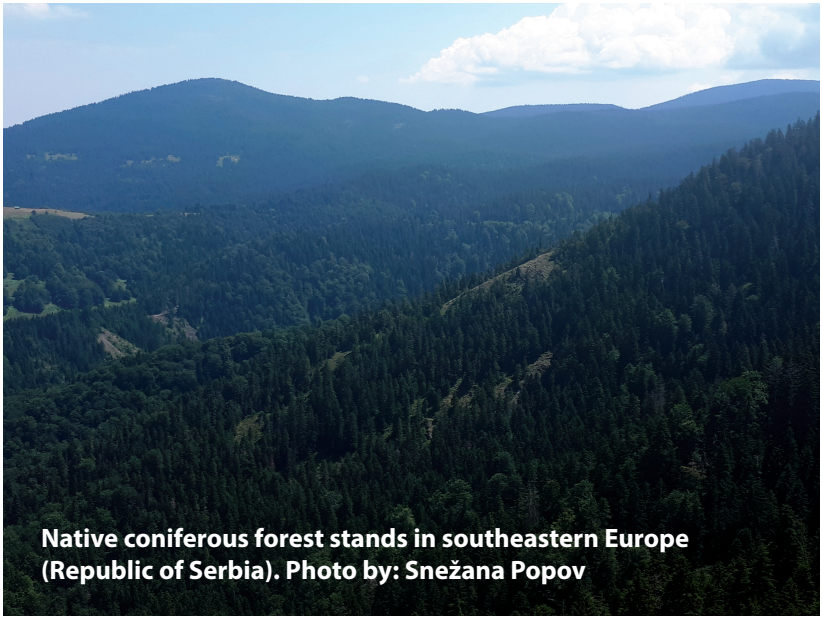
International observation networks, either specifically focused on forest ecosystems functioning (i.e., ICP Forests, FunDivEurope) or on the long-term change of a wide range of aquatic and terrestrial ecosystems (i.e., LTER) usually collect biodiversity data. However, given the geographical and the conceptual 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; Sitzia 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 several 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 comparability across studies 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 spatial 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 underlined that they hamper the understanding of forest biodiversity mechanistic response to management at multiple spatial scales.

Ecological data incompatibility is increasingly being solved by establishing common data platforms (Bruelheide 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 & 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



Native coniferous forest stands in southeastern Europe (Republic of Serbia). Photo by: Snežana Popov

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 (Moretti et al., 2017; Pérez-Harguindeguy et al., 2013; Sack et al., 2010).

The COST Action BOTTOMS-UP (CA18207) performed 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 discussed their similarities and differences (see Box 1). Based on this overview, several experts with different backgrounds developed a handbook of field sampling protocols 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 through graphical representation of 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 that are provided in the form of a handbook (paragraph 3.2).

The 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.

From data collection…

This handbook is based on the collection and harmonization of the vast majority of the available multi-taxon datasets in Europe (41 in total) that include data on multiple taxonomic groups, forest structure and forest management, and encompass 13 European countries.

Forest stand structure is 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 in this handbook. For structural data we focused on those measurements that are used to assess the main features of stand horizontal and vertical structure (Hui, Zhang, Zhao & Yang, 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).

Initially, we collected quali-quantitative descriptions of each sampling protocol to identify the main commonalities and sources of variation across datasets. This allowed us 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, standing trees, and lying deadwood.

The inclusion of all relevant information on a single table summarising the protocols used for 35 taxonomic groups across 41 datasets needed a long phase of iterative discussion with the dataset custodians through a Delphi-like technique (Mukherjee et al., 2015)(Fig. 1).

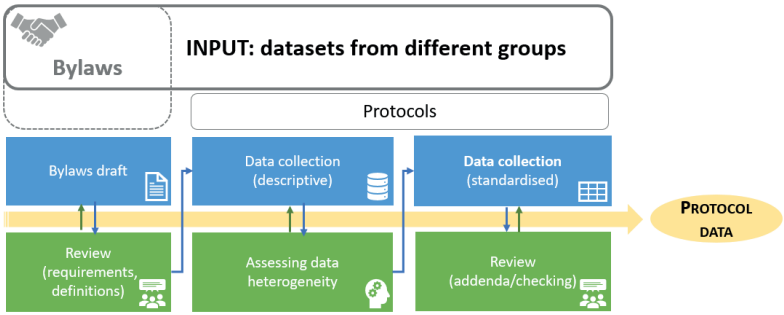


Figure 1: Workflow of protocol data harmonization. Blue boxes identify milestones; green boxes identify phases of common decisions and brainstorming; in yellow the outcome of the platform building process.

…to data visualization

Based on the table synthesizing the protocols, we analysed the share of plots across the variables describing the sampling methodologies and visualised this information through alluvial plots. 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 m^2). By following the flow of a specific taxonomic group, it is possible to identify the most common sampling approaches for that group (see Fig. 2 as an example).

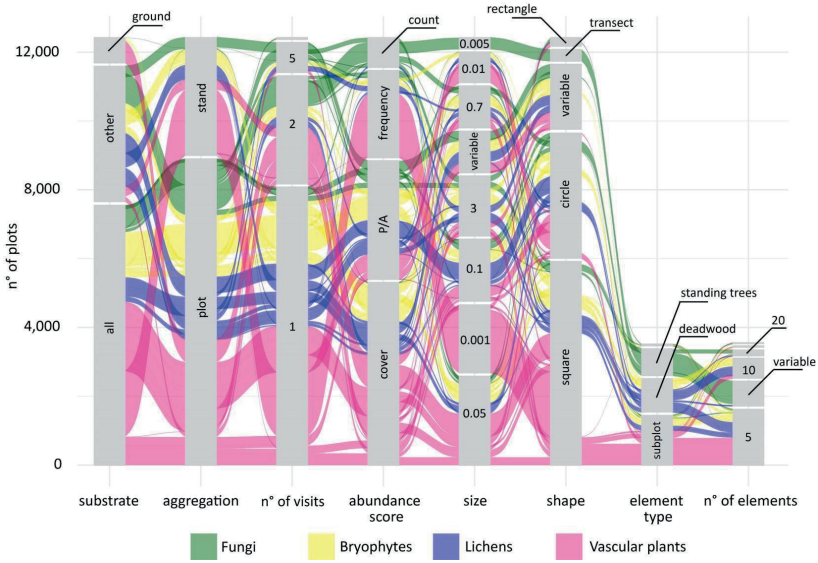


Figure 2. Alluvial plot synthesizing the methods for the sampling of sessile organisms across the total number of plots (12,418) in 41 studies. Columns from left to right report on: sampled substrates: 'ground' refers to taxa sampled only on ground, 'other' to protocols including taxa sampled on epiphytic/epixylic/epilithic organisms, 'all' to taxa sampled on all substrates; level for cross-taxon aggregation; number of visits within one year; type of abundance estimation (P/A is for presence/absence): sampling unit size (in hectares) and shape; type and number of nested elements. Only the upper limits of ranges are reported in the columns. Labels referring to less than 150 plots are not shown.

Mediterranean stone pine forest in central Italy.
Photo by: Giovanni Trentanovi

Norway spruce forest in western Bulgaria.
Photo by: Sabina Burrascano

2. WHAT TO SAMPLE?

The high degree of heterogeneity that can be found in the sampling protocols used in the studies on forest multi-taxon biodiversity is counterbalanced by consistent goals and similar sampling approaches. One of the commonalities is the object of sampling, that mostly focuses on those taxonomic groups that were often pointed out as potential biodiversity indicators for European forests (Oettel & Lapin, 2021). This underlines the indication value of these groups but also a certain degree of circularity that may lead to neglecting less studied taxonomic groups and ecosystem components.

The taxonomic groups that were sampled most often in multi-taxon forest biodiversity datasets are: vascular plants, beetles (either sampled across the whole Coleoptera order or limitedly to Carabidae), lichens, bryophytes, fungi, birds, bats, spiders and harvestmen. 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 flying 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 (see Box 2).

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 rarely sampled (Fig. 3) 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 their taxonomic identification.

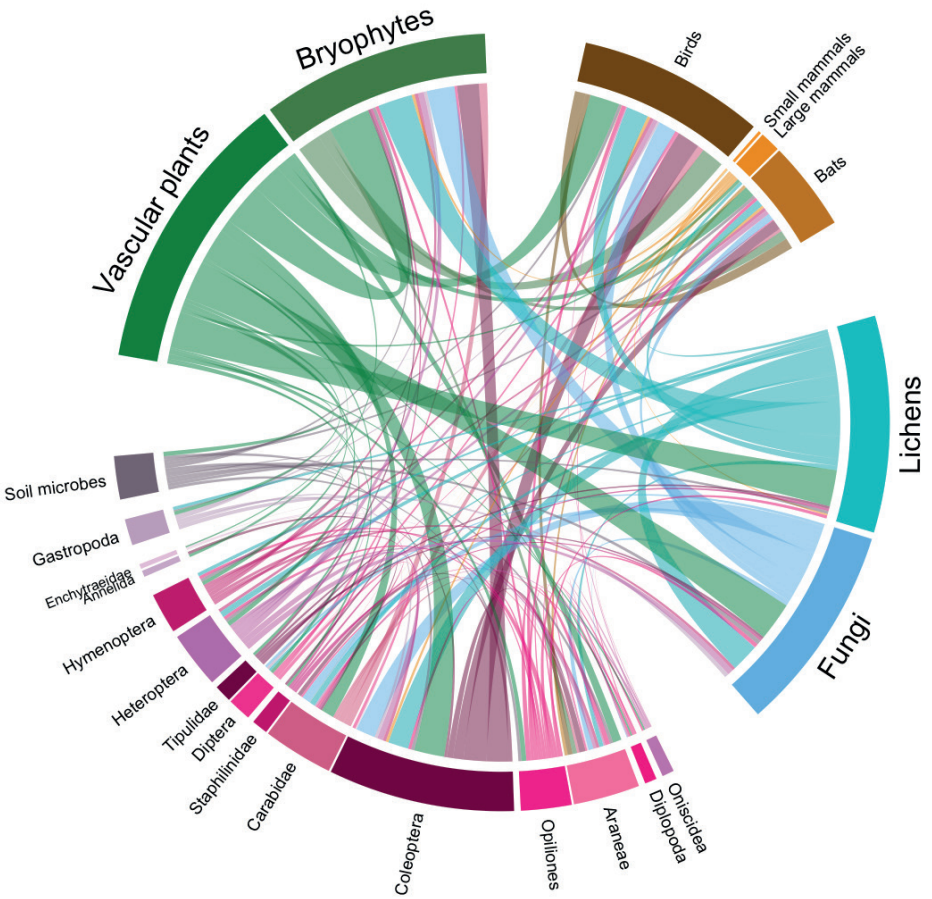


Figure 3. Extent of simultaneous and overlapped sampling for each possible pair of taxonomic groups across the plots/stands included in the 41 datasets. Sector and links width show the cumulative number of available plots with cross-taxon information for each taxonomic group and pair of groups, respectively. Taxonomic groups encompass various taxonomic ranks that may partly overlap (e.g., *Coleoptera* and *Carabidae*); those sampled in less than 60 plots are not shown.

Sampling methods for standing trees and lying deadwood 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. 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 nestedness of the sampling units 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).

What are we missing?

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). In fact, soil and litter invertebrates may have a great potential for future monitoring and assessment and may be sampled without adding sampling effort to the sampling of other invertebrates, although their identification will certainly require additional time and economic resources.

One of the reasons for the exclusion of these groups from multi-taxon studies 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., 2019) especially for those species with a distinct bank of propagules or other biological legacies (Frøslev et al., 2019).

We hope that future research on forest biodiversity will make the effort to overcome these difficulties and limitations to have a full picture of the composition and functioning of forest ecosystems.

Riparian willow forest and European beech forest in central Italy.
Photo by: Sabina Burrascano

3. HOW TO SAMPLE?

3.1 THE STATE OF THE ART

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.

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.

Sessile organisms were sampled visually, and their abundance was mostly estimated as cover or frequency (pseudo-abundance) across nested elements, rather than by counting individuals. 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 m^2. 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 substrate element.

The sampling unit (intended as a plot, see Box 3) 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. 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 these traps were

Conifer forest along the Samokovska river in the Republic of Serbia.
Photo by: Snežana Popov

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.

Among vertebrates, birds were by far those sampled in the highest number of plots mostly through point counts, 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. Forest structure sampling was based on sampling standing trees (including living and standing dead trees, snags and stumps), and lying dead wood (dead downed trees, coarse woody debris).

To calculate standing tree volume, the direct measurement of tree diameter and height is the most common adopted methodology. Tree height is sampled through either a fixed number of trees per plot (i.e., 1 to 50 trees) or a constant proportion of trees in each plot. Although both methods are biased (Zeide & Zakrzewski, 1992), given the great variability in plot size and tree densities, the constant proportion ensures a greater degree of comparability than the fixed number approach.

When recorded, tree vitality mostly followed Kraft (1884) or IUFRO standard classification (Nieuwenhuis, 2000) with respectively five and three classes. Some studies used a revised version of these classifications.

Most protocols used a plot-based method for sampling lying deadwood, mostly with diameter thresholds, plot size and shape consistent to the ones used for standing trees. Lying deadwood was sampled also through line intersect method with a threshold diameter lower than 10 cm (mostly 5 cm or 10 cm). 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 Söderström, 1988; Renvall, 1995).

Mediterranean holm oak forests in north-eastern Italy.
Photo by: Tommaso Sitzia

Plot vs. stand: at what scale should we aggregate multi-taxon data?

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 overlapping areas identified as one individual plot. This approach, i.e. plot aggregation level, allows for cross-taxon analyses and for the use of structural attributes as explanatory variables for biodiversity at the plot scale. In the other cases, different taxonomic groups and structural attributes were sampled either across a whole stand, without specific sampling units, or in plots that differed not only in size and shape, but also in their locations across the stand. This approach allows for full cross-taxon 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 & 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-taxon beta-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.

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 birds. Some studies using the stand aggregation level performed several revisitations, thus approximating a complete census that is substantially independent of a specific sampling design (Hofmeister et al., 2017).

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.

3.2 LOOKING FORWARD – OPERATING MANUAL

This handbook provides two standards for sampling forest multi-taxon biodiversity and structure (Fig. 4).

In the following paragraphs, we report the ecological relevance and indicator value of the taxonomic groups and structural variables that were most often considered in forest multi-taxon studies (*Reasons for sampling*).

Based on the critical analysis of the sampling protocols used in multi-taxon studies performed in Europe, on existing standards as well as on the expertise of the authors, we propose two standard methods for sampling and taxon and habitat specific tips (*How to sample?)*.

Furthermore, for each taxonomic group a specific list of references is reported that could help the reader navigate the vast literature on each group sampling methodologies.

The sampling we propose has to be intended as part of a multi-taxonomic approach since it is based on sampling units and elements that may be used for as many taxa as possible. This is the case for bryophytes and lichens, whose sampling approach is based on the same grids, and for ground-dwelling invertebrates, i.e., carabids and spiders and harvestmen, that may be sampled using the same pitfall traps. This will result in a certain degree of savings in equipment cost and setting time, and will allow for direct cross-taxon comparisons.

We defined two protocol standards designed as nested in a way that allows for direct and flawless comparison between them. This accounts for the fact that the choice of a specific standard will not

only depend on economic resources but also on the spatial scale at which heterogeneity can be detected in a specific stand or site, and on its biodiversity density. In this view, several plots sampled according to the second standard should be preferred over few according to the first standard where a fine scale horizontal heterogeneity and/or a high species density occurs. This choice will not affect the data comparability with studies that used a different standard as long as field crews associated each record to a specific subunit in the data entry. Researchers may also decide to switch across the two proposed standards for different taxonomic groups/structural elements in the framework of the same study.

For each taxonomic group/structural element a rough estimate of the time and people/experts needed is provided based on previous experiences. We also included ranges of sampling equipment costs in euros (< 100, 100-1,000, > 1,000) for each standard. An equipment cost < 100 euros is generally associated with sessile organisms that do not require specific sampling tools but only basic equipment, e.g., plastic bags, field manuals, lens and grids. Sampling of animals mostly requires traps or recorders that raise to higher equipment cost as compared to sessile organisms, except for birds, whose sampling on the other hand relies on a high degree of expertise of the field crew.

When designing multi-taxon fieldwork activities, it should be taken into account that multiple sampling activities in the same plot can result in substantial trampling by researchers, therefore we suggest limiting the access to one expert for each taxonomic group when possible, and to a single person managing traps for invertebrates.

Bog with *Pinus silvestris* in Latvia. Photo by: Thomas Campagnaro

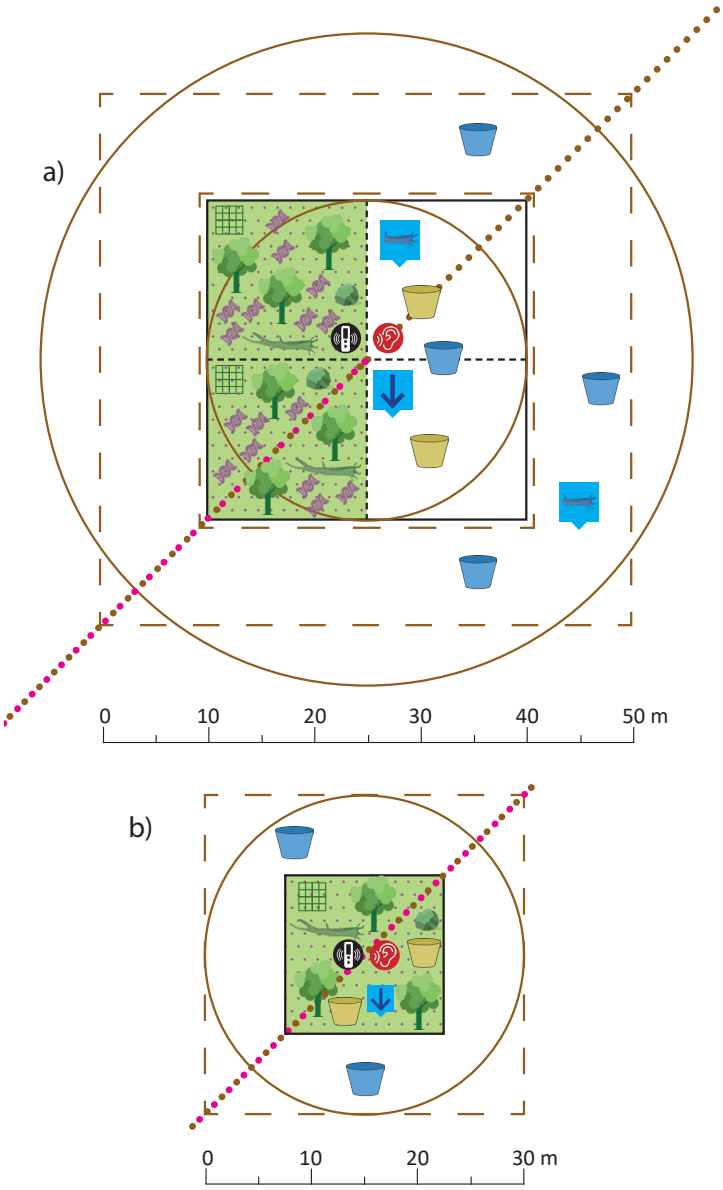


Figure 4 (a-b). Schemes of the sampling units for forest multi-taxon biodiversity and structure sampling according to the first (a) and second (b) standard. For the first standard (a), the right and left halves of the plot schemes report respectively the sampling methods used for sessile organisms and for invertebrates.

c)

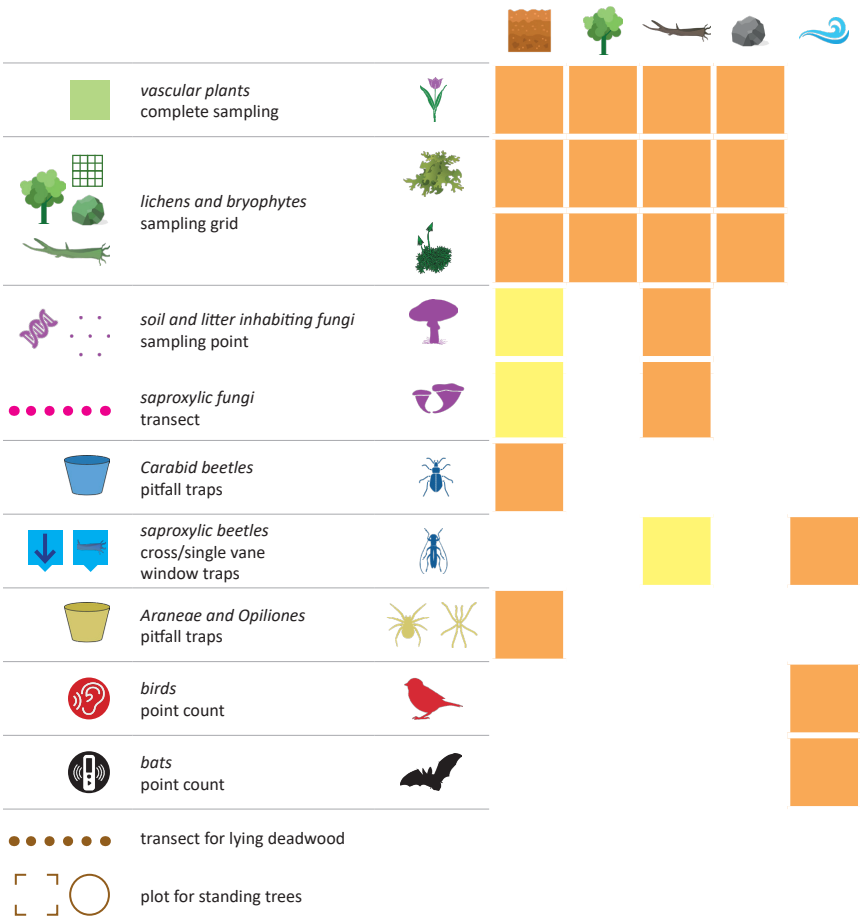


Figure 4 (c). Sampling substrates for each taxonomic group are represented in yellow (for the first standard only) and orange (for both standards). From left to right column headers represent: ground, standing tree, lying deadwood, rock and air.

The vernal species (*Scilla bifolia*) marking the beginning of spring in deciduous forests. Photo by: Sabina Burrascano

VASCULAR PLANTS

REASONS FOR SAMPLING

Vascular plants, including trees, shrubs and herbs, are by far the taxonomic group most commonly sampled in forests. This group is recognized as particularly suitable to assess forest biodiversity since it provides the physical structure for other organisms, makes up most of forest primary productivity, and plays a fundamental role in nutrient cycling. Vascular plants include a large number of habitat specialists distributed across broad environmental gradients that are used to detect forest habitat diversity (Standovár et al., 2006).

Overstorey trees (i.e., vascular plant layer over 3 meters height) are the bulk of forest biomass, as well as the component directly affected by management (Rackham, 2008). The shrub layer instead may be identified as between 1 and 3 meters height (Scheffer et al., 2014). Finally the understorey layer, here intended as the vegetation developing up to 1 meter height, makes up most of the plant species diversity in forests of the temperate zone (Gilliam, 2007) and was found to contribute substantially to ecosystem fluxes, i.e, productivity, nutrient cycling, evapotranspiration, to influence tree species regeneration, and to provide habitat and food to functionally important organisms (Landuyt et al., 2019).

Vascular plants are among the best known groups of organisms in terms of taxonomy. All these characteristics make vascular plants an ideal candidate for monitoring forest ecosystems and, for these reasons, they are also proposed as a surrogate taxonomic group of other important and less easily detectable taxa (Bagella, 2014; Burrascano et al., 2011; Hofmeister et al., 2019; Pharo et al., 2000).

HOW TO SAMPLE

In European forests, most vascular plants develop from the ground, and traditionally the abundance of tree, shrub and understorey species is estimated as their cover projected at the ground level. Therefore, the shape and size of the sampling unit is the main key choice for this taxonomic group. Most previous studies used square or circular plots, the latter being less common. Square sampling units have the advantage of allowing for an accurate delimitation of the sampling unit through a measuring tape starting from the coordinates of a vertex and are easier to subdivide into subplots. Circular plots instead may not be delimited at the ground level, therefore do not allow to accurately discriminate the extent to which species and individuals project their canopy within the sampling unit. The most frequent plot sizes range between 100 and

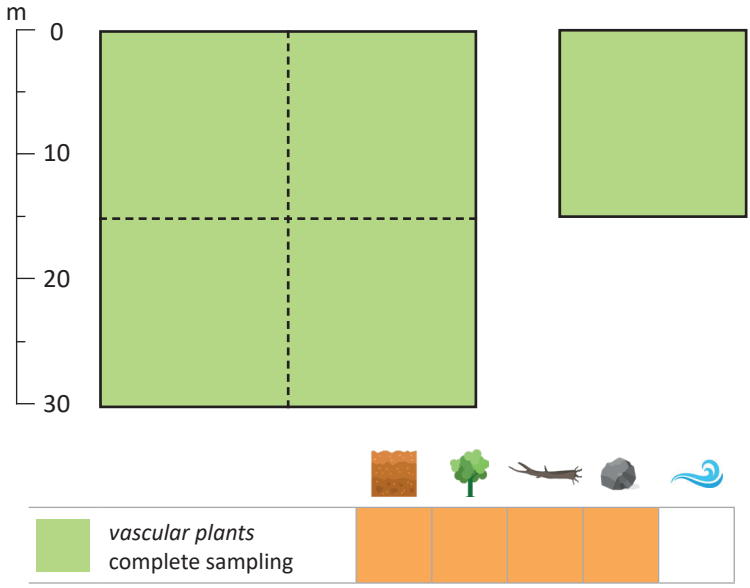


Figure 5. Sampling units (first standard left, second standard right) for vascular plants, and substrates to be sampled (bottom). Orange squares in the substrate scheme indicate that all the substrates but air have to be sampled for both standards.

1,000 m^2, even if this range is widened up to 1-20,000 m^2 by studies with nested designs with small plots or subplots scattered within a stand or very large plots respectively. The abundance data are usually recorded through ordinal scales, either based on percentage values or on the Braun-Blanquet (1964) classes.

What resulted from previous multi-taxon studies reflect past methodological comparisons reporting on the greater repeatability of plant censuses carried out in large plots as compared with small plots (Archaux et al., 2007). This is in line with the standard methods proposed for European forests by vegetation scientists (Chytrý & Otýpková, 2003), by the ICP Forests network (Canullo et al., 2020), and for forest habitats of Annex I of the Habitat Directive (e.g., Gigante et al., 2016), all suggesting the use of square plots larger than 200 m^2, and abundance scores based on Braun-Blanquet (1964) scale. This is here intended as modified by Westoff & van der Maarel (1978), i.e., splitting the value "2" in 2a (5-12%) and 2b (12-25%).

The sampling unit we propose as a first standard for vascular plants are 30x30 m square plots subdivided into four 15x15 m square subplots for an accurate assessment of each species cover to be performed separately in each subplot. For the second standard only one 15x15 m square plot will be surveyed (i.e., the same area of one of the first standard subplots). Also in the case of the second standard (15x15 m plot), we recommend that the plot is subdivided into four quadrats during species detection and cover estimates. Even if only one abundance value per plot will be kept after data processing, quadrats will substantially improve the accuracy of sampling and of cover estimates. We suggest a minimum of 30 minutes to be spent in each subplot as reported in specific literature (Archaux et al., 2006).

As for comparability of abundance values across standards, Braun-Blanquet scale can be easily transformed into percentage by using mid-values (van der Maarel, 1979). However, for analytical purposes, the percentage cover estimation is more appropriate and can be applied also to the second standard depending on the study objectives.

We strongly suggest recording separately species and abundance values for each of the three layers that are usually identified in European forests: overstorey, height greater than 3 meters; shrub, height between 1 and 3 meters; understorey, height below 1 meter.

This will allow to disentangle the functions of different vegetation layers, since these were found to be strongly complementary to each other in temperate forests (Landuyt et al., 2019). For studies that have a strong focus on patterns of understorey species diversity, in addition to the plot-level cover estimate, vascular plants, lichens and bryophytes should be sampled in the same soil grids proposed (see following paragraphs) to improve the comparability across taxonomic groups.

In many forest types, intra-annual variation in floristic composition and plant cover values could be considerably high (Korb & Fulé, 2008; Vymazalová et al., 2012). Early spring and summer seasons are considerably different, while it has been shown that autumn sampling does not have a strong impact in the assessment of understorey alpha-diversity (Vymazalová et al., 2012); thereby two visits per year across spring and summer were often used rather than a single survey. Since seasonality strictly depends on climatic domain, local climate, and weather differences across years (duration of snow cover, graduality of temperature shifts), the choice of performing one or two visits should be made for each individual study. Our suggestion is to merge the species lists deriving from two surveys performed in two seasons on the same plot and year and report the maximum cover value recorded for each species.

The bulbous species (*Crocus neglectus*) in the understorey of a European beech forest. Photo by: Sabina Burrascano

	First Standard	Second Standard
Target taxonomic level	Species/subspecies	Species/species aggregate
Plot shape	Square	Square
Plot size	30x30 m (900 m^2)	15x15 m (225 m^2)
Type of elements within the plot	Subplot	-
Number of elements	4	-
Element size	15x15 m	-
Abundance score	Percentage cover for each species in each layer	Braun-Blanquet scale for each species in each layer
Time needed (min.)	60-120/plot	30-60/plot
Number of visits and season	2/year, (early) spring and summer	1/year, early summer
Persons needed	2	1
Experts needed	1	1
Equipment costs (€)	<100	<100

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The epiphytic lichen *Lobaria pulmonaria* on a large maple tree.
Photo by: Philippe Janssen

LICHENS

REASONS FOR SAMPLING

Lichens constitute a highly paraphyletic group of fungi species (mainly Ascomycota) that form stable symbiotic relationships with cyanobacteria and/or algae that represent an ecologically defined group. Despite their limited biomass, lichens represent a significant component of forest habitats, supporting a considerable number of ecosystem functions (Asplund & Wardle, 2017; Giordani et al., 2012). In particular, forest lichens contribute to regulate the nitrogen cycle, constitute refuge and hunting sites for small invertebrates, regulate the temperature and the availability of water in epiphytic and epilithic substrates (Porada et al., 2013, 2018). Rare epiphytic lichens are often associated with specific microhabitats of old trees (Fritz & Heilmann-Clausen, 2010) and other old-forest structures (standing and lying deadwood) (Hofmeister et al., 2016). Due to their biological characteristics and to the different forest ecological niches they occupy, lichens are excellent indicators of environmental conditions (Ellis, 2012), and they are largely used to verify the sustainability of forest management (Brunialti et al., 2020; Moning et al., 2009; Nascimbene et al., 2013).

HOW TO SAMPLE

Most multi-taxon studies mainly focus on epiphytic lichens, and in few cases extend to those colonizing deadwood. As for other sessile organisms, plots of defined shapes (circular or square) and size were usually taken into consideration, and, similarly to bryophytes, nested elements were selected (i.e., one to ten living trees) with different methods for the assignment of species abundance scores.

Overall, the approaches of previous studies are in line with the current processes of standardization of protocols for monitoring lichens (see Giordani & Brunialti, 2015) that account for two main sources of uncertainty: i) the sampling error related to the high variability of lichen response to macro- and microenvironmental factors (Cristofolini et al., 2014; Matos et al., 2017), and ii) the non-sampling error depending on the taxonomic knowledge of the sampling expert(s), as well as on lichen species detectability (Brunialti et al., 2012; Giordani et al., 2009).

The general recommendation for lichen sampling is to include nested elements for different substrates: living trees, deadwood, rocks and soil. For rocks and soil, a 50x50 cm sampling grid, divided into 25 10x10 cm quadrats, is used. On living trees, 4 10x50 cm sampling grids (each split into 5 10x10 cm quadrats) are located parallel to the tree trunk, at the four cardinal directions, between 100

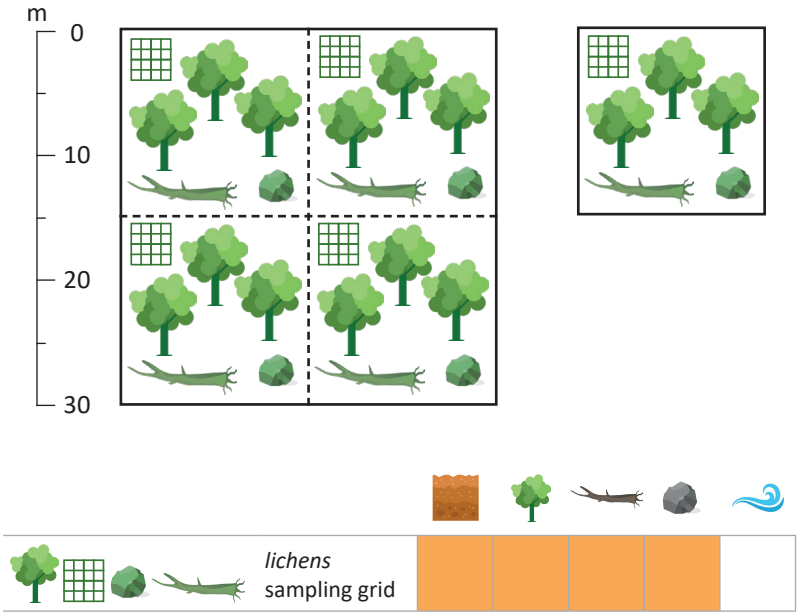

Figure 6. Sampling units (first standard left, second standard right) for lichens, and substrates to be sampled (bottom). Orange squares in the substrate scheme indicate all the substrates but air have to be sampled for both standards. The green square grid refers to the sampling of ground lichens.

and 150 cm from the ground. If, within a plot, standing trees with biodiversity relevant features occur, e.g., over-mature/dying trees, sporadic tree species, trees close to forest gaps, etc., these should be sampled to allow the detection of rare lichen species (Vondrák et al., 2018). On the other hand, if a substrate (rocks or deadwood) is missing within a plot, it is important to record that sampling on that substrate was not performed due to the absence of the substrate.

	First Standard	Second Standard
Target taxonomic level	Species	Species or morpho-functional groups
Plot shape	Square	Square
Plot size	30x30 m	15x15 m
Type of elements within the plot	grid (25 quadrats) -> soil grid (25 quadrats) -> rocks grid (5 quadrats) -> living trees grid (9 quadrats) -> dead-wood	grid (25 quadrats) -> soil grid (25 quadrats) -> rocks grid (5 quadrats) -> living trees grid (9 quadrats) -> dead-wood
Number of elements	4 grids for soil, rocks and deadwood (1 for each sub-plot), and 12 standing trees (3 for each subplot)	1 grid for soil, rocks and deadwood and 3 living trees
Element size	50x50 cm -> soil 50x50 cm -> rocks 10x50 cm -> living trees 30x30 cm -> deadwood	50x50 cm -> soil 50x50 cm -> rocks 10x50 cm -> living trees 30x30 cm -> deadwood
Abundance score	Frequency in standard sampling grids	Frequency in standard sampling grids
Time needed	120-360/plot	30-90/plot
Number of visits and season	1/year, no seasonality	1/year, no seasonality
Persons needed (min.)	2	1
Experts needed	2	1
Equipment costs (€)	<100	<100

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The moss *Rhytidiadelphus triquetrus* mostly associated with late-successional forests. Photo by: Péter Ódor

BRYOPHYTES

REASONS FOR SAMPLING

The special morphological and physiological characteristics of bryophytes enable them to colonize various substrates in forests, such as tree bark, decaying wood, or rocks, which are less favorable for vascular plants. This means that the bryophyte community is largely determined by the quantity and quality of these substrates. In fact, many species are directly related to specific substrates; therefore, the species composition varies substantially across different substrates that have different limiting environmental drivers (Smith, 1982). The bryophytes included in this work belong to two separate phyla, i.e., mosses (*Bryophyta*) and liverworts (*Marchantiophyta*) that are usually considered together in ecological studies due to their similar life history, photosynthetic and ecophysiological structure (Goffinet & Shaw, 2009).

Terrestrial bryophytes differ depending on litter and forest type, since they establish a permanent layer with few species in coniferous forests, which is missing from broadleaf forests because of the inhibitory effect of broadleaf litter (Márialigeti et al., 2009). Furthermore, terrestrial assemblages are strongly connected to fine-scale soil disturbances, like "pit and mound" formations in natural forests dynamics (von Oheimb et al., 2007).

Although epiphytic (living on bark) and epixylic (living on decaying wood) assemblages considerably overlap, both are influenced by microclimatic conditions (Táborska et al., 2020), distance to the forest edge (Hofmeister et al., 2016) and landscape factors (Löbel et al., 2006). Epiphyte diversity depends mainly on tree species composition, tree size and age distribution (Király et al., 2013; Mezaka et al., 2012), while the main limiting factor for epixylic assemblages is the amount, quality, and continuity of deadwood (Ódor et al., 2006).

Since these variables are strongly modified by human land use history of forests, these organisms are very sensitive to the forest management regime (Hofmeister et al., 2015; Kaufmann et al., 2017; Müller et al., 2019). Although epilithic species are mainly determined by the amount and quality of the rocky substrates, they are also sensitive to some management-related factors such as tree species composition and microclimate (Patiño et al., 2010; Weibull & Rydin, 2005).

HOW TO SAMPLE

Because of their strong dependency on substrates, the sampling methodologies of epiphytic, epixylic, epilithic and terrestrial bryophyte assemblages are different (Smith, 1982). Terrestrial bryo-

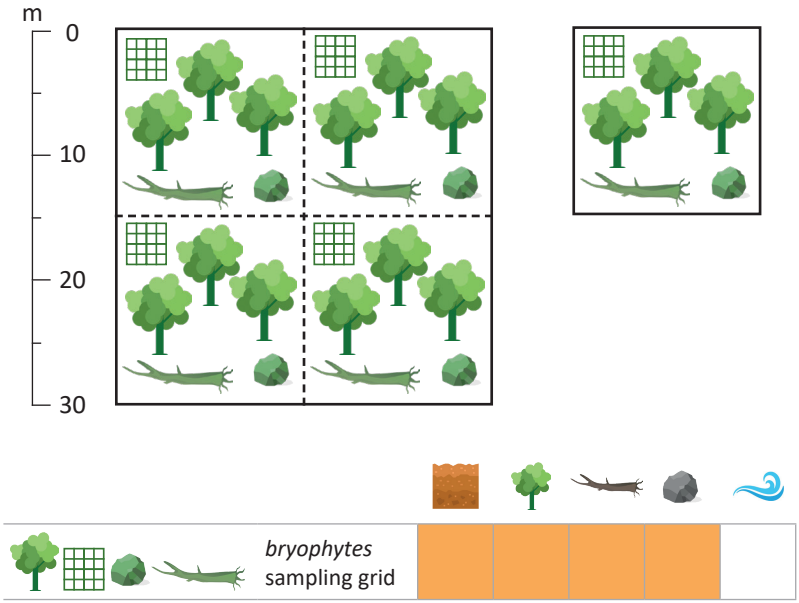

Figure 7. Sampling units (first standard left, second standard right) for bryophytes, and substrates to be sampled (bottom). Orange squares in the substrate scheme indicate all the substrates but air have to be sampled for both standards. The square grid refers to the sampling of ground bryophytes.

phytes were often surveyed by plot-based methods often connected to the sampling of vascular plants (Márialigeti et al., 2009). For the other assemblages, the sampling is based on selected units of the substrates (trees, logs, and rocks) that suppose a nested design within the plots (or stands). The sampling of the selected substrate units either cover the whole unit (entire logs, trunks) or subplot(s), or transect(s) within the unit. Epiphytic bryophytes are surveyed usually only on the lower 2 m of the trunks for practical reasons; whole tree inventory is applied only in studies specifically focused on vertical distribution (Fritz, 2009). Abundance may be quantified either as cover (related to the entire surveyed area) or as presence/absence on the substrate units, rising to frequency values on plot level (pseudo-abundance). Most of the bryophytes of these specific substrates are perennial, which means that one careful inventory throughout a year satisfies the scientific standards. There are some short-lived terrestrial species related to disturbed soil surfaces which can occur on relatively short periods of the year, but usually terrestrial assemblages are also surveyed only once.

Some previous forest multi-taxon studies recorded general plot level species list (with ordinal score abundance estimation). Many studies focused on trees (selecting all or a subset of trees within the plots), and only one focused on epixylic (log inhabiting) bryophytes. Even if only one study made separate samplings for different substrates, we deem this approach as the most appropriate since it is the only one that would provide information on different environmental (and management) drivers and allow for comparability across studies even when not all substrates are sampled. Based on this reasoning and on the multi-taxon approach of the handbook, the sampling here proposed for bryophytes is perfectly overlapped with the one proposed for lichens. It is interesting to note that, among the relevant substrates, rocks are mostly neglected during bryophytes sampling since this substrate is missing from many forest types and is not strictly related to management factors. As for lichens, we recommend that if a substrate is missing within a plot, it is important to record that sampling was not performed due to the absence of the substrate.

	First Standard	Second Standard
Target taxonomic level	Species	Species
Plot shape	Square	Square
Plot size	30x30 m	15x15 m
Type of elements within the plot	grid (25 quadrats) -> soil grid (25 quadrats) -> rocks grid (5 quadrats) -> living trees grid (9 quadrats) -> deadwood	grid (25 quadrats) -> soil grid (25 quadrats) -> rocks grid (5 quadrats) -> living trees grid (9 quadrats) -> deadwood
Number of elements	4 grids for soil, rocks and deadwood (1 for each subplot), and 12 standing trees (3 for each subplot)	1 grid for soil, rocks and deadwood, 3 living trees
Element size	50x50 cm -> soil 50x50 cm -> rocks 10x50 cm -> living trees 30x30 cm -> deadwood	50x50 cm -> soil 50x50 cm -> rocks 10x50 cm -> living trees 30x30 cm -> deadwood
Abundance score	Frequency in standard sampling grids	Frequency in standard sampling grids
Time needed (min.)	120-360/plot	30-90/plot
Number of visits and season	1/year	1/year
Persons needed	2	1
Experts needed	2	1
Equipment costs (€)	<100	<100

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The fungus *Oudemansiella mucida* finding its way out of a bark crowded with epiphytic mosses and lichens. Photo by: Sabina Burrascano

FUNGI

REASONS FOR SAMPLING

Fungi constitute a biological kingdom with at least 1.5 million species worldwide (Hawksworth & Lücking, 2017). They play a number of fundamental roles in forest ecosystems, as decomposers of deadwood and plant litter and as biotrophic symbionts, including endophytic and mycorrhizal fungi associated with forest trees and herbs (Heilmann-Clausen et al., 2015). Fungi associated with deadwood (saproxylic fungi) are most frequently included in inventories of forest biodiversity but also ectomycorrhizal fungi and leaf litter and humus saprotrophs are commonly considered (Dvořák et al., 2017; Kutszegi et al., 2015).

The focus on saproxylic fungi originates from deadwood being among the habitat features most strongly affected by forest management (Burrascano et al., 2013; Christensen et al., 2005). With their fundamental role in wood decay, they are among the most obvious indicators of biotic and abiotic processes related to deadwood (Halme et al., 2017). Ectomycorrhizal fungi have an equally important role in forest ecosystems, being intimately linked to tree growth and health (Sapsford et al., 2017). They are especially relevant to assess the effect of intense silvicultural regimes, such as tree retention clearcuts (e.g., Sterkenburg et al., 2019) and intensively thinned beech forests (Müller et al., 2007), and to investigate the effects of environmental pollution and climate change on soil biology (e.g., Kjøller et al., 2012). It is important to note that 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 fruiting bodies larger than 1 mm.

Fungi pose several challenges for sampling. Firstly, sampling often relies on reproductive structures that for most species are ephemeral, irregular and somewhat unpredictable in appearance (Lodge et al., 2004). Hence, a single sampling campaign will at best uncover a fraction of the true macrofungal diversity, and even extensive sampling campaigns spanning many years may not yield complete species lists (Abrego et al., 2016; Ruldoph et al., 2018; Straatsma et al., 2001). As a trade-off between unpredictability and sampling feasibility, most of the reviewed forest multi-taxon studies have included two samplings per plot/stand during the same year,

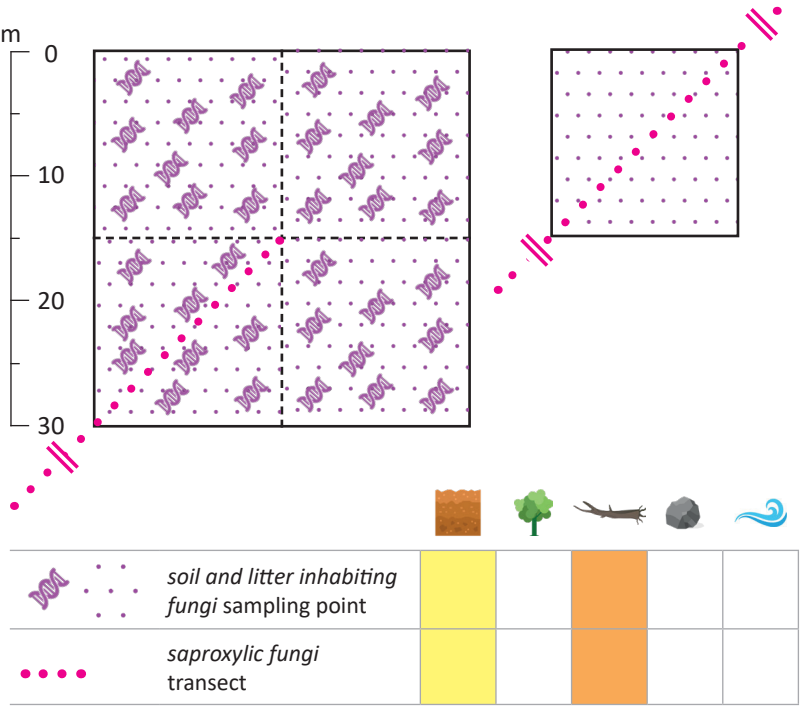

Figure 8. Sampling units (first standard left, second standard right) for fungi, and substrates to be sampled (bottom). The DNA symbol refers to the sampling of ground fungi through environmental DNA to be performed in the first standard only (yellow squares); the dotted line refers to the sampling of fungi on lying deadwood across both standards (orange standards).

mainly in spring and autumn. This strategy is hardly optimal to recover fungal diversity (Halme & Kotiaho, 2012), and when possible two samplings, early and late in the peak autumn season, should be combined over successive years (or within one year) to be cost-effective. The issue of undersampling is largest for macrofungi producing agaricoid reproductive structures and smallest for perennial polypores (Halme & Kotiaho, 2012).

Differently from other sessile groups, fungal species abundance is mostly recorded as the count of occupied units for species occurring on deadwood, or as the count of reproductive structures. While the first approach gives insights into the number of reproductive individuals per species, the second approach gives insight into the number of reproductive structures produced, but not the number of fungal individuals they represent. Sampling is usually separated between substrate types. Typically, ground and deadwood elements are differentiated, and often monitored using different protocols. Importantly, size thresholds for inclusion of reproductive structures vary widely among studies. This is especially true for ascomycetes (both discomycetes and pyrenomycetes) where reproductive structures smaller than 5 mm are sometimes excluded from surveys. In the same manner corticioid fungi are rarely fully included in surveys, especially among the soil and litter dwelling species. The agaricoid reproductive structure is most prominent among soil-living fungi (ectomycorrhizal and decomposing) that, for this reason, are particularly prone to undersampling based on reproductive structures. Sampling of fungal communities by eDNA based protocols is rapidly developing as an alternative to surveys based on reproductive structures. Although this approach needs the allocation of extra funds (not estimated here) as compared to traditional sampling approaches, it has been shown to be cost effective for soil-living fungi, and to provide a much better reflection of the true fungal diversity (Frøslev et al., 2019). For saproxylic fungi, the benefits of using eDNA based protocols are less prominent and fruit-body surveys can still be considered cost effective (Runnel et al., 2015). With the use of additional primers, the same samples used for fungal surveys can be investigated for many other groups of soil-dwelling organisms (e.g., Brunbjerg et al. 2019).

Soil and litter inhabiting fungi		
	First Standard	**Second Standard**
Target taxonomic level	Species or OTUs (Operational Taxonomic Units)	Species
Plot shape	Square	Square
Plot size	30x30 m	15x15 m
Type of elements within the plot	subplots, sampling points per subplot for litter and soil eDNA	-
Number of elements	4 (subplots), 8 (sampling points) per subplot	-
Element size	15x15 m, 0.2 liter sample	-
Abundance score	Presence/absence per subplot (reproductive structures); read count per plot (eDNA)	Presence/absence
Time needed (min.)	60/plot for fruit bodies + 60 /plot for soil samples	30/plot
Number of visits and season	3 surveys/plot in spring, summer and autumn for fruit bodies (eDNA samples collected at last survey)	3 surveys/plot in spring summer and autumn for fruit bodies
Persons needed	2	1
Experts needed	1	1
Equipment costs (€)	<100	<100

Saproxylic fungi		
	First Standard	**Second Standard**
Target taxonomic level	Species	Species
Plot shape	-	-
Plot size	-	-
Type of elements within the plot	Transect	Transect
Number of elements	2	1
Element size	50 m length for lying deadwood + 10 m buffer (5 m on each side) for standing deadwood	50 m length for lying deadwood + 10 m buffer (5 m on each side) for standing deadwood
Abundance score	Presence/absence per deadwood item with diameter > 10 cm intersecting the transect if lying or in the buffer area if standing	Presence/absence per deadwood item with diameter > 10 cm intersecting the transect if lying or in the buffer area if standing
Time needed (min.)	60-90/survey (including corticoid fungi and smaller ascomycetes) and similar time for ID work	30-60 /survey (excluding corticoid fungi and smaller ascomycetes) and similar time for ID work
Number of visits and season	3 surveys/transect, early and late autumn	3 surveys/transect, early and late autumn
Persons needed	2	1
Experts needed	1	1
Equipment costs (€)	<100	<100

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A wood-inhabiting fungus (*Mycena renatii*). Photo by: Sabina Burrascano

A jewel beetle (*Eurythyrea austriaca*) whose larvae develop on senescing conifer trees. Photo by: Ondrej Kameniar

COLEOPTERA

REASONS FOR SAMPLING

Insects make up the dominant part of the biodiversity of forest fauna and are represented in every level of trophic networks (Nageleisen & Bouget, 2009). Coleoptera represent the largest insect order, and are used as indicators of ecosystem stability (Niemelä, 2000), and of the impact of management on forest ecosystems (Niemelä, 1999). Among forest Coleoptera, those most often included in multi-taxon studies are Carabidae and saproxylic beetles. The latter include those species that depend, at least for part of their life cycle, upon wounded or decaying woody material from living weakened or dead trees (Stokland et al., 2012). Saproxylic beetles are crucial in a conservation perspective, since they represent an important part of the total forest biodiversity (Grove, 2002; Vallauri et al., 2005), and the vast majority of the beetles protected under the EU Habitats Directive 92/43/EEC.

Carabid species include both generalist and specialist predator species, with some species more sensitive to environmental changes than others (Rainio & Niemelä, 2003). Carabid conservation gained importance in the last decades, and the ecology of threatened and non-threatened species is studied to define conservation and management guidelines for several habitats (Kotze et al., 2011). Deadwood-associated species in general, and saproxylic beetles in particular, are increasingly targeted in forest biodiversity conservation, since they may represent structural biodiversity and sustainable management indicators (Bouget et al., 2013).

Both Carabidae and saproxylic beetles are useful indicators in forest ecosystems (Lachat et al., 2012; Rainio & Niemelä, 2003), their seasonal activity, abundance, species richness, diversity, and composition give hints on biotic responses to forest management and forest disturbance also in relation to the availability of different

microhabitats and/or deadwood typology (Niemelä, 1999; Siitonen, 2001; Toïgo et al. 2013).

HOW TO SAMPLE

Pitfall traps (PT) and window flight traps or flight-interception traps (WT) are the most commonly used passive collective methods for beetles (Iannuzzi et al., 2021).

PT yield large captures of epigean arthropods (Nageleisen & Bouget, 2009; Woodcock, 2005) and are a highly effective sampling method (Ward et al., 2001; Hoekman et al., 2017) for capturing ground dwelling Coleoptera (e.g., Carabidae and some saproxylic species). PT allow to detect changes in local populations, with the possibility to pool data from long-lasting monitoring programmes covering different activity periods, up to the entire season (April-October). PT should be roofed to prevent contamination with debris and leaves. The traps should be checked every two weeks or monthly (Elek et al., 2018; De Smedt et al., 2019). The same PT can be also used for Araneae and Opiliones (see the following paragraph for details on trap use and management).

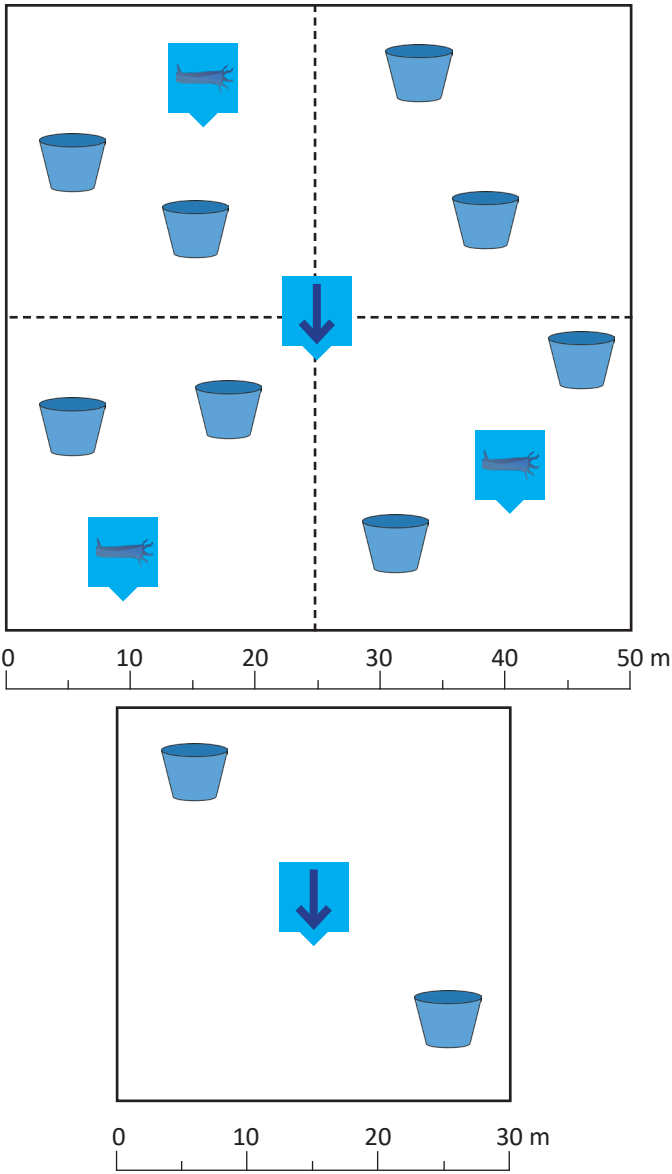
WT capture individuals that are intercepted during the flight by a vertical obstacle (on hitting the obstacle, the individual falls into a funnel below the transparent panels and ends up in the collection container with liquid preservatives); the obstacle consists of one (single vane traps) or two perpendicular transparent panels (cross-vane or multidirectional traps) of 20x30 or 40x60 cm. Approximately 60% of flying beetle fauna can be intercepted with WT which is considered a fairly representative sample of saproxylic beetles (Siitonen, 1994). WT should be hung from branches at approximately 1.5 m above the ground. Due to the multi-taxon approach followed in this handbook, we suggest using WT with an additional funnel above the transparent panels with a container at its end if the study aims at sampling and studying also Diptera and Hymenoptera simultaneously to Coleoptera (Knuff et al., 2019). Depending on the project goals and budget, additional traps may be hung at higher heights, i.e., 15-25 m depending on the dominant tree height, to include canopy-dwelling beetle sampling (Röder et al., 2010).

The traps should be used across the activity season (April-November) in order to enable the catch of rare species. In previous multi-taxon studies, the number of traps per plot varied from one to four, but mostly only one trap was used. Traps were checked every 2 weeks or monthly (Bouget et al., 2013; Franc & Götmark, 2008; Janssen et al., 2016; Kozák et al., 2020; Kraut et al., 2016; Sabatini et al., 2016; Vandekerkhove et al., 2016).

We also suggest the use of trunk window traps (Franc et al., 2007), single vane WT attached in proximity to trunk microhabitats (e.g., fungi, tree hollows) or deadwood (e.g., snag, log) that are more sensitive to specific saproxylic assemblages.

Several other methods were used in a minority of studies, such as glue rings (Vandekerkhove et al., 2016), substrate sampling (Chamagne et al., 2016), Winkler-Berlese extractors (Janssen et al., 2016), transects (Avtzis et al., 2018; Campanaro et al., 2016), and eclectors (Sabatini et al., 2016).

A longicorn beetle (*Rosalia alpina*) whose larvae develop in senescing or recently dead wood. Photo by: Daniel Kozák



Carabid beetles pitfall traps	■				
Saproxylic beetles cross/ single vane window traps			■		■

Flight interception trap for beetles.
Photo by: Elena Haeler

(Left page) Figure 9. Sampling units (first standard top, second standard middle) for *Coleoptera*, and substrates to be sampled (bottom). The pot symbol refers to the sampling of ground carabids through pitfall traps to be performed in both standards (orange squares), the square bubbles to the window traps to be placed in the center of the sampling unit across both standards (orange standard) or on relevant deadwood elements in the first standard (yellow square).

Carabid beetles		
	First Standard	**Second Standard**
Target taxonomic level	Species	Species or genus
Plot shape	Circular or square	Circular or square
Plot size	2826 m^2 (30 m radius) or 2500 m^2 (50x50 m)	706.5 m^2 (15 m radius) or 900 m^2 (30x30 m)
Type of elements within the plot	Pitfall traps	Pitfall traps
Number of elements	8	2
Element size	Opening 10 cm diameter	Opening 10 cm diameter
Abundance score	Activity-density	Activity-density
Time needed (min.)	60 for trap setup + 10 for emptying	30 for trap setup + 10 for emptying
Number of visits and season	Every two weeks, from April to September	Monthly, from April to September
Persons needed	2	2
Experts needed	1	1
Equipment costs (€)	<100	<100

Saproxylic beetles		
	First Standard	**Second Standard**
Target taxonomic level	Species	Species or genus
Plot shape	Circular or square	Circular or square
Plot size	2826 m^2 (30 m radius) or 2500 m^2 (50x50 m)	706.5 m^2 (15 m radius) or 900 m^2 (30x30 m)
Type of elements within the plot	cross-vane window trap and single-vane window traps	cross-vane window trap
Number of elements	4 (1 cross-vane window trap in the center of the plot and 3 single-vane window traps at the most relevant dead-wood habitats present (snag, log, hollow, up to stumps)	1 (in the center of the plot)
Element size	-	-
Abundance score	Activity-density	Activity-density
Time needed (min.)	60 for trap setup +20 for emptying	30 for trap setup + 10 for emptying
Number of visits and season	Every two weeks, from April to September	Monthly, from May to August
Persons needed	2	2
Experts needed	1	1
Equipment costs (€)	100-1,000	<100

A longicorn beetle (*Saperda scalaris*) whose larvae develop on senescing or dead trees. Photo by: Sabina Burrascano

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A harvestman (*Nemastoma bimaculatum*) usually found below rocks, and woody debris, and within litter. Photo by: Jinze Noordijk

ARANEAE AND OPILIONES

REASONS FOR SAMPLING

Spiders (Araneae) and harvestmen (Opiliones) are the largest arachnids in temperate forests. Both are generalist predators and can influence prey populations, thereby influencing trophic interactions and subsequently ecosystem processes such as nutrient cycling and litter decomposition (Clarke & Grant, 1968; Lawrence & Wise, 2004). Additionally, together with Carabid beetles they are the most numerous predatory macro-arthropods in forest ecosystems (De Smedt et al., 2019), with harvestmen having a large proportion of species with a strong affinity to forest habitat. Spiders and harvestmen are good indicators of forest structural complexity, tree species richness and composition, management practices, and natural disturbance dynamics (Ampoorter et al., 2020; Černecká et al., 2017; Elek et al., 2018; Samu & Sárospataki, 1995; Schall et al., 2018).

HOW TO SAMPLE

Spiders and harvestmen are commonly sampled through pitfall traps, which are especially efficient in temperate regions (Tourinho & Lo-Man-Hung, 2021). The size of the pitfall trap is important (Lange et al., 2011) and mostly larger traps (diameter about 10 cm) are used. The use of a funnel inside the trap can limit the number of small vertebrates as by-catch and will not influence the total catch of arachnids (Knapp & Ruzicka, 2012; Lange et al., 2011). The trapping fluid also influences the size of the catch (Knapp & Ruzicka, 2012) and nowadays glycol is most often used. The most common mixture is ½ glycol and ½ water (car antifreeze can be used). It is important to use a roof above the pitfall trap to prevent rain from diluting the solution and to prevent fallen leaves from filling up the

trap. The pitfall traps used for spiders and harvestmen may be the very same ones used for Carabid beetles.

A plot or stand should always be sampled with more than one individual pitfall trap (preferably from two to five) since pitfall traps sample a very local community especially for smaller species. Forest plots can be circular or square with a surface of 100-900 m^2. Pitfall traps can be placed in a row or a square spaced two to five meters apart. It is recommended to empty the traps after 14 days and refill them with trapping fluid for another 14 days. In this way it is possible to account for bad weather events in a 14-day period (extremely wet or dry). Ideally, individuals of the different traps are treated separately, but for processing efficiency, the catch of more traps (four if the sampling here proposed is followed) can be pooled in the field. Timing is crucial since both taxa show strong phenological patterns (Harvey et al., 2002; Wijnhoven, 2009). Spiders and harvestmen should only reliably be identified in their adult stage. Most spiders are adults during late spring, but a significant amount (e.g., many species from the Linyphiidae and Araneidae family) have adult peaks later in the year. Different soil dwelling harvestmen (e.g., Trogulidae and Nemastomatidae) have adults year-round, but most species (despite a few spring species) have their adult peak in late summer. Therefore, we propose to sample spiders and harvestmen during at least two time periods in the year, i.e., late spring and late summer.

Species living in understorey vegetation are difficult to sample using pitfall traps, therefore suction sampling is often used as a complement. Suction sampling should be carried out at the same time as pitfall captures. A motorized hand-held suction sampler

(e.g., Samu & Sárospataki, 1995) can be used for 60 seconds around each pitfall trap sampling as much microhabitats as possible, e.g., lower branches of trees, forest understory vegetation, tree trunks and terricolous mosses (e.g., Samu et al., 2014).

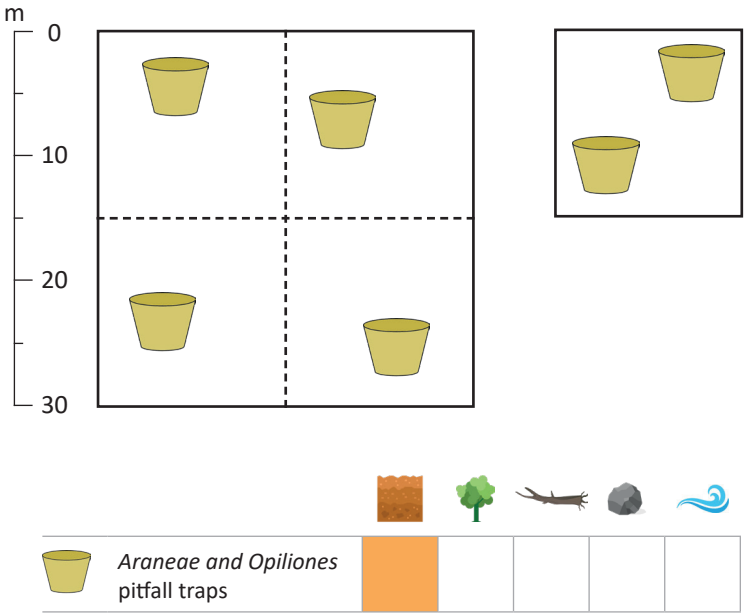

Figure 10. Sampling units (first standard left, second standard right) for *Araneae* and *Opiliones*, and substrates to be sampled (bottom). The pot symbol refers to the sampling of spiders and harvestmen through pitfall traps to be performed in both standards (orange square). The ground is the only sampled substrate.

A spider (*Pardosa saltans*) occurring in woodland edges and clearings.
Photo by: ARABEL - Gilbert Loos

Araneae and Opiliones		
	First Standard	**Second Standard**
Target taxonomic level	Species	Species
Plot shape	Square	Square
Plot size	30x30 m	15x15 m
Type of elements within the plot	Pitfall traps, suction sampling	Pitfall traps
Number of elements	4	2
Element size	-	-
Abundance score	Activity-density	Activity-density
Time needed (min.)	60/plot	15/plot
Number of visits and season	One month sampling between late spring to (late) summer, emptying every two weeks	One month sampling between late spring to (late) summer, emptying every two weeks
Persons needed	2	2
Experts needed	1-2 (depending on taxonomic coverage)	1-2 (depending on taxonomic coverage)
Equipment costs (€)	100-1,000/>1,000 depending on suction method	<100

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Boreal owl (*Aegolius funereus*), a nocturnal raptor which inhabits mainly coniferous forests. Photo by: Matej Ferenčík

BIRDS

REASONS FOR SAMPLING

Birds are among the most sampled taxonomic groups worldwide, with a vast data availability, notably thanks to generalized breeding bird surveys and citizen science (e.g., Jiguet et al., 2012). Birds have generally large vital range and relatively good dispersal abilities, but some species are typical forest species that rely on structural tree features and more generally forest environment (Bouvet et al., 2016; Laiolo et al., 2004; Paillet et al., 2018; Regnery et al., 2013). Some groups (e.g., woodpeckers) even act as ecosystem engineers that modify the environment through their excavating activities and condition the presence of other cavity-dependant species (Cockle et al., 2011). As such, both forest landscape features and local forest structure have an influence on the bird community.

HOW TO SAMPLE

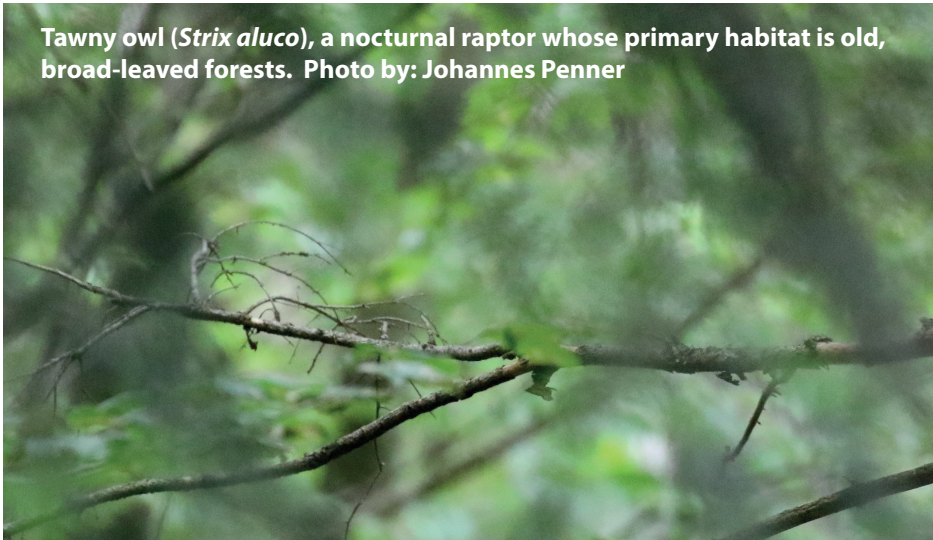
The most classical way to sample birds is by point-counts of a certain time, and this is the approach used in a large majority of previous multi-taxon studies. All birds heard or seen during the amount of time spent on the spot are noted. The index sampled is an activity-abundance estimation that can be translated into a number of individuals using estimates of detection probability.

Breeding bird surveys generally used a point-count of 5 to 20 minutes duration. The sampling starts after a pause of at least 2 minutes after arrival on the sampling plot, so that the animals are accustomed to the presence of the observer. The completeness of the sampling directly depends on the sampling duration, even if it is important to report that most species are detected within five minutes and the number of additional species decreases with durations

(e.g., Leu et al., 2017). The number of visits per year varies from one to 15, but in most cases ranges from two to five. The revisitations allow to cover the community as much as possible by repeated point-counts over the year (i.e., spring birds vs birds more active in the summer). The distance and direction of the sampled individual to the center of the plot may be noted to calibrate detectability models (distance sampling). Noting the distance (eventually by classes, e.g., < 25 m, 25-50 m, >50 m) also allows for selections depending on the purpose of the study.

In some cases, and with the development of acoustic sampling and semi-automatic species determinations, point-counts may involve automatic recorders and ex-post species determinations. Such protocols, as well as those to some species-groups (e.g., transects for woodpeckers), complete the overview of bird sampling methods in multi-taxon studies.

Point counts with a limited duration (e.g., 5 or 10 minutes) are traditionally used in national breeding bird surveys and allowed to incorporate citizen science in massive data acquisition (e.g., Jiguet et al. 2012). This standard is well developed and data are comparable across a wide range of situations. As such, the two standards here presented echo those already spread worldwide.



Tawny owl (*Strix aluco*), a nocturnal raptor whose primary habitat is old, broad-leaved forests. Photo by: Johannes Penner

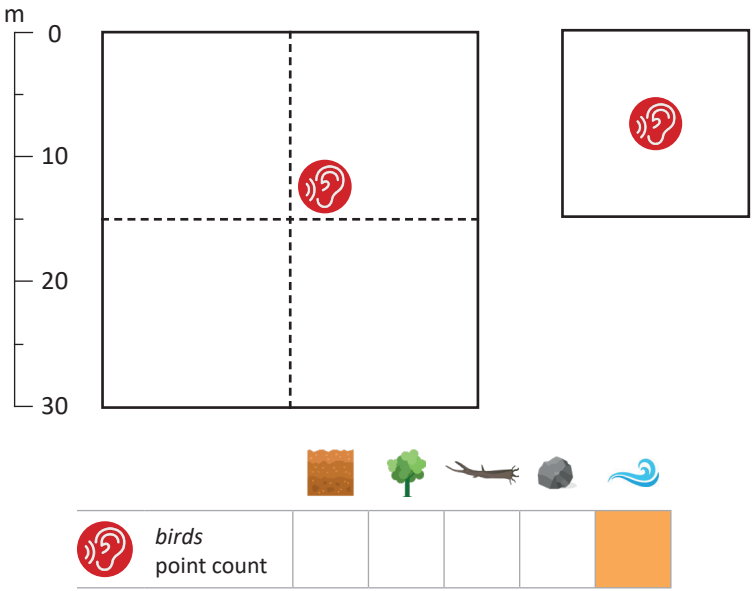


Figure 11. Sampling units (first standard left, second standard right) for birds, and substrates to be sampled (bottom). The ear symbol refers to the sampling of birds through point-counts (i.e. all birds heard or seen in the spot) to be performed in both standards (orange square). The air is the only sampled substrate.

Birds		
	First Standard	**Second Standard**
Target taxonomic level	Species	Species
Plot shape	Circle	Circle
Plot size	Radius up to 100 m, including distance estimation	Up to 100 m
Type of elements within the plot	-	-
Number of elements	-	-
Element size	-	-
Abundance score	Activity-density (including detection probability estimation)	Activity-density
Time needed (min.)	20/plot	5/plot
Number of visits and season	2/year, in spring and summer	2 /year, in spring and summer
Persons needed	1	1
Experts needed	1	1
Equipment costs (€)	<100	<100

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Three-toed woodpecker (*Picoides tridactylus*) feeds on larvae of tree-dwelling insects. Photo by: Daniel Kozák

The Bechstein's bat (*Myotis bechsteinii*) a European forest-dwelling bat species. Photo by: Chris Damant

BATS

REASONS FOR SAMPLING

Bats are highly mobile species that occur in forest ecosystems and nest or roost in tree cavities and hollow trees (e.g., Kalcounis-Rupell et al., 2005; Regnery et al., 2013; Zellweger et al., 2013). Knowledge on their ecology, social behaviour, habitat preferences and relation to forest management and biodiversity-friendly measures remains relatively limited (Basile et al. 2020; Bouvet et al., 2016; Paillet et al., 2018; Regnery et al., 2013). As a mobile group with complex social interactions, they depend on local forest characteristics as well as larger scale - up to landscape - features (Le Roux et al., 2017). The interest to study this group also derives from some forest specialist species of conservation concern.

HOW TO SAMPLE

Bats are recorded by point-counts, using their echolocation calls (heterodyne and time expansion) resulting in an estimate of species activity-density that can be translated into the number of individuals if the species detection probability is known. Manual or automatic ultrasonic detectors associated with a portable recorder were used to a similar extent across previous studies. This approach allows to analyse unknown and unsure heterodyne signals with a dedicated software or other statistical approaches (e.g., deep learning). Bat activity is assessed in terms of number of contacts per minute. A contact is either a single signal or a short sequence of signals over a maximum duration of 5 seconds. Each bat count may be carried out alone or by a team of experienced chiropterologists. Duration of the sampling may vary from 30 to 60 minutes or even more, generally one to three times a year (e.g., April–May, June–July

and August–September) to cover the activity of bats over the year. Recording should occur at sunset on nights with no rain or wind and with temperatures above 5°C. No recording should occur within 5 days of a full moon since moonlight can negatively impact the amount of signalling (Römer et al., 2010).

Point counts from the ground may not cover the whole community of bats since echolocation calls may be targeted and limited to zones above the canopy. A costly way to improve detection of bats is to sample at different heights from ground to canopy (Müller et al., 2013), but this approach multiplies the effort for sampling and for the treatment of all the accumulated data.

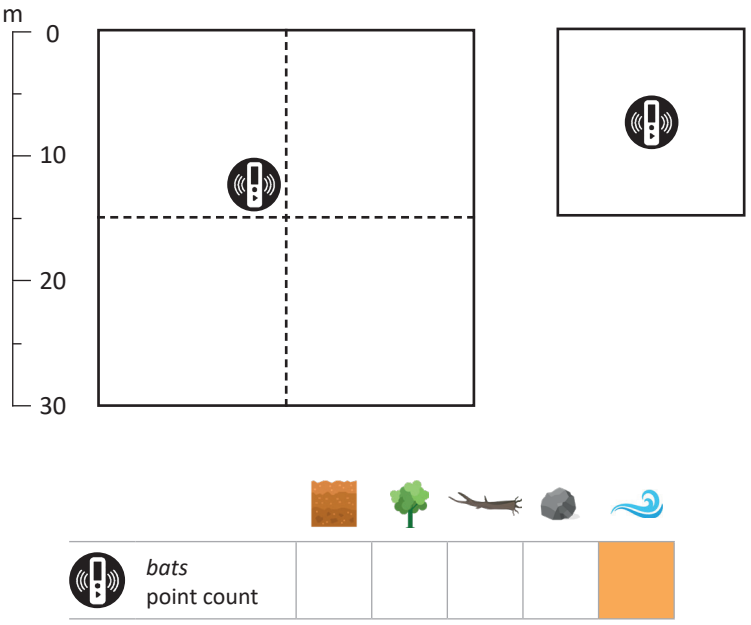

Figure 12. Sampling units (first standard left, second standard right) for bats, and substrates to be sampled (bottom). The portable recorder symbol refers to the sampling of bats through point-counts (with manual or automatic ultrasonic detectors) to be performed in both standards (orange square). The air is the only sampled substrate.

Bats		
	First Standard	Second Standard
Target taxonomic level	Species	Species or genus
Plot shape	Circle	Circle
Plot size	Usually 20-30 m radius, depending on the local cluttering of the vegetation	Usually 20-30 m radius, depending on the local cluttering of the vegetation
Type of nested elements	-	-
Number of elements	-	-
Element size	-	-
Abundance score	Activity-density	Activity-density
Time needed (min.)	45/plot	30/plot
Number of visits and season	3/year, in spring and summer	2/ year, in spring and summer
Persons needed	1	1
Experts needed	1	1
Equipment costs (€)	100-1,000/>1,000	100-1,000/>1,000

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The Barbastelle (*Barbastella barbastellus*) a European forest-dwelling bat species.
Photo by: Chris Damant

Mixed silver fir and beech forest in central Italy.
Photo by: Thomas Campagnaro

FOREST STRUCTURE: LIVING TREES AND DEADWOOD

REASONS FOR SAMPLING

With forest structure we refer to the patterns and relationships of biophysical elements within the forest three-dimensional system. It is the driver and result of ecosystem processes and biological diversity (Gadow et al., 2012). Therefore, knowledge about forest structure is crucial for understanding history, current condition, and future of forest ecosystems (Spies, 1998).

In the handbook, by standing trees we mean living trees, dead standing trees, snags, and stumps (height<1.3 m); while, with lying deadwood we consider fallen logs and branches. Living standing trees are the forest components *par excellence* and, therefore, are essential to describe and understand forest conditions (Hui et al., 2019). A number of parameters sampled from standing trees can be used to directly describe stands (e.g., number of standing alive trees) or to derive indices used in forest management. Deadwood, all woody material that is no longer living, is greatly affected by silviculture practices (Merganičová et al., 2012; Rondeux & Sanchez, 2010), and in turn influences patterns and processes in forests. It is habitat for a variety of wildlife (Lassauce et al., 2011), and can influence natural regeneration dynamics, nutrient cycling and geomorphological processes (Harmon et al., 2004; Müller & Bütler, 2010; Radu, 2006; Stokland et al., 2012).

HOW TO SAMPLE

A wealth of textbooks and reviews focus on field methods for sampling the elements of forest structure (e.g., Hui et al., 2019; Ron-

deux & Sanchez, 2010). However, a synthesis of these methods in studies dealing jointly with forest structure and biodiversity is still lacking. Activities towards the standardization and harmonization of protocols have mostly focused on national forest inventories (Rondeux et al., 2012; Vidal et al., 2016; Winter et al., 2008) or on single forest features (e.g., tree related microhabitats; Larrieu et al., 2018). Analyzing forest structure means making decisions about plot shape, size and sampling strategy (Curtis & Marshall, 2005; Kershaw et al., 2017). Depending on the study objective any combination of these three factors may be selected.

Regarding plot shape, circular plots are the best option to minimize edge length to area relation; they are easy to deploy in the field as only one center coordinate and one radius is needed. However, in large plots, the distance to the center may be difficult to establish if tree density is high and the spatial pattern is not regular. Square plots are relatively easier to establish, but more time consuming as four points need to be correctly located. In general, quadrangular shapes best integrate with remotely-sensed optical data and they could be easier to use for long-term monitoring. However, subjective bias in the selection of edge trees has been observed in squared plots (Paul et al., 2019). If the terrain is steep or irregular, e.g., terraced slopes, decisions on horizontal projection plot must be made. Plot size for structural analyses usually ranges from 0.1 to 1 ha, although small plots as 0.04 ha are also found as subplots within larger ones. Very large forest plots (>1 ha) have been long ago believed to provide highly detailed information on tree communities ecology and demography in the tropics (Condit, 1998), and are also used in temperate forests (e.g., Král et al., 2017; Kraus et al., 2018; Needham et al., 2016). However, increasing plot size will decrease the relative variability of stand structure, but even a single one-hectare plot can be poorly representative of a stand structure (Král et al., 2010).

Sampling strategy ranges from census of all trees and species in fixed-area plots to probabilistic sampling based on tree size in variable-size plots or relascope sampling (i.e., angle count sampling). Fixed-area plots are valid for individual plot and stand level analyses, and a nested approach (i.e., with concentric plots of different sizes) is commonly applied to increase measurement efficiency by reducing effort in measuring high numbers of small trees over large

Beech snag with polypores perennial fruit bodies.
Photo by: Elena Haeler

areas. Variable-size plots provide unbiased estimations at the stand level and they are faster to measure and cheaper. However, tree neighborhood analyses cannot be conducted in these plots.

Deadwood has increasingly received attention in forest structure surveys in the past decades. Field measurements usually focus on coarse woody debris (diameter>10 cm) and seldom on fine woody biomass (diameter<10 cm). Different sampling approaches have been applied for deadwood such as the fixed-area (Gove & Deusen, 2011), line-intersect (Warren & Olsen, 1964; Van Wagner, 1968) and point and transect relascope sampling (Ståhl, 1998; Gove et al., 1999). The optimal dimension or number of transects and plots varies depending on forest conditions but it should be preferred to sample a larger number of small areas or transects rather than few large ones (Nemec & Davis, 2002; Woldendorp et al., 2004; Korboulewsky et al. 2021). When coarse woody debris is extremely scarce, a nested scheme for fine woody debris may be applied (Korboulewsky et al., 2021).

Field activities include measurements of diameters, heights, lengths, and decay classes depending on whether standing or lying elements are considered. Diameter thresholds are commonly set but there is a large variability: inclusive approaches do not apply any threshold but most studies use a 5-10 cm threshold, although those used in typical forest inventories are generally higher. The height of living trees is not always measured for each tree, but for a proportion of trees. Diameter at breast height and height are commonly measured for standing dead trees and snags; whereas, the diameter at the top section and height are usually recorded for stumps. Total length and the diameter intersecting the line transect are measured for fallen logs and branches (lying deadwood). Here, we recommend assigning tree vitality and deadwood decay classes for each sampled woody element, following respectively a three or five/six stage classification (Kraft, 1884; Maser et al., 1979; Nieuwenhuius, 2000).

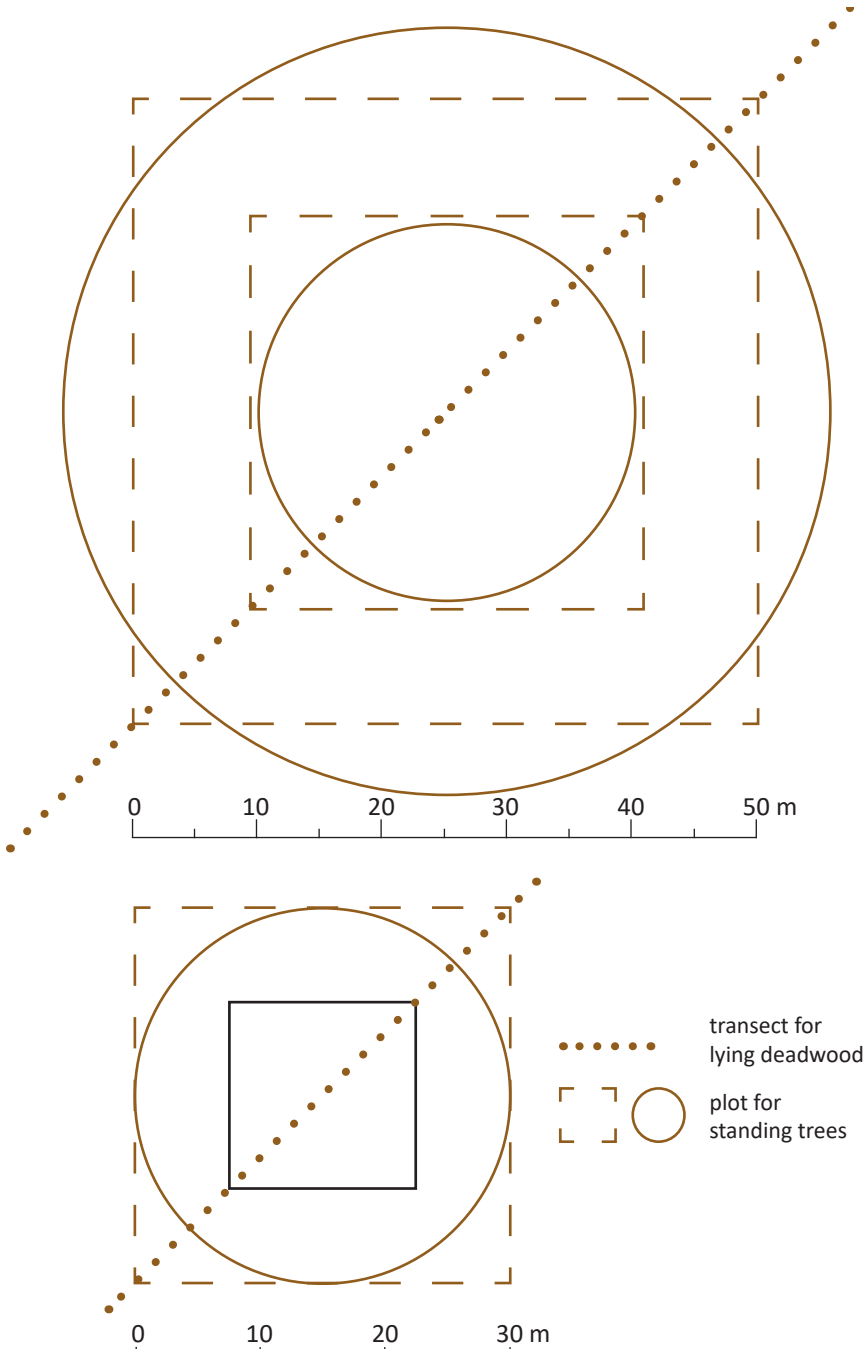

Figure 13. Sampling units (first standard top, second standard bottom) for forest structure (living trees and deadwood). The sampling unit shape can be chosen on the basis of forest stand characteristics and study objectives.

Standing trees (*)		
	First Standard	**Second Standard**
Target taxonomic level	Species	Species
Plot shape	Circular or square	Circular or square
Main plot size	2826 m2 (30 m radius) or 2500 m2 (50x50 m)	706.5 m2 (15 m radius) or 900 m2 (30x30 m)
Nested plot size	706.5 m2 (15 m radius) or 900 m2 (30x30 m)	-
Diameter threshold (main plot)/(nested plot)	>10 cm/ >5 cm	>5 cm
Height/length	All	30% of standing trees
Time needed (min.)	90-120/plot	60-90/plot
Number of visits and season	1/year, spring or summer for deciduous forest stands, for conifer stands better before the onset of the growing season or after	1/year, spring or summer for deciduous forest stands, for conifer stands better before the onset of the growing season or after
Persons needed	2	2
Experts needed	1	1
Equipment costs (€)	100-1,000/>1,000 depending on the method for height measurement	100-1,000/>1,000 depending on the method for height measurement

* When applying the first standard, it will be important to record which are the trees with a threshold diameter >10 cm sampled in the nested plot (706.5 m2 or 900 m2) to permit a consistent comparison with the second standard.

Lying deadwood		
	First Standard	**Second Standard**
Target taxonomic level	Species	Species
Plot shape	Line transect	Line transect
Main plot size	2 transects of 50 m length	1 transects of 50 m length
Nested plot size	-	-
Diameter threshold (main plot)/(nested plot)	>5 cm	>10 cm
Height/length	All >1 m intersecting the transect	All >1 m intersecting the transect
Time needed (min.)	60-90/plot	45-60/plot
Number of visits and season	1/year, spring or summer for deciduous forest stands, for conifer stands better before the onset of the growing season or after	1/year, spring or summer for deciduous forest stands, for conifer stands better before the onset of the growing season or after
Persons needed	2	2
Experts needed	1	1
Equipment costs (€)	100-1,000	100-1,000

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Scots pine forest in southern Finland.
Photo by: Tommaso Sitzia

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4. GLOSSARY

Alpha diversity: refers to the diversity within a particular habitat (Whittaker 1977) usually expressed by species richness (number of taxonomic groups), evenness (distribution of abundances of the groups), or both.

Beta diversity: the use of the term beta diversity has changed through time from a relatively abstract concept to a measure to be used in several research applications. First discussed by Whittaker (1960) as "The extent of change in community composition, or degree of community differentiation, in relation to a complex-gradient of environment, or a pattern of environments" and then as the among-habitat differentiation in a landscape (Whittaker 1977), with recent development and clarifications of this concept (Tuomisto 2010).

Biodiversity indicator: any of the indicators belonging to the criterion C4 (Maintenance, Conservation and Appropriate Enhancement of Biological Diversity in Forest Ecosystems) for the assessment of Sustainable Forest Management (FOREST EUROPE 2020).

Biodiversity proxy: a single or several taxonomic groups which are used as an indirect measure for the approximation of the whole biodiversity.

Bryophytes: 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 ecophysiological structure (Goffinet & Shaw, 2009).

Cross-taxon analysis: a comprehensive analysis which includes several taxonomic groups.

Cross-taxon congruence: spatial covariation of diversity patterns of different taxa

Data platform: repository for the storage of data.

Dataset: a homogeneous range of data sampled through the same protocols by a given research group.

Deadwood decay stage: a number which represents any stage of decay of non-living woody biomass (not litter) defined by applying one of the several extant classification systems.

Epiphytic organisms: organisms growing above the ground, supported non parasitically by another plant or object.

Epixylic organisms: organisms living on decaying wood.

Forest inventory: the systematic collection of data on the forestry resources within a given area. It allows assessment of the current status and lays the ground for analysis and planning (FAO 2020).

Forest type: a category of forest defined by its composition, and/or site factors (locality).

Handbook: a manual which is intended to provide ready reference covering a particular subject.

Harmonization: the process aimed to combine data from different sources and improve the comparability of variables from separate studies, reducing study heterogeneity.

Harvestmen: any arachnid of the order Opiliones (or Phalangida), having a small rounded body and very long thin legs.

Home range: area traversed by the individual in its normal activities of food gathering, mating and parental care (Burt 1943).

Lichens: plantlike organisms that consist of a symbiotic association of algae (or cyanobacteria) and fungi.

Line Intercept Sampling: sampling method aimed at the estimation of lying wood volume, first described by Van Wagner (1968), which consists in recording the diameter of every piece of wood intersected in a line of known length.

Lying deadwood: deadwood fallen on the ground, it does not include stumps.

Meta analysis: examination of comparable data from a number of independent studies.

Multi-taxon: a dataset that includes a minimum of three taxonomic groups representing the animal kingdom and at least one of the kingdoms of plants and fungi (i.e., fungi or lichens).

Multi-taxon biodiversity: any application of the biodiversity definition when including several taxonomic groups. In forest ecology, the importance of considering multiple taxonomic groups lies in the different influence that each taxon has on many ecological processes.

Nested design: a research design in which levels of one factor are hierarchically organized as within levels of another factor.

Open science: an approach to the scientific process that focuses on spreading knowledge as soon as it is available using digital and collaborative technology.

Pan-European Region: includes Eastern Europe, Caucasus and Central Asia (EECCA), South Eastern Europe (SEE), as well as Western and Central Europe (WCE) (EEA 2006).

Plot: concretely delimited forest area as part of a fieldwork to which sampling units for one or more taxon groups are referred, and of which geographical coordinates are known. This is the elementary unit of structural, environmental and taxon data collection.

Pseudoreplication: pseudoreplication happens when the conditions to have a true replication are not met. A true replication is defined as taking multiple independent samples from a particular location, whereas each sample is located sufficiently far away from the other (the exact distance depends on biological process).

Sample grain: the size of the elementary sampling unit (Burrascano et al. 2018).

Sampling extent: the geographical area included in the survey (Burrascano et al. 2018).

Sampling protocol: any procedure used to select units from the study population to be measured. The goal of the sampling protocol is to select units that are representative of the study population with respect to the attribute(s) of interest. The sampling protocol deals with how and when the units are selected and how many units are selected.

Saproxylic organisms: organisms dependent on dead wood to complete their life cycle.

Sessile: permanently attached to a substratum.

Silviculture: the science of controlling the composition, structure, and dynamics of forests.

Site: homogeneous geographical area across which different management systems or developmental stages may occur. Within each site data are collected in one or more plots or stands.

Stand: specific forest area, which is sufficiently uniform in species composition, age distribution, and condition as to be distinguishable from the forest on adjoining areas. It represents the unit for which the same silvicultural management is prescribed (Van Laar & Akça 1997).

Stand structure: the spatial arrangement of the various components of the forest ecosystem (McElhinny et al. 2005) including biotic, e.g., trees, and abiotic elements, such as soils and streams.

Standing trees: living and dead trees or part of trees (snags and stumps) that have not fallen on the ground.

Structural attributes: include measures of abundance, relative abundance, richness and size variation related to forest ecosystem components (McElhinny et al. 2005).

Sustainable development: defined as development that meets the needs of the present without compromising the ability of future generations to meet their own needs.

Sustainable forest management: a dynamic and evolving concept, which aims to maintain and enhance the economic, social and environmental values of all types of forests, for the benefit of present and future generations (FAO 2020).

Symbiotic relationship: a close ecological relationship between the individuals of two (or more) different species, which may benefit both species, only one species at the other's expense, or neither species.

Taxonomic group: a unit (or taxon) of any rank (Domain, Kingdom, Phylum, Class, Order, Family, Genus, and Species) designating a group of organisms.

Taxonomic identification: The recognition of the essential characteristics of an organism which can be used to assess if the organism belongs to a defined taxon.

Tree Related Microhabitat: A Tree related Microhabitat (TreM) is a distinct, well delineated structure occurring on living or standing dead trees, that constitutes a particular and essential substrates or life site for species or species communities during at least a part of their life cycle to develop, feed, shelter or breed (Larrieu et al. 2018).

Tree vitality: vitality has been considered as the tree's ability to grow under the condition the system finds itself (Shigo, 1991). However, tree vitality is a theoretical concept that cannot be directly measured, which is why it has been commonly described using tree health indicators.

Trophic network: a network of organisms in an ecological community that are linked to each other through the transfer of energy and nutrients.

Vagile: able to move freely.

Vascular plants: also called tracheophytes, refer to plants that have specialized conducting systems which include xylem and phloem.

Silvo-pastoral landscape in central Italy dominated by beech with scattered conifer plantations. Photo by: Giovanni Trentanovi

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Sustainable Forest Management (SFM) is crucial for biodiversity conservation. Although it should be assessed by monitoring the diversity of multiple taxonomic groups, 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 through heterogeneous sampling approaches that hamper the identification of general trends, and the broad-scale inference for designing SFM.

The COST Action BOTTOMS-UP (CA18207) established a network of researchers involved in 41 projects on European forest multi-taxon biodiversity across 13 European countries.

We provide an overview of the sampling approaches to multi-taxon biodiversity, standing trees and deadwood in the form of an operational handbook for nine different taxonomic groups and for the sampling of standing trees and lying deadwood. For each of these forest components, we provide two standards that differ in spatial scale and effort, and give specific instructions for the comparability across standards, taxonomic groups and studies.

This handbook derives from an effort of networking and synthesis and represents a pragmatic synthesis and an important step forward to direct monitoring of forest biodiversity, in Europe and elsewhere.

ISBN 978-88-31222-50-1