**A handbook for forest multi-taxon and structure sampling**

This handbook provides two standards for sampling forest multi-taxon biodiversity and structure.

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 (*How to sample?)*.

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 much 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 any 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.



Fig. SI3-01. *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. Sampling substrates for each taxonomic group (c) are represented in yellow and orange for the first and second standard respectively.*

**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)](https://www.zotero.org/google-docs/?I4nVCy). 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)](https://www.zotero.org/google-docs/?Lbp2iX) 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 other functionally important species [(Landuyt et al., 2019)](https://www.zotero.org/google-docs/?neB1lQ).

Moreover, vascular plants are one of 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 were 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 1,000 m2, even if this range is widened up to 1-20,000 m2 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 previous 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 m2, 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 the after data processing only one abundance value per plot will be sufficient. This would 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)](https://www.zotero.org/google-docs/?bphfII). 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 ⲁ-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.

|  |  |
| --- | --- |
|  | **Vascular plants** |
|   | **First Standard** | **Second Standard** |
| **Target taxonomic level** | Species/subspecies | Species/species aggregate |
| **Plot shape** | Square | Square |
| **Plot size** | 30x30 m (900 m2) | 15x15 m (225 m2) |
| **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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## Lichens

## *Reasons for sampling*

Lichens represent an ecologically defined group of biotrophic fungi associated with algae or cyanobacteria that enable an autotrophic lifestyle. 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). They are largely used to verify the sustainability of forest management (Brunialti et al., 2020; Moning et al., 2009; Nascimbene et al., 2013).

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## *How to sample*

Most multi-taxon studies mainly focus on epiphytic lichens, and in few cases extend to those colonizing deadwoods. 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 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)](https://www.zotero.org/google-docs/?flreeD). 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.

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| --- | --- |
|  | **Lichens** |
|   | **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) -> rocksgrid (5 quadrats) -> living treesgrid (9 quadrats) -> deadwood | grid (25 quadrats) -> soilgrid (25 quadrats) -> rocksgrid (5 quadrats) -> living treesgrid (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 and 3 living trees |
| **Element size** | 50x50 cm -> soil 50x50 cm -> rocks10x50 cm -> living trees30x30 cm -> deadwood | 50x50 cm -> soil50x50 cm -> rocks10x50 cm -> living trees30x30 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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## 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).

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 deciduous forests because of the inhibitory effect of deciduous 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 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 bryophytes 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.

|  |  |
| --- | --- |
|  | **Bryophytes** |
|   | **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) -> rocksgrid (5 quadrats) -> living treesgrid (9 quadrats) -> deadwood | grid (25 quadrats) -> soilgrid (25 quadrats) -> rocksgrid (5 quadrats) -> living treesgrid (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 -> rocks10x50 cm -> living trees30x30 cm -> deadwood | 50x50 cm -> soil50x50 cm -> rocks10x50 cm -> living trees30x30 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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**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)](https://www.zotero.org/google-docs/?smTfDd).

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).

*How to sample*

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, 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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## 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). Carabid and 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 given that the vast majority of the beetles are 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 management).

WT capture individuals that are intercepted during the flight by a vertical obstacle (on hitting the obstacle, the individual falls into a funnel 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 hanged 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 hanged 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).

|  |  |
| --- | --- |
|  | **Carabid beetles** |
|  | **First Standard** | **Second Standard** |
| **Target taxonomic level** | Species | Species or genus |
| **Plot shape** | Circular or square | Circular or square |
| **Plot size** | 2826 m2 (30 m radius) or 2500 m2 (50x50 m) | 706.5 m2 (15 m radius) or 900 m2 (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 m2 (30 m radius) or 2500 m2 (50x50 m) | 706.5 m2 (15 m radius) or 900 m2 (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 deadwood 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 |

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### Araneae and Opiliones

### *Reasons for sampling*

Spiders (Araneae) and harvestmen (Opiliones) are the largest group of 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 with pitfall traps, which is 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 harvestman 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 m². Pitfall traps can be placed in a row or a square spaced two to five m apart. It is recommended to empty the traps after fourteen days and refill them with trapping fluid for another fourteen days. In this way it is possible to account for bad weather events in a fourteen-day period (extremely wet or dry). Ideally, individuals of the different traps are treated separately, but for processing efficiency, the catch of a plot 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).

|  |  |
| --- | --- |
|  | **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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## Birds

### *Reasons for sampling*

Birds are among the most sampled taxonomic groups worldwide, with a vast data availability, notably thanks to the 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 min. duration. The sampling starts after a pause of at least 2 min. after arrival on the sampling spot, 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 5 min., 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 min.) 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.

|  |  |
| --- | --- |
|  | **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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## 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 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 min. 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.

|  |  |
| --- | --- |
|  | **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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## 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 geomorphological processes, nutrient cycling and natural regeneration dynamics (Harmon et al., 2004; Müller & Bütler, 2010; Radu, 2006; Stokland et al., 2012).

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### *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; Rondeux & 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 feature (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 a center coordinate and a 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 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).

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|   | **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, all season for conifer stands | 1/year, spring or summer for deciduous forest stands, all season for conifer stands |
| **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.

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|   | **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, all seasons for conifer stands | 1/year, spring or summer for deciduous forest stands, all seasons for conifer stands |
| **Persons needed** | 2 | 2 |
| **Experts needed** | 1 | 1 |
| **Equipment costs (€)** | 100-1,000 | 100-1,000 |

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