PASTFORWARD
Sampling Protocols 2015-2016

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SCOPE

This sampling protocol aims at providing a consistent methodology to collect harmonized, high-quality and comparable vegetation resurvey data at the visited study regions within the PASTFORWARD project. Harmonization of procedures is essential to enhance comparability of vegetation data between the different sites that will be revisited in the Spring & Summer of 2015 & 2016. The protocol includes details on (i) the plot relocation and evaluation of general stand and plot characteristics as well as the plot-specific sampling of (ii) vegetation resurvey data, (iii) data characterizing site conditions, (iv) data characterizing present and past stand composition and (v) data characterizing present and past availability of key resources in and productivity of the understorey. Finally, part (vi) will summarize the protocol per plot in a chronological way.

The measurements and analyses of the collected data will allow gaining a more in-depth and process-based understanding of the (interactive) impacts of the main drivers of global change in forests (i.e. climate warming, land-use change & atmospheric deposition) on the herb layer communities under study. This is the final goal of the observatory part of PASTFORWARD.

1. PLOT RELOCATION

Plots will be relocated as detailed as possible by making use of several supports (depending on the availability per study region) including

(i) GPS coordinates of the plots,
(ii) descriptive maps and notes from previous data collectors
(iii) potential markers that remained from earlier surveys (i.e. for permanent plots) and
(iv) previous vegetation surveys.

When the plot has been relocated as accurately as possible, we will first mark the **original plot** used in the first survey, in order to perform a vegetation survey that can be exactly compared over time (see part 2). However, since we want to be able to make spatial comparisons in vegetation composition as well, we will standardize the measurement protocol by setting out standard plots of **10x10 m²**, nested in a larger **20x20 m²** plot in all visited plots (**figure 1**). The centre of the original plot should remain the centre of both standardized plots and we will try to put as much of the original plot surface into the standard plot sizes. How the original plots will be marked in the field, depends on whether they are semi-permanent or permanent and whether it is still necessary to mark them with measuring tape and poles, or if they are clear enough in the field already (by means of dotted trees for example). The standard plots will be set out using measuring tapes and a prisma to create right angles. The 20x20 plot will only be used for determining basal area (see part 4) and choosing the dominant trees and age trees to core, whereas the 10x10 plot will be used for a vegetation survey, as well as humus and soil sampling, herb biomass sampling, densitometer measurements and herb shoot length measurements. Before starting the measurements, GPS coordinates of the plot centre are logged.

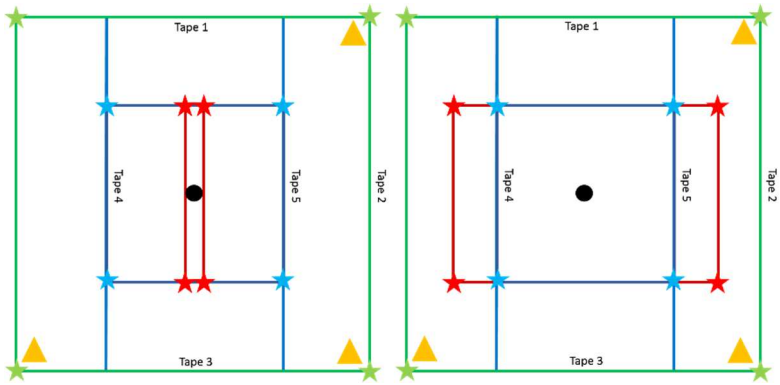

Figure 1. Two examples of the three plot sizes to be used during sampling: the original plot size in *red*, standard plot 10x10 m² in *blue*, standard plot 20x20 m² in *green*. Black dots represent the plot centre, stars indicate where poles are placed (plot corners), triangles indicate where a prisma is used for right angles and measuring tapes (1->5) are put down on the ground for clear visualization of the plots during sampling. **Left**: original plot < 10x10 plot; **Right**: original plot > 10x10 plot.

Before starting with the vegetation resurvey, general **plot** characteristics are written down including the **centre** GPS **coordinates**, **date**, **surveyors** and **writers'** names, **relocation possibility** or difficulties, **accessibility** (distance from other land cover), **original plot size**, **slope** (flat: <5%/ca.3°; medium: 5-15%/ca.3-9°; steep: >15%/ca.9°), **altitude** and **exposition** (main slope : N/E/S/W?) of the plot.

Also in this phase, a general **plot** description is written down, a visual assessment of **potential stand development** is performed (based on the plot situation!) and several **management** variables are assessed. The plot description includes noting shortly which main species and layers can be distinguished in the tree and shrub layer and noting remarkable things (e.g. "large regenerating groups of beech"). Potential stand development means writing down what seems to us a plausible development of the stand based on the plot situation, e.g. "probably former coppice history, now left unmanaged"). This can be based on a number of things that we see in the field and is not necessary the correct history but can help with the historical reconstruction in a later stage of PASTFORWARD. Finally, assessment of forest management in the field is done by describing the following characteristics:

(i) **stand type** (even-aged vs uneven-aged) [**stand**-level]
(ii) **stand form** (uniform vs non-uniform) [**stand**-level]

(iii) **stand species** (monoculture vs stemwise or groupwise mixture) [**stand**-level]
(iv) degree of **canopy closure** (scoring in %) [**plot**-level 20x20 m²]
(v) **stand vitality** (bad (many dominant species are dead or show disease symptoms); medium (some individuals around are dead or show disease symptoms but the main stand vitality of dominant species is good); good (no signs of dead or diseased specimens) [**stand**-level]
(vi) **number of stumps** (note if multi-stemmed) is counted (DBH>7.5) [**plot**-level 20x20 m²]
(vii) presence of **driving tracks** is given a score from 0 (no visible tracks) to 1 (some signs of tracks but very shallow) to 3 (obvious, recent tracks and destruction of biomass visible) [**plot**-level 20x20 m²]
(viii) standing and lying **dead wood** is counted: all present dead trees in the plot with DBH>10cm and with the stem base (if standing) or the stem itself (if lying) lies within plot borders.
(ix) visible **signs of natural disturbances** are noted: drought, storm, disease (e.g. ash dieback; dutch elm disease), browsing and grazing damage [plot-level 20x20 m²]
Some info on specific diseases occurring:
-Ash dieback (*Chalara fraxinea)*: symptoms are leaf loss & crown dieback, starting from the outside mostly -> can occur with Ash trees (Fraxinus excelsior)
-Dutch elm disease (DED) spread by the elm bark beetle: symptoms are earlier withering of leaves in summer, dieback of branches, ultimately leading to root dieback -> can occur with Elm trees

Finally, some **overview photos** are taken that are representative of the general plot situation.

Concerning duration: we expect at least **30'** per plot are needed for this first part of the fieldwork.

2. VEGETATION RESURVEY [original plot & 10x10 plot]

= description of vegetation composition at plot-level

A. SAMPLING

A first vegetation survey will be performed in the **original plot**, following the definitions of **tree, shrub and herb layer** as used from the **first, earliest survey**. We will always start with the tree layer and work down to the herb layer. Before estimating species cover, we will walk systematically through the plot to be sure we identified all present species in the forest layers. Therefore, we start in one corner and walk along the plot border lines, after which we restart in the same corner to walk both diagonal lines.

Then, per forest layer, the area covered by each species (i.e. species cover) is estimated visually as percentage of the total (plot) area. Two methods can be used for this estimation: first, estimating whether pushing all present individuals of a species towards a corner of the plot would cover about 1/4th, 1/2nd, 3/4th (etc.) of the plot area and second, estimating the area covered by a certain species, and divide this by the total plot area.

The **moss, litter and bare soil layer** is also assessed, but here, only total coverage is estimated and no species-specific cover values (for the moss).

When species are not (easily) identifiable, they are put in a field **herbarium** for later determination and are given a general name and number: U_plotnr_x. This code is used in the sampling sheet instead of species name until determination is certain.

Vegetation surveys are performed according to the **horizontal projection** method: everything that is horizontally projected inside the plot surface, is surveyed. This means that when a tree or shrub has its trunk inside the plot area, but part of the crown projection outside the plot area, only the part of the projection of the crown within the plot area should be estimated for species cover. Similarly, trees outside the plot area with parts of their crown projections inside the plot area should also be assessed, but only the projections inside. The same accounts for herb species.

For this first vegetation survey, the definitions of tree, shrub and herb layer will of course vary per region since they were surveyed by different people per region and had nothing in common with each other at that time. For the 2015 campaign, an overview of the **definitions and plot sizes used in earlier surveys** is given in the table hereunder. T = tree layer, S = shrub layer, H = herb layer.

Study region	Size orig plots	Layers definition (T, S, H), scale, remarks
1. Wytham (UK)	100 m² (10x10)	- T: Estimate of cover in height band **over 2.5m** across diagonal, split by species. Estimated along diagonal of 10 x 10m plot; >2.5 m height (so fairly shrubby!) - S=H (!): **Up to 1m**, including scramblers such as Rubus, Rosa, Solanum dulcamara, Lonicera, Ribes, but excluding taller shrubs such a Ligustrum, Viburnum, Euonymus and seedlings of woody species. 1974 presence absence for most species, plus frequency in subplots across diagonals; score 1 present in plot, plus one for each of thirteen 0.1 sub plot across diagonals - Presence/absence within the 10x10 plots - Frequency measurements in 14 subplots across diagonals: the number of circlets in which the species was rooted was measured - dominance scores for the species cover were measured in 1991, 1999 and 2011, but not in 1974
2. Warburg (UK)	? email	?
3. Lyons-la-foret (FR)	? email	
4. Tournibus (BE)	100 m² (10x10)	T: **>7m** height; S: **1-7m** height; H: **<1m** height (incl. all ferns/bramble) Braun Blanquet scale old and new surveys [+=0.5,1=3,2=15,3=37.5,4=62.5,5=87.5]

5. Binnen-VL (BE)	150 m² (10x15)	T: all woodies in the **top canopy**; S: all woody species **>1m** height and not in the canopy (i.e. subcanopy); H: **<1m** height Londo scale old and new surveys (see forestreplot sheet)
6. Prignitz (DL)	Between 15x15 m (=225 m²), 15x20m (=300m²) or in less cases even 20x20m (=400m²). Very rare cases only 10x10m (=100m²)	I used no fixed height to define herb, shrub and tree layer. The tallest herb species (even if it was 1,3m of height) was used to define as herb layer all species not higher than 1,3m. Shrub and tree layer (and first and a second if present) were defined stand specific; if a clear structure of three woody layers occurred than I take the real heights of each. Thus, in the first case it can be that shrubs reaches only a height of 3 to 5 m in another of 8 to 12 m, while in the first case second tree layer reaches 8-12 m but in the second 15-18, and in the first case the first tree layer (because it is a medium old stand of about 80-90 yrs) may be only 18-20m, while in the second of 22-25 m (visually estimated!). First stand: shrub layer: 3-5m; 2. Tree layer: 8-12m and 1. Tree layer: 18-20m, and second stand: shrub layer: 8-12m (very old corylus avellana for example); 2. Tree layer: 15-18m and 1. Tree layer: 22-25m – ok? Cover was estimated over the whole plot using the scale of Barkman, Doing and Segal (1964). Do you know it? However, some species occurring as small patches have been estimated as follow: looking for a representative patch – estimating its size – counting number of patches and multiply it with estimated size of the representative patch.
7. Brandenburg (Uckermark) (DL)	? email	
8. Göttingen (DL)	100 m² (10x10)	T: all woody species with a height > 5 m; S: all woody species between 0.5m and 5m, including Rubus species; H: all species (also woody, Rubus species) < 0.5m height, herbs, grasses and ferns were always considered as 'herbs' (even if > 0.5m); M: all moss species on mineral soils Braun Blanquet scale old and new surveys
9. Mramor (CZ)	? email	
10. Devin Wood (CZ)	??	T: all individuals of woody species taller than ca. 4 m (actually merged from Ti (emergent tree layer), Tii (main tree layer), Tiii (lower tree layer)); S: all individuals of woody species taller than ca. 1 m not exceeding ca. 4 m; H: individuals of herbaceous species of any height Scale for old relevés: Zlatník; scale for new relevés: Braun-Blanquet; transformed into median percentage values.
11. SLK1 (SLK)	? email	
12. SLK2 (SLK)	? email	

After the first, a second vegetation survey will be performed, now in the **standard 10x10** plot and using our **own definitions** of tree, shrub and herb layer for all plots and regions to be able to make spatial comparisons. These are the following:

- **Tree layer cover** (T: % of sampling area (total and species-specific))

The tree layer (T) includes all tree species **taller than 7 m**.

- **Shrub layer cover** (S: % of sampling area (total and species-specific))

The shrub layer (S) includes all woody species with a height **between 1 and 7 m**.

Note:

-Depending on the species of course, some flexibility is required for the height boundaries. For example, *Corylus avellana* is consistently put in the shrub layer although reaching heights > 7 m since this remains a shrub and never grows into a tree, whereas *Sorbus aucuparia* may occur as tree or shrub and can grow between 5-15 m high. In this

case, putting *S. aucuparia* in the shrub or tree layer depends on its structural appearance in the plot. The general rule of thump for difficult species is based on logical interpretation in the field and knowing the species' habitus and structural function.

- **Herb layer cover** (H: % of sampling area (total and species-specific))

The herb layer (H) includes all vascular plant species (i.e. woody (e.g. *Rubus* spec) and non-woody (e.g. *Anemone nemorosa*) with a height **≤ 1 m**.

Notes:

-Several herb species (e.g. *Urtica spec, Rubus spec, Pteridium aquilinum, even some grasses)* may grow larger than 1 m and into the theoretical shrub layer, however they should always be put in the herb layer since their structural function remains in the understorey of forests and not in the shrub layer.

-Other difficulties may arise with species such as *Vaccinimu myrtilis* and *Calluna vulgaris*, but the same rule applies here: these species structurally appear as part of the herb layer, hence should be put in this layer, even if they grow above the 1 m height boundary.

-In case of a creeper on the ground (e.g. *Hedera helix),* there is no problem (herb layer), but in case of a creeper growing on a tree, it is put in a separate layer called "**Lianas**". We expect these will be rare situations, hence we will just mark these species under the herb layer and put "liana" as remark so we can create this layer afterwards.

-Saplings and seedlings of trees will be put in the appropriate layer, depending on their height. For example, a group of regenerating *Ash* trees of only 0.5 m tall, will be put in the herb layer, whereas *Ash* saplings of about 2 m tall are put in the shrub layer.

Besides these three layers, cover of the following layers are also estimated per plot:

- **Moss layer cover** (M: % of sampling area (total))

The moss layer (M) includes bryophytes and lichens. No individual species are identified.

- **Litter layer cover** (L: % of sampling area (total))

The litter layer (L) is the top layer of the soil and consists of dead organic material such as leaves, bark, needles and twigs that have fallen on the ground. Decomposition of this material takes place in the litter layer.

- **Bare soil cover** (B: % of sampling area (total))

The bare soil layer (B) comprises the soil not covered by herbs, shrubs, trees, moss or litter.

B. SAMPLING EQUIPMENT

-Data sheets and writing equipment
-GPS
-Necessary maps and notes
-Small herbarium press for species that could not be identified in the field
-Plant floras & photo guides
-Poles to delimit plot corners
-Camera for overview photo (+spare batteries)
-Measuring tapes (for the 10x10 and 20x20 plots)
-Prisma

C. DURATION & TIME OF SAMPLING

We should take in consideration that the understory vegetation contains both spring- and summer-flowering species. Spring-flowering communities are dominated by vernal species, whilst grasses, sedges and rushes rather dominate summer-flowering communities. Because of these phenological differences, we will determine an optimal sampling time per region, based on a latitudinal gradient, to cover both communities. We expect **1h30' per plot** is definitely needed for the vegetation resurvey part, performing 2 surveys (in original plot and standard 10x10 plot).

3. SITE CONDITIONS [10x10 plot]

= description of soil and humus characteristics

A. SAMPLING

Site conditions will be determined by a detailed quantification and chemical description of the (i) **humus layer** as well as a detailed (ii) morphological, (iii) physical and (iv) chemical description of the **soil**. All the following will be done in the **10x10 m²** plots.

- **Humus layer (organic)**

First, to characterize the **humus type** in the field, humus will be classified according to the Jabiol key (1995). However, besides a visual estimation, biomass and chemical composition (N, P, Al, base cations) of the humus layer will be quantified later in the lab. To achieve this, in each plot, samples will be taken of the ectorganic horizons (**OL** and **OF-OH**). The OF and OH layer are sampled together, since it is very difficult to distinguish between these two. This will be done as a mixed-humus-sample: a subset of 2 locations where soil is to be sampled will be used to sample humus as well, and both samples will be kept separately (**figure 2**). For this, a 20x20 cm² wooden frame (or 25x25 cm², depending on availability) is put on the ground on each location. Before sampling, the ground vegetation present above the litter and humus layer should be removed with a garden shear. The ectorganic horizons are then carefully removed from within the frame (using a knife/trowel for the borders) and put in a labelled plastic pot of 250 mL or in a ziplock bag if there is too much litter for a plastic pot. After the samples are taken, we can assess the **depth** of the two layers OL and OF-OH.

Notes:

-Since taking full samples from the two locations may sometimes comprise a large volume and weight, we will only take a **subsample** (1 full plastic pot) home with us from both OL and OF-OH, but weigh the full and subsamples of OL and OF-OH after each field day, calculating the results afterwards back to the original sample.

-It is very important here to sample the three organic layers and not the mineral soil. The three layers (OL, OF and OH) can be distinguished as follows: OL is the litter layer, and will mostly be the smallest part, that consists of larger pieces that are not yet degraded (still fully intact). OF is the next layer, where you can still distinguish different pieces, but these pieces have started to degrade. OH is an amorphous layer, where you can no longer distinguish different pieces.

- **Soil (mineral)**

Second, to achieve a **morphological** description of the soil, a **soil profile description** is performed. To do this, the soil profile is sampled by means of a soil auger, going to a depth of 5**0 cm**. After the soil auger is pulled out of the soil, the surface is leveled out with a knife. Measuring tape is held next to the sample, and a photograph is made in order to double check later if necessary. Diagnostic horizons (O, A, B, E, C, R) are identified, as well is their thickness (mm) and depth of occurrence (cm) noted. Other important characteristics to describe per horizon are clear signs of waterlogging or reduction (i.e. gley) and illuviation and eluviation of specific materials (aluminum, silica, gypsum etc.).
Meanwhile, to achieve a **physical** description of the soil, **soil texture** is determined. This is a measure of the relative proportion of the various soil particle size fractions (clay, silt, sand) in soil. First, a quick and **visual assessment** is made in the field by means of the finger test and based on the FAO soil texture triangle (Appendix 2). For this test, you take a sample of mineral soil in the palm of your hand, after removing the >2 mm fraction and evaluate several features when touching/kneading it. Just rough estimates are given due to the nature of this technique ("mainly" sand, silt or clay or some combination of two if it remains uncertain). Therefore, a detailed **lab-based analysis** of soil texture will also be required. The top soil samples collected as described in the next paragraph can be used for this. These analyses will be performed by the Institute of Forest and Nature Research (INBO).

Finally, to achieve a **chemical** description of the soil, samples at various soil depths are collected in order to derive variables as pH, SOM, SNC, P (N & P: see §4), base cations (CEC) and Al concentrations. That is, after removal of the organic soil layers, mixed-soil-samples at **[0-10] (2x**: 1 kept fresh in the freezer and 1 dried), **[10-20]** and **[20-30]** cm are collected with the soil auger and stored in separate pots per interval (subsamples are taken along

the diagonals: **figure_2**). Also important: the [0-10] **fresh** topsoil sample will be collected in twofold, one to be dried immediately vs. one to be frozen until "fresh" analyses are performed on them. The fresh samples will be collected in ziplock bags, to facilitate storage and clearly distinguish between the fresh and dry samples, whereas all other soil samples will be collected and stored in plastic 500 mL pots. Also important: the fresh soil samples will be **weighed** at the end of each sampling day when weighing the humus samples. Besides the more general chemical analyses (CNS, pH, SOM and specific N & P analyses), also a topsoil PLFA, 16s analysis, Biolog ecoplates analyses and soil SOM fractionation with spectroscopy will be considered for the fresh topsoil samples. In the center of the plot, two samples in the [2.5-7.5] interval will be taken by means of Kopecky rings, to determine bulk density afterwards. Both samples will be mixed into 1 container.

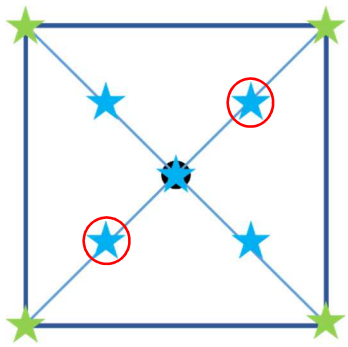

Figure 2. Soil and humus sampling location. In the standard 10x10 plot, soil samples (0-10 (2x); 10-20; 20-30) are collected starting in the centre and moving along the diagonals (*blue stars*). If the pots per interval are not full yet, more samples are taken in the corners (*green stars*). In two locations where soil is sampled, humus (OL, OH-OF) is sampled as well – using a separate pot per location (for example *red circles*).

B. SAMPLING EQUIPMENT

-Data sheets and writing equipment (incl. permanent marker)
-Wooden frame 20x20 cm^2 inner size
-Garden shear
-Soil auger
-Hammer
-Trowel
-Permanent marker to write labels
-Camera for soil profile photo (+spare batteries)
-Kopecky rings
-Moisture for finger test
-Plastic pots (for soil biomass: 3 per plot (intervals [0-10] dry; [10-20]; [20-30] cm depth))
-Ziplock bags (for soil biomas [0-10] fresh: 1 per plot)
-Plastic pots/ziplock bags (for humus biomass: 4 per plot (OL & OF-OH)
-Plastic pot (250 mL) for Kopecky-sample
-Larger box + scale for weighing the humus sample
-Knifes (sharp knife + kitchen knife)
-Freezer

C. DURATION & TIME OF SAMPLING

We expect that about **one hour per plot** is needed for the site conditions characterization part.

D. COLLECTION, TRANSPORT AND STORAGE

The ziplock bags with (fresh) **soil samples** from [0-10] cm should be stored in a freezer as soon as possible, in order to stop the ongoing N processes and should always be transported in a cooling box.

There is a dry and clean space required for storage of the plastic pots with **humus** and **soil** samples per plot ([0-10] dry, [10-20] and [20-30] cm depth). These samples should be air-dried before being sent to the laboratory. To achieve this, the pots are stored side by side and opened. Every three days, their content is turned to dry better.

Eventually, pots are closed, transported to the laboratory and put in the drying oven as soon as possible to avoid the samples getting mouldy.

4. STAND STRUCTURE [20x20 plot]
= description of distribution of trees by species, size and location within a stand

A. SAMPLING

We will complement the plot-level vegetation resurveys (see part 2) with a detailed description of the past and present **canopy** species composition and structure. The measurements consist of two parts (basal area and stand development reconstruction) and are both performed in the **20x20 m²** plot.

First, the **present** forest stand structure and composition will be characterized by measuring **DBH** of **all** trees and shrubs with **DBH>7.5 cm** in the plot. This can be used to determine stand density (BA and stem density) and mean diameter.

Notes:

- The same **instrument** should always be used for measuring DBHs: either a caliper or a measuring tape.

- We will take **two DBH measurements** per tree, at different sides, and calculate the average afterwards to avoid a too large measurement error.

- We will note whether a tree stands on a plot **border**/corner, so that we should consider this when calculating basal area: ½ and ¼ of the basal area of a border and corner tree should be taken into account respectively.

- When there are **multi-stemmed** individuals in the plot, every stem with DBH>7.5 cm is measured and it is noted which stems belong to the same tree/individual.

Second, the past stand (i.e. based on the plot-situation) development **history** will be reconstructed by means of growth ring analyses on **increment cores** of a number of selected trees per plot. Our goal is to (a) reconstruct at least **the last 150 years** for **minimum 2 dominant trees** per plot and to (b) complement this information with development reconstruction for **minimum 1 younger trees/shrubs** from the lower forest layers. The dominant trees' growth ring series will allow to reconstruct past stand development and the role of natural (e.g. climatic variation, competition) and human (e.g. management-related) disturbances therein. We are mainly interested to reconstruct these management-related disturbances from the increment cores: that is, we want to reconstruct the cutting (/thinning) history and hence associated pulses of light availability created from these canopy openings that reach the herb layer. Since these natural or biotic disturbances will also have influenced growth of these trees over the past century, this should not be neglected (for instance by calculating competition indices). The growth-ring information will be complemented by detailed, historical information at plot-level collected in another part of the project, allowing us to substantiate the findings from these growth ring analyses.

a) The selected dominant trees should belong to the **dominant/co-dominant** tree **layer** ànd at the same time be a **locally dominant** tree **species** in the plot. Measurements for these trees include (i) 2 DBH measurements (as above), (ii) 3 height measurements from 2 locations (with Vertex), as well as (iii) recordings of their neighbourhood (i.e. all trees and stumps within 9m distance from the tree with DBH>7.5) and of course (iv) **2 (!)** increment cores per individual are cored at a 90° angle from each other at BH. If for some reason, the cores cannot be taken at BH, the diameter at the height of coring should also be noted. The neighbourhood recordings are important to determine a distance-weighted competition index (Heygi's CI) for the dominant trees, since local competition for light influences tree growth rates as well.
b) The selected "**age trees**" should belong to the lower forest layers (lower D classes) and are cored to determine their age, e.g. to know at what time over the last 150 years these trees entered the plot or to find out the last cutting time before abandonment of a historical coppice management. Measurements for these trees include (i) 2 DBH measurements (as above) and (ii) **1 (!)** increment core per individual at BH.

Notes:

-To core or not to core?

Since taking increment cores of hardwood species requires some physical effort, we will only be able to core **maximum 5 or 6 trees per plot**. Depending on the plot situation and the workload (e.g. coring 2 dominant hornbeams is much more intensive than coring 2 dominant oaks), **minimum 2, maximum 3 dominant trees** should be cored and **minimum 1, maximum 3 age trees** should be cored. In certain situations, for instance

where there is presumably a history of coppice(-with-standards), it is important to core **at least 2 age trees**, since the lower D classes in such a plot will contain the trees that were cut systematically and their **age** may indicate the when this historical management system was abandoned.

We cannot standardize which **tree species** will (have to) be **cored**. This also depends on the plot-specific situation, but we should aim to core at least one of those species that is locally dominant in the plot.

We should be cautious when determining which (co-)dominant trees to core per plot, since this depends very much on the plot-specific situation. For instance, when a giant old oak tree is present in the plot, but it is surrounded by higher beech trees, this does not mean we should core the beeches and ignore the oak. It is clear that in this situation, the oak has been there for a very long time, but was ultimately outcompeted from the upper canopy by the beeches. In this case, the oak can tell us a lot about the stand history, so should definitely be cored here. Another example could be that when there are not so many dominant trees of importance within the plot borders, whereas a clearly dominant tree is found at 1 m outside of the plot borders, we should core beyond the plot boundaries.

-How to core?
Two tree-ring series per individual will be cored at a 90° angle from each other and at stump height for the dominant trees, whereas one tree-ring series will be cored for trees in the lower diameter classes, since we are only interested in their age and not in the whole development.

B. SAMPLING EQUIPMENT

-Data sheets and writing equipment
-Vertex and calipers for measuring heights, distances and diameters
-Pressler increment bore and corers (5/12 mm diameter) + paper straws to store them
-Working gloves

C. DURATION & TIME OF SAMPLING

-DBH (all trees & shrubs >7.5 cm) inventory may take at least half an hour per plot.
-9 increment cores are taken maximum per plot (max 3 dom trees, max 3 age trees). We expect this to take at least 1 hour per plot (1 person). In the meantime, the neighbourhood and height of the dominant cored trees is measured by the other two persons, which may take up to 1 hour per plot, depending on the number of trees and stumps.

D. COLLECTION, TRANSPORT AND STORAGE

Cores should not be glued on mounts and/or sanded when they are also used for isotope analyses (see part 5). Pinching the samples between wooden boards with slots enables long storing and safe shipping. However, since we also want to use the samples in the CT scanner, it is best to store them in paper straws. Once in the laboratory, the cores should be used for non-destructive analyses (growth ring analyses) first, and after that destructive analyses (isotope & C and N analyses) can be performed.

5. RESOURCE AVAILABILITY [10x10 plot]

= Description of present and past key resources (light, N, P) available in and productivity of the understorey

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A. SAMPLING

- **Light**

First, we will use a spherical densitometer in five locations – i.e. where soil samples are taken (**figure 2**) – to derive a reliable estimate of canopy openness, i.e. a robust proxy for **current** light availability at the forest floor. **Second**, the logged GPS coordinates of the plot centre is already logged (see part 1) and will (potentially) allow us to use Remote Sensing data to achieve a measurement of **historical** canopy changes.

Our ultimate goal is to define relations between *basal area*, (*current*) *light availability based on canopy species cover* and (*current*) *light availability based on spherical densitometer measurements* and to use these to determine historical light availability based on historical canopy cover data (from previous surveys) and to interpret historical basal area values.

- **Nutrients**
    - **Nitrogen**

Quantifying **present** N stocks will comprise several analyses. First, we will determine total N, $N0_3$-N and NH_4^+-N availability as well as net N mineralization rates on the [0-10 cm] **topsoil samples** (see part 3). Second, **herb layer biomass samples** will be used for quantification of aboveground nutrient concentrations (total N and P conc and N:P ratios) and for isotope analyses of N15, C13 and O18. For this, we will sample specimens from the **3 most dominant species** per plot (i.e. the species with **highest cover (%)** in the plot). We will store the biomass in paper envelopes and depending on the species, make sure we collect enough to preserve at least 1 g dried sample for all analyses. We will only collect aboveground and non-woody material (leafs, flowers, fruits and shoots).

Quantifying **past** N stocks can be reliably determined by assessing δ15N (isotope analyses) in both the **increment cores** and the mineral **topsoil samples** (collection of these: see above). At the same time, C13 and O18 isotope analyses will be performed on the tree cores to derive information on past climate (e.g. on heat and drought stress). This can be done in collaboration with the laboratory of Pascal Boeckx (http://www.isofys.be).

    - **Phosphorus**

Quantifying **present** P stocks will comprise several analyses as well. First, the mineral **soil samples** (see part 2) will be analyzed for total P, plant-available (Olsen) P as well as for the different P fractions through the Hedley sequential fractionation (labile P, slowly cycling P, occluded P).

Past P stocks may be quantified by means of a newly-developed technique by a Swiss laboratory using laser rays on specific locations in increment cores to determine past resource peaks. However, this method is still under development and since increment cores are already collected in our protocol, we will follow-up on this technique in the coming years, in case it would become available for use.

- **Productivity**

To quantify herb biomass productivity in the plots through allometric relations, we will gather **mean shoot length data** of the most representative species in the plot. The estimation model PhytoCalc (see e.g. Heinrichs et al 2010 Eur Journal of Forest Research) allows a non-destructive quantification of dry weight of understorey plants in Central European forests by using the relationship between **species biomass, species cover** (see part 2 veg resurvey) and **mean shoot length** (this part). Hence, for this, we still need to measure the shoot length of 10 random individuals of **all the dominant species (i.e. with a cover >5%)** in the plot. Shoot length in this context describes the "elongated length of a shoot including inflorescences and leaves" (see **figure 3** for examples). So depending on the plot situation (containing both flowering and non-flowering individuals and higher and lower individuals), we will sample a number of individuals to get a mean value that represents the plot situation. For instance, 5 non-flowering and 5 flowering individuals. For the shoot length of the other (non-dominant) species, we will use a height value implemented in PhytoCalc for the morphological growth forms they belong to. All species from the understorey may be used for these measurements, including both understorey herbs, grasses, tree

seedlings, reptant species etc. Finally, validating our Phytocalc estimations with some real observed data outside of the plots will not be done during the field campaign due to lack of time, but may be performed later in a similar forest.

Figure 3. Examples of shoot length measurements to determine understorey productivity. The whole plant body is stretched along the ruler.

B. SAMPLING EQUIPMENT

-Data sheets and writing equipment
-Spherical densitometer
-Clipping shears to clip understorey vegetation
-Paper envelopes to store biomass
-Scale
-Measuring tape/ruler to measure shoot length of the understorey species

C. DURATION & TIME OF SAMPLING

Densitometer measurements, herb biomass samples and shoot length measurements should be taken when most of the understorey plants have emerged, and since our field campaigns in 2015 and 2016 starts in Spring, should be OK. We expect about **45'** per plot should be enough for this fieldwork part: duration for the increment cores and soil samples are already calculated in part 3 and 4 hence these three measurements are the only remaining things to do here.

D. COLLECTION, TRANSPORT AND STORAGE

Ideally, the soil samples taken to determine inorganic forms of N ($N0_3^-$N and NH_4^+-N) should be frozen and analyzed as soon as possible for valid results. This is because N processes continue in the fresh soil, hence air-drying can lead to small but significant increases in exchangeable $N0_3^-$N and NH_4^+-N, leading to misinterpreted results. However, some delay is nearly impossible to avoid, because samples must be transported between study regions during a long field campaign spread over Europe and to the laboratory at the end of the field campaign where they have to be sieved and subsampled before performing all analyses. However, at all times during transport, fresh samples will be put in a cooling box and for far regions, we will send them to the lab via the post.

The herb biomass samples should be oven-dried as soon as possible (in the lab drying oven at 65°) and stored in a dry place before transportation to the laboratory.

6. SUMMARY OF SAMPLES/MEASUREMENTS TO DO PER PLOT (CHRONOLOGICALLY)

STEP 1 (3p together/2p+1p)

Setting out the **plots** (original, 10x10, 20x20), **GPS logging** the plot centre. Evaluating the **general stand and plot characteristics** (see part 1). Taking **overview photos** of the plot.

STEP 2 (3p together)

Vegetation survey in original plot and in 10x10 plot and determining (i) all dominant species in the understorey (cover>5%) to perform **shoot length measurements** on 10 random individuals per species (10x10 plot!) and (ii) the 3 most dominant species (species with highest cover%) to **collect herb biomass** samples (one full paper envelope) of these 3 species (10x10 plot!).

STEP 3A (2p)

Basal area measurements in the 20x20 plot and afterwards determining which trees to core (by 1 person who is now taking soil samples). During this time, the other person collects soil samples and these 2 p will take a look for **soil profile description, soil texture and photos** of the soil profile.

STEP 3B (1p)

Taking 4 **soil samples** in 10x10 plot (fill whole 500mL pot (dry samples)/ziplock bag (fresh samples)) (! Kopecky vs not Kopecky).

Whoever finishes first with basal area/soil samples, can perform the 5 **densitometer measurements** where the soil samples are taken (centre+4 locations, figure 2).

STEP 4A (2p)

Determining the DBH of the trees (also note which species!) to core as well as additional **measurements** (height, neighbourhood) for the **dominant trees**.

STEP 4B (1p)

Taking the **increment cores** in 20x20 plot and storing in paper straws (2 cores per dominant tree, 1 core per age tree).

STEP 5 (3p)

Taking the 4 **humus samples** in 10x10 plot on 2 of the soil samples locations (fill the content of the wooden frame into small pot 250mL or ziplock bag if too much).

STEP 6

After each field day, **storing** the samples in a good way (leave open to dry, in freezer etc.) and **weighing** humus and fresh soil samples (0-10).