FutMon (Life+) field protocol:

Sampling procedure for evaluation of nutrient budgets in vegetation in FutMon intensive monitoring plots and more intensive foliage surveys (D2)

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1. Background

Even though understorey vegetation usually represents a relatively minor component of the whole biomass of high forests, it can play an important role in the annual biomass production and hence also in the nutrient cycling of the forest ecosystem. Furthermore, compared to the tree foliage (especially from evergreen trees), the litter of deciduous dwarf shrubs, herbs and grasses has a faster rate of decomposition. This means that the litter produced by the understorey vegetation is likely to have a major impact on soil microbiological processes, possibly with a different seasonal pattern to the canopy trees. In addition, vegetation cover protects the soil from erosion and alters its moisture content and temperature. This is particularly important in complex forests with tall shrub undergrowth (> 2m ht), such as may be found under stands of deciduous oak or ash. Consequently knowing the nutrient content and biomass of different components of standing ground vegetation is essential to be able to quantify nutrient budgets in different compartments of forest vegetation. Together with information about deposition, soil water and soil nutrients nutrient budgets in forest vegetation will help to gain understanding of nutrient cycling in forest ecosystems.

The aim of this protocol is to present a methodology to yield comparable and reliable information about living biomass and nutrient concentrations of ground vegetation in FutMon intensive monitoring (IM) plots at Pan-European level. Together with ICP Forests manual part IV (Sampling and analysis of needles and leaves) this protocol will help to evaluate the nutrient budgets needed in FutMon Action D2. Estimates of biomass per species may be expanded to plot level through the results of the ground vegetation surveys (i.e. species list and cover/abundance values). Ideally different species present in each IM plot should be analysed separately but, especially in species rich plots, this would not be economically feasible. Therefore, in this protocol species have been amalgamated into seven groups on the basis of their structure and/or function so that species that have presumably similar life cycles, decomposition rate etc. belong to same functional group (see details in chapter 3.4). The underlying goal of the methods below is to have reliable estimates of

living biomass and nutrient concentrations of these species groups expressed per known ground surface area.

2. Definitions

Here ground vegetation refers to:

- ground layer (terricolous bryophytes and lichens) and
- field layer (all non-ligneous and ligneous plants < 0.5m height)

Ligneous plants exceeding 0.5m height belong to:

- shrub layer (ligneous plants of > 0.5m height) or
- tree layer (ligneous plants of > 5m height)

Note that the "shrub layer" (i.e. ligneous plants exceeding the 0.5 meters) in the protocol means tree and bush species that exceed 0.5 m. Hence dwarf shrubs (e.g. Vaccinium, Calluna, Empetrum etc.) possibly exceeding 0.5 m belong to field layer and should thus be sampled and analysed.

3. Destructive sampling, biomass weighing and pre-treatment of living ground vegetation before chemical analysis

This sampling design pertains only to understorey vegetation of less than 50 cm height as recorded under the Level II protocol for site ecology. The field sampling should be undertaken by or under the supervision of an experienced plant ecologist.

3.1 Time of year.

Sampling should be aimed at attaining maximum biomass values at the peak growing period. This might require more than one sampling, for example spring for communities dominated by vernal ground flora (largely herbs) but later in summer for grasses, sedges and rush. Also evergreen vegetation shedding leaves in mid-summer will need to be sampled later, when new canopy is fully developed. Suitable sampling dates to collect all functional groups (see later in chapter 3.4) present

in the plot need to be consulted with an experienced botanist. In the final analysis dried (or frozen) samples collected during different dates can be pooled to one composite sample per functional group. In plots where it is not possible to sample all species in one sampling occasion the sampling area is marked (with plastic or wooden markers) and the same sampling area is used in the following sampling time. If more than one sampling is used it is not necessary to collect regrowth of species collected earlier. Hence if grass species that have been already collected have regrown by the time other (e.g. evergreen) species are to be collected, these grass species can be ignored.

3.2 Sampling design

Sampling of standing ground vegetation is performed in FutMon IM plots in places where this does not interfere with other activities in the plot, such as deposition and soil water collection, or assessment of ground vegetation. Suitable areas within the IM plot are e.g. in the surroundings of subplots used for vegetation assessment leaving, however, a large enough buffer zone around the area used for vegetation assessment, so that there is no disturbance by e.g. trampling or secondary succession. If possible, sampling should be established no less than 10m outside the sample plot used for vegetation assessment and its buffer zone and in areas similar to the conditions inside that area; so called site type representative sampling.

Ground vegetation is sampled using a frame of known area (see chapter 3.3). The frame is placed on the ground and all above ground parts of the vegetation passing through the frame are cut at ground level using scissors, loppers or a pair of shears. Hence a "projectional" approach should be adopted: parts of plants (of plants rooted inside the frame) which grow outside of the frame are omitted from the study; likewise parts of plants which grow into the frame (of plants rooted outside the frame area) are included. The number of the frames (and hence the total area sampled) need to be carefully recorded and reported. The samples of each frame are stored separately in plastic bags or durable paper bags to be transported to a laboratory for a further preparation. It is recommended to measure the shoot length of at least the 5 most abundant species within the frames. These results are submitted using the form XX2009.GBH in order to allow biomass modelling on the basis of vegetation height in the coming studies.

3.3 Number of sampling units and quantity of sampled material

The number of sampling units (i.e. the number of the frames in which the vegetation is collected) within each IM plot depends on the area of frame used. A minimum total area requirement is 2 m^2 that can be achieved e.g. by collecting ground vegetation from four sampling units (frames) of an area of 0.5 m^2 or eight frames of an area of 0.25 m^2. It is recommended that the bigger frame is used for more robust vegetation. The area of 2 m^2 is enough only in case the vegetation on the plot is homogenous. A larger area (more sampling units or larger frames) needs to be collected in case the vegetation is very heterogeneous (a large number of different species) or the biomass of the collected samples is small (e.g. the coverage is scarce and dominated by small lichens or bryophytes). Because the total area needed for a representative sample depends on the ground vegetation diversity, the sampling should be done in co-operation with the persons responsible for the assessment of ground vegetation. The exact number of sampling units (i.e. the number of the frames within which the vegetation is harvested), the area of the frame, and the total area sampled (sum of the area of individual samples) must be recorded and reported.

The aim of the sampling is to have a representative sample of the ground vegetation in each plot. Hence, sampling units (frames) are positioned on the plot the way that gives statistically reliable estimate of different species in different conditions. However, unusual micro-topographic features, such as drains, paths, rides or animal disturbance should be avoided, so as to best represent uniform under-canopy conditions. Because IM plot design is different in different countries a common sampling design (e.g. random sampling, systematic sampling, cluster sampling etc.) can not be stipulated. Hence each country has to apply a method that fits to its plot design and will yield a representative sample of the vegetation in that particular IM plot. Whatever sampling design is used care must be taken that the sampling does not interfere other activities in the plot.

Different species are grouped in larger functional groups (to be submitted in field "sample number" with code 1-8, cf. chapter 3.4) for the element analysis. The amount of collected sample should be large enough so that for each functional group present on the plot the chemical element analyses can be carried out. The total amount of sample needed for the element analyses depends on the method in use. As a rule of thumb: 10 grams of fresh mass of plant material will give around 2-5 grams of dry mass of which about half is left after grinding which is enough for microwave wet digestion (preceding e.g. ICP or AAS measurements). However, in order to conserve samples for future use, to be able to do quality checks if needed etc. it is recommended to sample at least twice as much as the minimum requirement for the chemical analyses. If for some functional group there is not enough biomass for chemical analyses, only the biomass should be recorded, but no chemical

analyses are performed. In case there are two or more functional groups in the plot where the biomass is too small for individual chemical analyses, these groups can be pooled. In this case the biomass of these functional groups is recorded separately but the chemical analyses are reported for a group that is labelled "rest" (group number 8, cf. chapter 3.4 for the functional groups). If, despite the pooling, chemical analyses are still not feasible, the chemical analyses for these groups can be dismissed.

3.4 Assorting species into functional groups

If collected samples contain detritus this is carefully separated from the living biomass and removed. Samples of living ground vegetation of each sampling unit (frame) are separated into following seven different functional groups, dried and weighted (see below chapter 3.5).

1) Bryophytes (mosses, liverworts and hornworts)
2) Lichens
3) Ferns (all Pteridophytes)
4) Grasses (Poaceae), including sedges (Cyperaceae) and rushes (Juncaceae)
5) Herbs
6) Deciduous shrubs, including deciduous tree seedlings <50 cm height*
7) Evergreen shrubs, including evergreen tree seedlings < 50 cm height*
8) Rest**

* In cases of functional groups 6 and 7 (shrubs) the minimum requirement is to analyze foliage. If stems are chemically analysed they are separated from foliage. The biomass of foliage and stems are recorded separately. The results for stems are reported in the formats with sub code "b" (i.e. 6b for stems of deciduous shrubs and 7b for stems of evergreen shrubs). In case chemical analyses for the stems are not performed, only biomass results are reported.

** Group "rest" (code 8 in the formats) is used in case two (or more) groups are pooled for the chemical analysis due to small amount of available biomass. The biomass results are, however, reported for the actual functional groups.

3.5. Biomass weighing

After individual species are pooled into the above functional groups each of these are oven dried (keeping samples from different frames separated) at a maximum of 80°C, for at least for 24 hours. The presence of woody material requires longer drying period. After the drying, the dry mass of

each functional group is measured. After recording the weight of 80°C dried samples (functional groups within each frame) the following steps are to be follow:

1) In the case that there is not enough material per functional group within a IM sample plot (i.e. when the samples of individual frames are pooled) to do the chemical analyses (including grinding, microwave digestion etc. described in chapter 3.3) different functional groups can be pooled to form the group "rest" (cf. chapter 3.4). If even the pooling, to form the group "rest", will not yield enough material per plot to do the chemical analyses, the functional groups should be kept separate and dried at 105 °C.
2) In the case that there is enough material for chemical analyses the samples (a functional group or group "rest") is ground to fine powder following the procedure described in the ICP Forests manual (Part IV).
3) Two subsamples of the ground material are then taken for:
    a) drying a known amount of material in 105 °C (to determine the moisture content of the 80°C dried samples), and
    b) to be used for chemical analyses
4) Element concentrations and biomass are then reported on dry weight of 105 °C

3.6. Pretreatment before chemical analyses

In cases where the weight of the total pooled sample of a given functional group is sufficiently large, a subsample (e.g 10 to 20 g dry weight, depending of the analysis method in use) is ground to obtain homogenous powder that is treated and analysed as in case of chemical analyses of foliage samples (see Part IV in the manual of ICP Forests). If there is not enough material for chemical analyses only the biomass results are reported. Element concentrations are reported by reference to 105°C-dried material (cf. Table 1 in Part IV in ICP Forests manual).

3.7 Data submission

The data on Laboratory Quality Assurance ("XX2009GB.LQA") will be submitted together with the reduced plot file ("XX2009.PGB") and the data files ("XX2009.GBM" and "XX2009.GBO").

4. Sampling methods for more intensive foliar survey (Foliage D2)

Mandatory on D2 plots

The methods described in the ICP Forests manual (Part IV) are valid here with one exception. In case of evergreen species, foliage age classes older than C+1 are also sampled and analysed. Hence if the species collected for analysis of element concentration has older foliage classes than C+1, these are collected and analysed the same way as C and C+1 foliage classes. Foliage age class should be considered to be present when there are more than 50% of the original leaves/needles present in the annual shoot (i.e. less than 50% have been shed). The number of the foliage age classes present are recorded and reported in the data forms. Foliage age classes older than C+1 can be chemically analysed separately or by using a combined sample (all foliage age classes older than C+1 together). When the data is submitted to the database only one value for the older than C+1 foliage is reported, i.e. if foliage age classes (older than C+1) are analysed separately a mean is computed and reported.

In order to allow for the submission of further tree numbers (only 5 tree number may be submitted with .FOM yet) it is foreseen that those further tree numbers will be submitted in a second data line (or record, respectively) with the same analysis results for nutrients, biomass, etc. as those in the first respective data line.

The data on Laboratory Quality Assurance will be submitted together with the reduced plot file ("XX2009.PLF") and the data files ("XX2009.FOM" and "XX2009.FOO") using the form "XX2009FO.LQA".