# FunDivEUROPE

**Functional significance of forest biodiversity in Europe**

Project number: 265171

# Sampling of soil for assessment of soil nitrogen stocks

FunDivEUROPE (FP7) field protocol

V1.0

Last update: 11th March 2011

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# 1 Introduction

Recent studies have shown that tree species mixtures may have both additive and non-additive effects on decomposition rates for organic matter. Soil nitrogen stocks are strongly affected by tree species, because species produce litter of differing quality and quantity, thereby influencing decomposition processes and nutrient residence times in the humus layer. Furthermore, deposition of nitrogen varies between species and thereby also influences the soil nitrogen stores. However, little is known quantitatively about the effects of tree diversity on nitrogen stocks and cycling. This task will deliver quantitative estimates of N stocks in explicit and extensive gradients of forest diversity with the aim of assessing the N retention potential by conversion from single species to mixed species stands.

# 2 Scope and application

The assessment will focus on forest floors and the upper part of mineral soils (0-40 cm) as these soil compartments contain the overwhelming part of soil N stocks at the European level. We aim to test the following hypotheses: 1) Soil N stocks are strongly influenced by the N-cycling-related traits of the dominant species, but additional variation can be explained by tree species diversity; 2) nitrate leaching can be predicted from sets of indicators (deposition, soil C/N ratio), which are in turn influenced by tree species.

# 3 Objectives

The aim is to show whether soil N stocks increase with tree species diversity and whether more nitrogen is allocated to the mineral soil in diverse compared to single-species forests. Soil N stocks will be related to N input from above and belowground litter production and based on data on litterfall N (see "Fine root biomass", page **Fehler! Textmarke nicht definiert.**; "Fine root production", page **Fehler! Textmarke nicht definiert.** and "Litter production and element fluxes", page **Fehler! Textmarke nicht definiert.**), the fractional annual N loss of forest floors by decomposition, i.e. the litterfall N/forest floor N ratio, will be estimated as an index of N turnover. A multivariate analysis of tree species diversity effects on soil N, C/N and leaching will be performed. This work will rely on soil data from the Experimental and Exploratory Platforms, and the EU BioSoil and Level II monitoring as found in the Forest Focus/FutMon database (Inventory Platform of FunDivEUROPE). Environmental covariates of sites such as geology and soil type, temperature and precipitation will be included in the analysis to account for confounding factors. The work on leaching of nitrate by use of the Inventory Platform will be strongly coordinated with the work on leaching in "Soil water content", page **Fehler! Textmarke nicht definiert.**.

# 4 Location of measurements and sampling

Please consult ICP-forests website for basic sampling procedures for fixed depth samples in Level I plots as described the ICP Forest manual, section 4.1.2

http://www.icp-forests.org/pdf/FINAL_soil.pdf

We basically follow the mandatory sampling scheme for Level I plots with the following exceptions:

- Forest floors will not be separated in sublayers;
- We sample nine subsamples per plot instead of five for preparation of one composite sample for chemical analysis.

## 4.1 Field sampling design

Soil N stocks will be determined from forest floor samples and soil cores extracted from two Experimental plots (BIOTREE and Satakunta) in summer-autumn 2011 and all Exploratory plots during summer-autumn 2012. The sampling will be co-ordinated with the root biomass sampling task, coordinated by Metla (Leena Finér and Timo Domisch) and is fully coordinated with the carbon stock task. *For the Experimental plots: depending on the design for baseline sampling at experiment start, the sampling design may be revised for these plots. Application of the same methodology is crucial in repeated inventories to address soil C changes.* At the BIOTREE site at Kaltenborn, 15 soil cores were sampled from each "plot" in 2004 (Scherer-Lorenzen et al., 2007).

## 4.2 Number of replicates

Nine forest floor and nine core samples of mineral soil per plot (see sampling scheme below). Forest floors will be sampled from 25 x 25 cm plots using a wooden frame or similar. A frame of 25 x 25 cm is laid on the forest floor and all living plant parts are clipped off within the frame. The forest floor is then removed by hand or by knife if it has the morphology of a thick mor layer. After removal of the forest floor, one core sample will be taken down to 40 cm in mineral soil. Cores are sampled with a long corer (inner diam. 36 mm, length 50 cm or longer). If this is not possible due to the stone content of the soil, the sampling can be at 10 cm-intervals by using a smaller core (inner diam. 38 mm, length 10 cm). Mineral soil core samples will be divided by fixed depth into five composite samples to comply with the sampling scheme used in the ICP Forests/BioSoil soil data base: forest floor (if existing), 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm (Cools & de Vos, 2010). The nine replicate samples serve to represent the soil conditions across the core plot. Analyses of soil N will only be done on a composite sample.

## 4.3 Sampling scheme

Forest floor and mineral soil samples will be taken systematically from a grid established on the plots. We plan to divide the 30 x 30 m core plot into nine 10 x 10 m subplots in which one sample of forest floors and one soil core will be sampled. A similar design will be applied as far as possible to Experimental plots depending on plot size and sampling design of previous soil inventories. Later on, forest floor as well as mineral soil samples will be pooled into one sample/plot by layers giving five subsamples/plot.

### 4.4 Sampling equipment

The following tools or other material are needed for carrying out the sampling: two soil corers (10 and 50 cm length), plastic bags, knife, scissors, ruler, spoon, spatula, impact free hammer, field sampling protocol and template, permanent marker pens, labels and bags for transportation.

### 4.5 Frequency of sampling

In the Experimental Platform sampling will be done once in 2011 (Aug–Sept–Oct) on the BIOTREE (16 plots) and the Satakunta experiment (38 plots).

In the Exploratory Platform, sampling will be done once during the project period in Aug–Sept–Oct 2012.

In both cases sampling must be done **BEFORE** the onset of leaf litterfall.

### 4.6 Sample collection, transport and storage – quality control in the field and between plots and sites

Soil samples need to be stored in cold storage (approx. +5° C) before transportation to Metla/Finland. If the storage or transportation time is long (> 2 weeks) the samples have to be stored in a freezer (approx. -18° C), otherwise there is a risk that the roots start to disintegrate.

All she soil samples will be sent to Finland (Timo Domisch, Finnish Forest Research Institute, Joensuu Research Unit, Yliopistokatu 6, FI-80101 Joensuu, Finland). After root extraction the soil samples will be sent to Denmark for bulk density, C and nutrient analyses.

## 5 Measurements

In the lab, the nine forest floor samples will be dried at 55° C and weighed before grinding in a Retsch mill and mixing to one composite sample per plot. A subsample will be dried to 55 and later to 105° C for correction of dry mass. Mineral soils will be dried at 55° C and later sieved through a 2 mm sieve to separate and weigh the coarse fraction and the fine fraction, respectively. Based on these mass values and the volume of the corer, bulk density will be determined. The fine fraction will subsequently be mixed by depth to establish one composite sample of mineral soil per depth per plot. A subsample will be ground in an agate mortar to fine powder prior to chemical analysis.

Nitrogen content of forest floor material and mineral soil will finally be measured by dry combustion, i.e. the Dumas method (Matejovic, 1993).

## 6 References

Cools, N., De Vos, B. 2010. Sampling and Analysis of Soil Manual Part X, 208 pp. In Manual and Methods and criteria for harmonizing sampling, assessment, monitoring and analysis of the effects of air pollution on forests. UNECE, ICP Forests. Hamburg. (http://www.icp-forests.org/pdf/FINAL_soil.pdf)

Matejovic, I., 1993. Determination of carbon, hydrogen, and nitrogen in soils by automated elemental analysis (dry combustion method). Commun.Soil Sci.Plant Anal. 24: 2213-2222.

Scherer-Lorenzen, M., Schulze, E.-D., Don, A., Schumacher, J., Weller, E. 2007. Exploring the functional significance of forest diversity: A new long-term experiment with temperate tree species (BIOTREE). Perspectives in Plant Ecology, Evolution and Systematics 9: 53-70.