FunDivEUROPE

Functional significance of forest biodiversity in Europe

Project number: 265171

Sampling of leaves and twigs

for Carbon and Nitrogen element analyses, stable isotope composition, chlorophyll fluorescence, and insect and pathogen damage

FunDivEUROPE (FP7) field protocol

V1.0

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Content

1 Introduction

The overall goal of FunDivEUROPE is to quantify the effects of forest biodiversity on ecosystem functioning and services in major European forest types. The foliage of trees in a forest as the major compartment associated with carbon fixation from the atmosphere is important to understand the element and water cycle in forest ecosystems, and thus important provisioning and supporting services of forests. In addition, leaves are habitat of (herbivorous) insects and targets of pathogens – being both part of the forest biodiversity and drivers of community dynamics – can also impair forest stability and production functions. Invasive species can directly influence diversity and ecological processes, potentially affecting the delivery of ecosystem services. To understand ecosystem functioning as affected by biodiversity, sampling and analysis of needles and leaves (partially together with the twigs they are attached to) is essential. Depending on the temporal resolution required as well as on the processes examined, analyses and thus sampling have to be performed at different times during the growing season.

2 Scope and application

The objective of this part of the manual is to provide harmonized and standard procedures for the sampling and analysis of leaves and needles at the Experimental and Exploratory sites of FunDivEUROPE. The protocols are as close as possible adapted to the ICP Forests protocols for leaf and needles sampling (Rautio et al. 2010). Due to the partially different objectives and analyses performed, however, a complete compliance cannot be achieved.

A harmonised leaf and needle sampling protocol for FunDivEUROPE is necessary to allow comparability between sites and species. In addition, the procedure guarantees that different measurements, which have to be linked to obtain a complete picture of diversity and function, are performed on the same material. Procedures are intended for application at both Experimental and Exploratory sites and will also be applied on the highly instrumented plots (HIPs) of both platforms. All partners and site managers of FunDivEUROPE should follow the methods described here to minimise any potential bias by the sampling procedure. The parameters measured on the foliage samples by the different partners are listed in table 1.

Table 1: List of parameters measured on the tree core samples by the different partners.

Task	Task leader(s)	Partner responsible	Parameter determined	Additional information
T.3.9	Arthur Gessler, Damien Bonal, André Granier	ALU-FR, INRA	Stable isotope composition ($\delta^{13}C$, $\delta^{18}O$)	In total organic matter (dried)
T.3.6	Kalliopi Radoglou	NAGREF	N content and N resorption	

Task	Task leader(s)	Partner responsible	Parameter determined	Additional information
			efficiency	
T.3.2	David Coomes	UCA	C content	
T.3.4	Filippo Bussotti	UFI	Chlorophyll fluorescence	Fresh material - leaves attached to (small) twigs
T.4.3	Hervé Jactel	INRA	Insect damage	
T.4.6	Helge Bruelheide	SLU	Fungal pathogen infection	

3 Objectives

The objectives of foliar sampling and analyses are to assess water use efficiency, N and C physiology, photosynthetic parameters of the leaves together with the effects of herbivorous insects and fungal pathogen damage as affected by diversity patterns.

4 Location of measurements and sampling

The sampling will be done in the Experimental and Exploratory sites (including the highly instrumented plots - HIPs) of FunDivEUROPE.

4.1 Number of replicates

Five field replicates (i.e. different individuals) per species will be sampled on all plots

Estimated samples per campaign:

Experimental sites: WP3: 100 plots x 2.5 (average of species per plot) x 5 replicates = 1250 trees sampled); **WP4**: ca. 1000 / site × 4 sites;

Exploratory sites: 300 plots x 2.5 species (average per plot) = 750 x 5 replicates = 3750 trees sampled.

4.2 Sampling scheme

General scheme

For all analyses twigs with leaves and needles from the south exposed sun crown will be collected. For the assessment of insect and pathogen damage four branches of the two target tree species, will be cut at two opposite directions x two heights: at the lower part of tree crown and at mid- or upper crown (depending on tree height). The damage assessment will be made on 25 fresh leaves per branch according to the protocol developed for experiments.

On coniferous trees herbivory and pathogen damage will be assessed on 10 shoots, from each yearly needle cohort separately, on each sampled branch.

For chlorophyll fluorescence measurements the leaves and needles will still have to be connected to the twigs/twiglets to keep them hydrated. The samples, separated per tree, will be put in plastic bags, labelled with the data and number of the plot and tree, finally arranged in a thermic bag (cooled, but not frozen), with kitchen paper to kept them well watered. At the end of each working day, samples will be delivered to a close depository. The measurement of chlorophyll fluorescence will be done the day after (or the same evening) in a dark room. For insect herbivory and pathogen damage an additional sampling height in the lower crown is required.

In an autumn campaign leaves will be collected from litter traps in order to determine N absorption capacity.

Plant material harvested
For Chlorophyll fluorescence measurements as well as for the sampling of leaf material for C and N element analyses, stable isotope composition, and insect and pathogen, the following leaf material according to the ICP forest protocol (Rautio et al. 2010) is used:

Deciduous species: Sampling is done on current year leaves or needles.

Evergreen species: Sampling of both the current year needles or leaves and the second year needles or leaves.

Technical sample procedure
The preferential sampling techniques are the harvest of twigs/twiglets with extension loppers but alternatives might apply when this technique is not applicable.

If the access to the sun crown is possible from the forest floor with extension loppers (up to 8-10 meters), twigs with leaves and needles will be cut with this equipment.

If the tree height exceeds the range of extension loppers, shooting with a rifle is the next preferred option. If this is not possible due to legal reasons, tree climbing and the use of flexible saws shot with a crossbow into the canopy are the next possible alternatives. It is necessary in all cases that twig and leaf material as defined above is harvested with all techniques applied.

4.3 Sampling equipment

Extensions loppers (8-10 m), rifle, tree climbing equipment (harness, ropes), flexible saw and crossbows.

4.4 Frequency of sampling

2011: Experimental sites

Two campaigns:

- Main campaign summer (July/August) for: C,N, isotopes, fluorescence, insect and pathogen damage;
- Campaign end of the growing season for: fluorescence (at selected sites) and leaves collected in litter traps for N absorption capacity.

2012: Exploratory sites

Two campaigns:

- Main campaign summer (July/August) for: C,N, isotopes, fluorescence, insect and pathogen damage;
- Campaign end of the growing season: fluorescence (f: only in Mediterranean conditions. A third winter assessment is possible on Mediterranean evergreen species) and leaves collected in litter traps for N absorption capacity.

A sampling campaign at a given site should not exceed two weeks (10 working days) in order to avoid strong weather differences. The total sampling period for all ecoregions should not exceed 1.5 - 2 month.

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

The most important task is to collect twigs from trees with the needles and leaves attached. For broadleaf species 20-30 leaves and for conifers an appropriate amount of needles (approx. 5 g fresh weight for broadleaves and conifers) is needed. The twigs with leaves and needles still attached will be transferred in plastic bags and stored under cool conditions. Chlorophyll fluorescence measurements will be performed after the samples have been stored in the dark for one night (dark adapted).

After the chlorophyll fluorescence measurements the leaves/needles from each tree are transferred into paper envelops labelled with the tree ID and dried for three days at 60°C. Dried plant material can be stored in a dry environment, preferentially in desiccator cabinets. In a next step samples will be homogenised with a ball mill and aliquots (transferred in 20 ml vials) will be sent around to the different labs, so we can guarantee that all partners measure the same material. The homogenised dry material can be also stored in desiccator cabinets or other dry environments. A storage space of 1-2 m^3 would be needed at each ecoregion. Pooling/binning of leaf samples – as required by the different groups – should be done after homogenisation if the amount of samples to be homogenised can be handled.

If the tree species is a target species for insect and pathogen damage assessment an additional 100 leaves from four branches have to be collected. In this case a subsample of 40 leafs, 10 per branch, per sampled tree will be collected, put into paper bags and dried (at

60°C), for further microscopic and DNA analysis to better characterize the leaf pathogen communities. If no oven is available this can be done by placing the samples in a desiccator or a plastic bag containing silica gel. The other 15 leaves per branch will be kept fresh for further damage assessment.

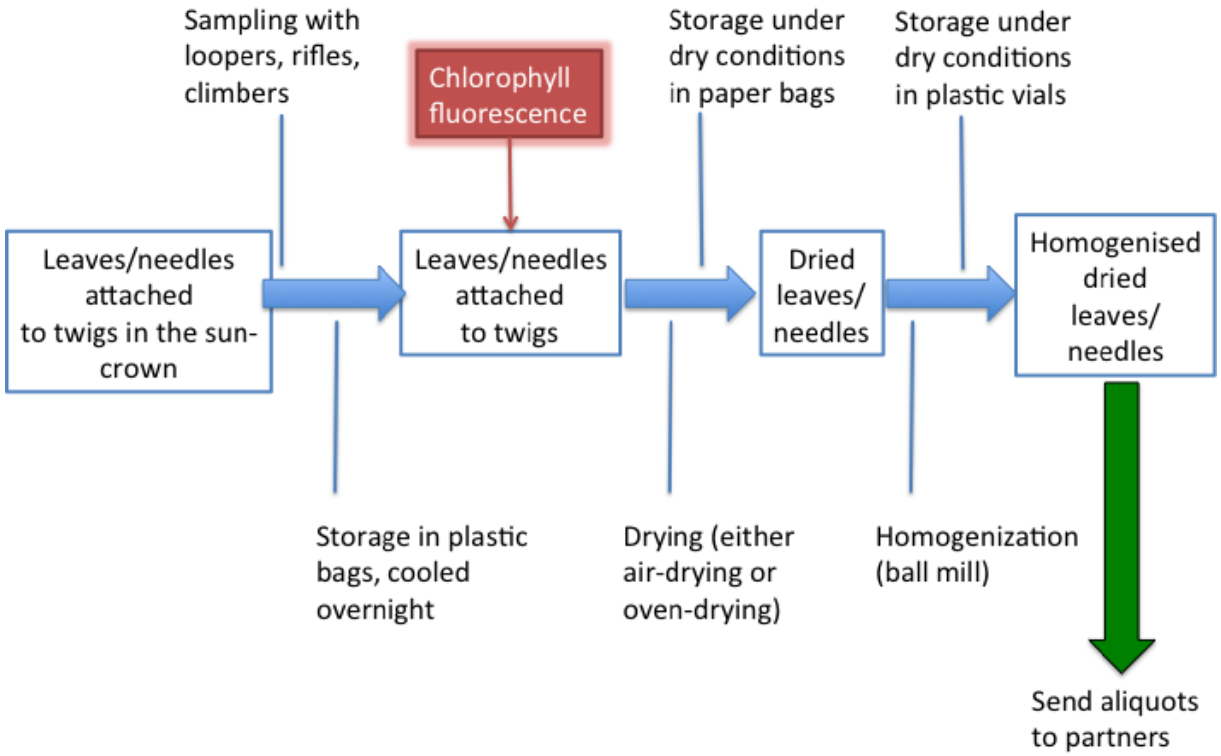

Figure 1: General sampling and handling scheme for leaves/needle samples.

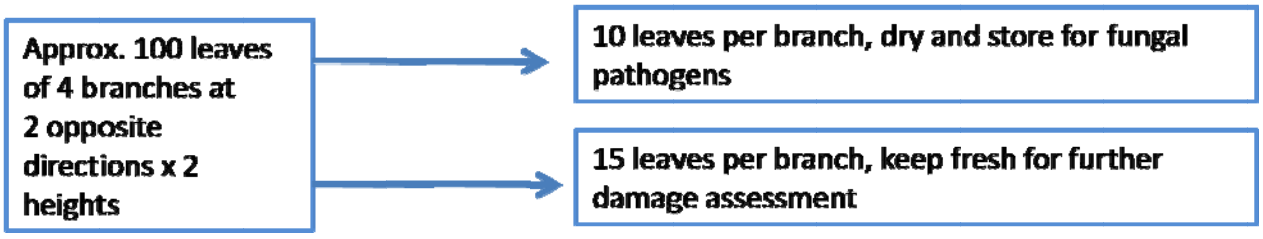

Figure 2: General sampling and handling scheme for „Insect and Pathogen assessment“: 2 target species per region.

5 Measurements

Chlorophyll Fluorescence: The measurement of chlorophyll fluorescence will be done on fresh leaves, one day after they have been collected (leaves dark adapted in plastic bags). Leaves from different branchlets of the same tree will be randomly selected, and measurements will be taken on 20-30 leaves per tree with Handy PEA and/or Pocket PEA fluorimeters (direct, or prompt, fluorescence). Standard procedures (time and intensity of illumination, gain) will be applied. All that procedures will be performed in a dark room. Data are treated at tree level. After fluorescence measurements are made, the samples will be stored for successive analysis.

Stable Isotope analysis: For $\delta^{13}C$ analysis 0.2-0.6 mg dried homogenised leaf sample material will combusted in an elemental analyser and for $\delta^{18}O$ (0.2-0.8 mg) analysis in a high temperature conversion/elemental analyser, both coupled to an isotope ratio mass spectrometer (IRMS). Carbon isotopic and oxygen values will expressed in the δ notation, relative to the Vienna Pee Dee Belemnite (VPDB) and to Vienna Standard Mean Ocean Water (VSMOW), respectively. Details of the method are given by Gessler et al. (2004)

Fungal pathogens: Pathogen load will be quantified as percentage of leaf area infected by foliar pathogenic fungi. Assessment of pathogen load will be performed by identifying all fungi species present on the sampled leaves, using microscopes, staining techniques, reference literature and reference collections, and then estimate their abundance per target tree based on the abundance on sampled leaves.

In addition, subsamples will be sued for massive parallel sequencing by pyrosequencing of fungal DNA-markers. This methodology will allow for huge numbers (10^5 - 10^6 individual determinations) of parallel species determinations.

6 Data sheet template

Use a new sheet for each plot.

General information:

Person responsible for sampling

Sampling date/time

Weather conditions (T_{air}, Rainfall, cloud cover)

Site

Plot Nr.

Diversity level

How collected (rifle/lopper/climber)

Sample ID	Tree No	Tree Species	Fluorescence measurement done (yes/no)	Leaf sample for pathogen (add "P" the ID)	Leaf sample for homogenisation (add "I" the ID)	Comment

7 References

Ammer CH, Ziegler C, Knoke T (2005) Zur Beurteilung von intra- und interspezifischer Konkurrenz von Laubbaumbeständen im Dickungsstadium. Allg Forst Jagdztg 176: 85-94.

Rautio P, Fürst A, Stefan K, Raitio H, Bartels U. 2010: Sampling and Analysis of Needles and Leaves. 19 pp. Manual Part XII. In: Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests, UNECE, ICP Forests Programme Co-ordinating Centre, Hamburg. ISBN: 978-3-926301-03-1. [http://www.icpforests. org/Manual.htm]

Gessler A, Rennenberg H, Keitel C. 2004. Stable isotope composition of organic compounds transported in the phloem of European beech - Evaluation of different methods of phloem sap collection and assessment of gradients in carbon isotope composition during leaf-to-stem transport Plant Biology 6: 721-729.