FunDivEUROPE

Functional significance of forest biodiversity in Europe

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Micro-organism sampling

FunDivEUROPE (FP7) field protocol

V1.0

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1 Introduction

Soil micro-organisms (including Fungi, Bacteria, Protists) form the dominant group of biota in terrestrial ecosystems, both in terms of biomass, number of individuals, and number of species. It is estimated that only 5% of the species is described (Jeffery et al. 2010). They are considered crucial for litter decomposition, nutrient turnover and soil fertility. It is known that soil type and tree species through their litter quality have a strong influence on the abundance and composition of soil micro-organisms. In general terms rich soils and nutrient rich litter would lead to dominance of bacteria, while poor soils and nutrient poor litter tends to give dominance to Fungi.

There are several methods to determine biomass and diversity of micro-organisms (Swift & Bignell 2001; Campbell et al. 2003, Classen et al. 2003, Gaublomme et al. 2006). In the FunDivEUROPE project we opted for fast medium-budget methods to gather information from a large amount of plots.

2 Scope and application

The idea is to investigate the effect of tree species diversity on biomass and functional diversity of micro-organisms. All these features will be further related to earthworm abundance and diversity, litter decomposition and humus form.

3 Objectives

The objectives of this task are to determine total microbial biomass (in g per g of soil) and to determine functional diversity of bacteria using Biolog ecoplates.

4 Location of measurements and sampling

4.1 Field sampling design

4.1.1 Number of replicates

The (30 x 30) m^2 plots are divided in nine (10 x 10) m^2 subplots. Five subsamples per horizon (organic and mineral) are taken, one in each corner and one in the centre 10 x 10 m subplot. The subsamples are put together in a composite sample for each horizon, which will be used for determination of microbial biomass and bacterial functional diversity.

4.1.2 Sampling scheme

The **Exploratory plots** are 30 x 30 m and subdivided in nine 10 x 10 m subplots. In five subplots a subsample is taken in the dedicated zone for soil biological sampling. In principle, within that dedicated zone, the subsample is taken randomly, but samples should not be taken close to tree stems, and whenever possible, locations in between trees of different species should be preferred over crowns of single species. The sampling sheet is filled in before taking the sample.

The sampling scheme in the **Experimental plots** can be different and will be decided together with the site managers, based on the used mixing pattern.

4.2 Sampling equipment

The following equipment is needed in the field:

- A half open corer (e.g. gouge auger with sampling cylinder with diameter 5-10 cm and height 10-25 cm
- Two transparent **polyethylene** bags of 3 litres per plot with a closing zipper and a label for writing sample code
- Garden shear
- Wooden frame of 25 x 25 cm
- A 20 or 30 cm plastic ruler
- Trowel
- Knife
- A cooling box at 4° C with a plastic recipient to put samples
- Disposable gloves (one pair per plot)
- The Micro-organism sampling data sheet
- A permanent marker
- A pencil
- A Set of sterilizing equipment:
    - Gas burner and lighter
    - Bucket with lid
    - Disinfectol* in spray bottle
    - Paper towel
    - Container for Disinfectol recovery (it can be filtered to be reused)
    - Distilled/deionised Water
    - Wire brush
    - A clean box to store sterilized equipment

* C_2H_5OH, denatured with up to 5% ether

The following equipment is needed in the field station (central place near the plots where samples are temporarily stored and pre-treated).

- Refrigerator for two plastic bags per plot; dark and temperature at 4° C
- Freezer to cool cold packs for field cooling box and for mailings to the lab

- Packing material to prepare mailings for courier

4.3 Frequency of sampling

Sampling is done only once in every plot, for the Experimental Platform in 2011, and for the Exploratory Platform in 2012. The period of sampling is spring and autumn, more specifically a period with humid soil conditions and positive temperatures. In spring sampling can start from the moment that all the snow is melted and no night frost occurs (probably March in Mediterranean, April in cold temperate and early June in boreal zone). In autumn, sampling can be done starting from full litterfall period onward (probably September in boreal, October in cold temperate and November in Mediterranean zone).

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

Sample collection

If ectorganic layer is thick (average 2 centimetre or more without fresh fallen leaves), follow procedure A; if ectorganic layer is thin (average <2 cm without fresh fallen leaves), follow procedure B. To recognize transition from ectorganic layer (OL, OF and OH horizons) to mineral soil, remark that OH is generally mat brown or black coloured, while mineral soil is more greyish and shiny (more details in the ICP Forests soil manual, annex 7, Cools & De Vos, 2010).

PROCEDURE A. Put a new pair of gloves. If ground vegetation is present, cut it away just above the ground with a garden shear on an estimated 20 by 20 cm square. Then remove freshly fallen undecomposed litter, as well as branch material of more than 0.5 cm diameter from the same area. Take now a sample with the corer. Use the knife to separate the ectorganic layer from the soil. Put the ectorganic sample in the labelled polyethylene bag for the composite ectorganic sample. Then use the ruler and the knife to cut off the upper 5 cm of the soil sample and put it in the labelled polyethylene bag for the composite soil sample. Repeat the procedure in all five subplots and add subsamples to the composite samples in the respective bags for ectorganic horizon and soil.

PROCEDURE B. Put a new pair of gloves. If ground vegetation is present, cut it away just above the ground with a garden shear on an estimated 20 by 20 cm square. Then remove freshly fallen undecomposed litter, as well as branch material of more than 0.5 cm diameter from the same area. Put the 25 x 25 cm wooden frame on the ground in the middle of the cleaned area. Then remove the ectorganic horizon from within the frame and put it in the labelled polyethylene bag for the composite ectorganic sample. Use trowel or knife to carefully scrape the bottom of the sample. Sample OL, OF and OH, but not the mineral soil (see above). Then the soil sample is taken in the middle of the area where the ectorganic horizon was removed, using the corer. Use the ruler and the knife to cut off the upper 5 cm of the soil sample and put it in the labelled polyethylene bag for the composite soil sample.

Repeat the procedure in all five subplots and add subsamples to the composite samples in the respective bags for ectorganic horizon and soil. When the ectorganic layer is absent or sparse, traces of the ectorganic layer on the soil surface are removed and only the mineral soil is sampled.

When sampling is finished, put the composite sample(s) in the cool box. The samples are ready for transport to the field station. Now the equipment must be cleaned before going to the next plot. Clean the core cutter with metallic brush to take away the soil. If necessary use water. Clean also the knife and trowel if used. Above the bucket, rinse the instruments with Disinfectol and set fire to them. (NOTE: beware for forest fire; verify compatibility of this procedure with local regulations). Place the sterilized instruments in the clean box. Recover Disinfectol from the bucket into the container. Afterwards it can be filtered for reuse. Throw used gloves in a disposal bag.

Sample storage
Sample bags are stored in plastic recipients in the refrigerator in darkness at 4° C (control temperature). Storage time: max. 4 days, then they have to be sent to the lab by courier.

Transport
At the end of the week all collected samples are taken from the refrigerator and packed together with cooling elements in an insulated box in order to bring or send them to the lab for analysis. Samples need to be kept at more or less 4° C. It is therefore recommended to experiment with the right amount and position of the soil samples and cool elements in the insulated box. Inappropriate packing of the soil samples easily leads to samples which are to warm or frozen.

5 Datasheet Micro-organism sampling

should contain:

- Date of sampling:
- Name(s) of sampler(s):
- Weather conditions (general weather description at the day of sampling: temperature, precipitation, cloudiness):
- Plot number:
- Diversity level:
- Observations made:
- Problems encountered (indicate for which subsample):

6 References

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