Fundamentals in Ecology

Practical guidelines

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1. Main objectives of the practical

In this course you will:

- Learn basic field and laboratory methodologies used to understand the structural and functional properties of terrestrial and freshwater communities and ecosystems.
- Visualize, analyze and interpret data obtained in the field and laboratory during the experiments.
- Report and discuss the results of the experiments.

2. General structure of the practical

The practical work will be carried out in two different types of ecosystems: in an **aquatic** and a **terrestrial** ecosystem (Figure 1). Although similar questions will be addressed for the two ecosystems, the unique nature of each ecosystem determines the choice of approaches and methodologies.

Terrestrial Ecosystem

Response variables:
Plant performance: stomatal conductance, chlorophyll content and fluorescence, growth, biomass allocation

Factors to test:
- Drought: water supply
- Heat: air temperature
- Other treatments from your suggestions

Additional measurements:
soil water content, soil temperature, air temperature, air relative humidity, photosynthetic active radiation

Aquatic Ecosystem

Response variable:
Leaf-litter decomposition

Factors to test:
- Physical habitat (*pool vs. riffle*)
- Litter quality (*labile vs. recalcitrant, deciduous vs coniferous plants*)
- Oxygen availability (buried in sediment vs streambed)
- Terrestrial vs. aquatic

Auxiliary measurements:
stream water temperature, electrical conductivity, pH, velocity, dissolved oxygen concentration

Figure 1: Short description of the terrestrial and aquatic experiments performed during the practicals in Fundamentals of Ecology

3. Study area

The selected terrestrial and aquatic study sites are located within the boundaries of the EPFL Campus (Figure 2). This selection has been chosen to minimize both travel time and logistics.

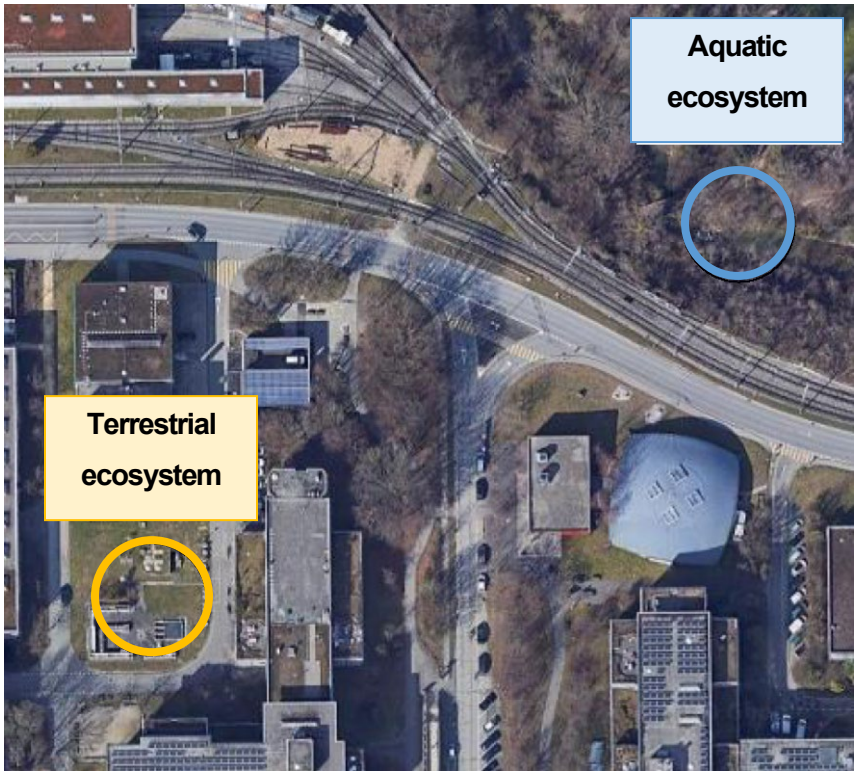

Figure 2: Location of study sites on the EPFL campus, including the river Sorge and its adjacent riparian forest.

4. Organization of groups, schedule, report and grades

Group organization

You will work in groups of 5 students on selected topics. The organization of the groups will be done before or latest during the introductory session on Moodle. Every group will repeatedly take measurements at each of the indicated dates (see timetable). The members of the group thus have to share responsibilities and workload.

Schedule

The practicals include theoretical and practical sessions to provide you all the information needed to design, perform and report an experiment in ecology (Figure 3).

The first sessions aim to introduce the importance of research in ecology, the experiments and demonstrate the field and lab equipment. You will then prepare the materials for the experiments: leaf litter bags for the decomposition experiment in the aquatic groups, and pots with plants for your own experiments in the terrestrial groups. Along with these practical aspects, you will learn the basic theory behind the measurements, visit the field sites to understand the experimental design and learn how to use the measuring devices.

After this initial phase, the groups will carry out field and laboratory work and obtain measurements independently, supervised by teaching assistants. Measurements will be taken four times over the course of the semester.

Between the measuring sessions, you will learn how to use the software R to organize, analyze and visualize the data generated during the experiments and how to write a scientific report. The last session will be reserved for questions and answers.

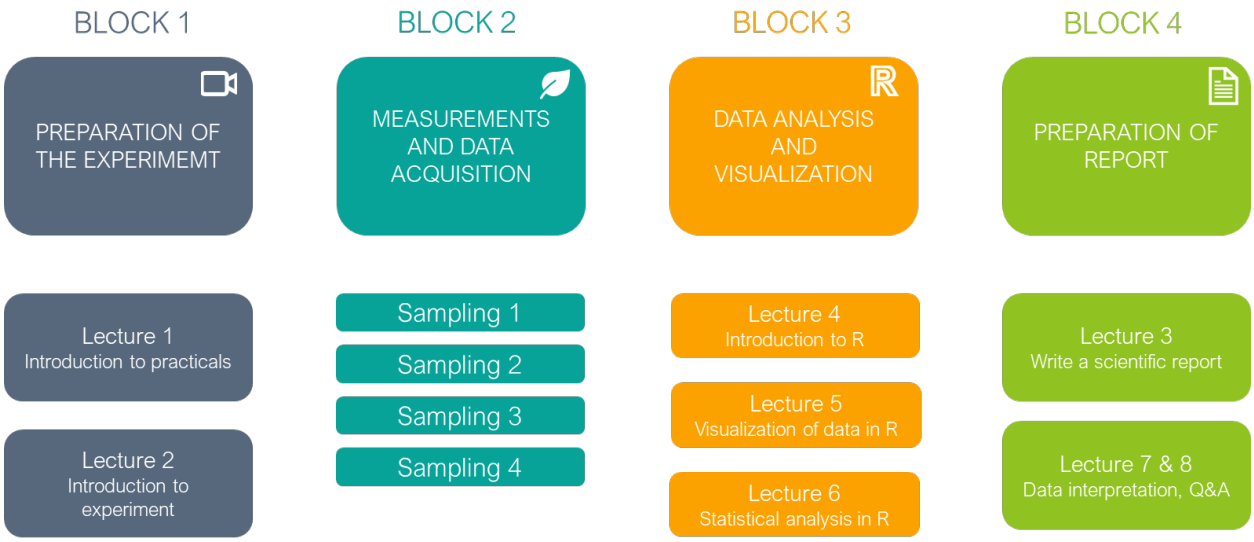

Figure 3 Schematic representation of the course layout. The course is organized in thematic blocks with multiple dedicated sessions per block.

Report

During the practicals you will learn how to write a scientific report. The format will be the same as a typical scientific paper, including: 1) an *Introduction* section, describing the scientific background of the study; 2) a *Material and methods* section, describing the experimental design, measurements performed and statistical analyses; 3) a *Results* section, including plots of data and describing the results of the statistical analyses; 4) a *Discussion* section, interpreting the results and describing their implications in a wider context, related to what you wrote in the introduction and building on what you learned during the lectures of Fundamentals in Ecology.

At the end of the semester, you will need to submit a scientific report (deadline indicated on moodle), including all the above-mentioned sections. The essay should be written in Times New Roman, 12-point font, structured in paragraphs and *should not exceed 5 pages*. For the Introduction and the Discussion, you will need to read and cite scientific literature (include at least 5 citations from scientific journals, as for example Limnology and Oceanography; Ecology). Rubrics will be uploaded onto Moodle to further guide development.

Grades

For the practical part of Fundamentals in Ecology, you will be graded based on the group performance in the written final report. The grade will be per group. The grade of the practicals will count for 40% of the final grade in Fundamentals in Ecology.

5. Aquatic Ecosystem: Experiment with leaf-litter decomposition

Background

There are two primary sources of energy in aquatic ecosystems: (1) photosynthesis by algae, mosses, and higher aquatic plants; and (2) imported organic matter from adjacent terrestrial ecosystems, either in particulate (e.g., leaves and other parts of vegetation) or dissolved form (i.e., dissolved organic matter, DOM). In small and shaded streams, there is usually insufficient light to support substantial instream photosynthesis. Thus, energy pathways are largely supported by imported (allochthonous) organic matter. In such streams, the bulk of imported organic matter usually enters as dead leaves during leaf-fall in autumn. Leaves falling into streams may be transported for short distances, but are usually caught by structures in the streambed forming "leaf packs". Leaf packs are then "processed or decomposed" by components of the stream community in a series of steps (Figure 4).

Litter decomposition is defined as the process through which dead organic material is broken down into particles of progressively smaller size and initially organic molecules are mineralized to their prime constituents: CO_2, H_2O, and mineral components (Swift et al., 1979). It is a complex process that involves: (1) leaching of soluble compounds, (2) fragmentation of litter into smaller sizes, (3) catabolism by decomposer organisms (for instance: bacteria, fungi and invertebrates). Therefore, it is influenced by the chemical and physical properties of the decomposing plant material (resource quality), physical forces acting on leave litter, the community of decomposers and by environmental conditions such as temperature and nutrient availability.

At early stages, leaves entering streams leach soluble nutrients into the water. After leaching, structural materials like cellulose and lignin remain, neither of which are easily digestible by most animals. Within a few days of entering the water, fungi and bacteria begin to colonize the leaves leading to a process known as "microbial conditioning" (Bärlocher and Kendrick 1975). Microorganisms produce a suite of enzymes that can digest the remaining leaf constituents and begin the conversion of leaves to smaller particles (Suberkropp and Klug 1976).

After about two weeks, leaves undergoing microbial conditioning begin to soften and fragment. The reduction in particle size from whole leaves to coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM) is generally thought to occur through the feeding activities of a variety of aquatic invertebrates collectively known as "shredders" or "detritivores" (Cummins 1974). The particles then serve as food for a variety of micro- and macroconsumers. Leaves may also be fragmented by physical or mechanical factors such as current and abrasion (Benfield et al. 1977, Paul et al. 1978).

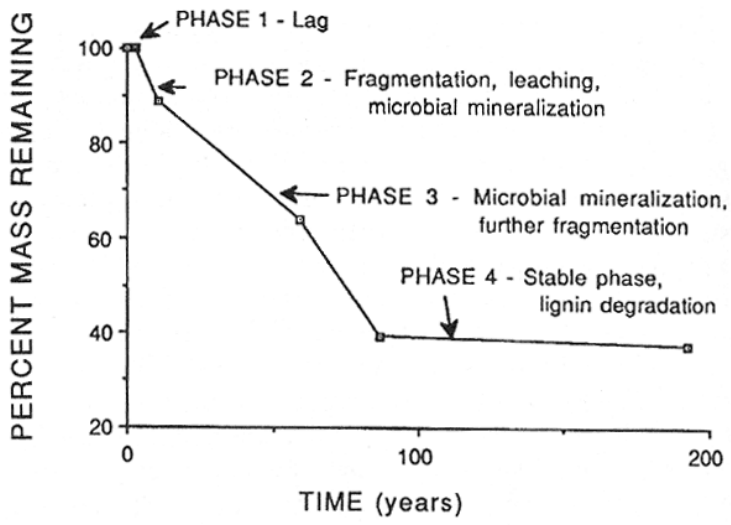

Figure 4 Phases of leave litter degradation

Leaves from different plant species break down at different rates. Differences in the rates at which "fast," "medium," and "slow" species break down in a particular stream appears to be mostly a function of initial physical and chemical properties of leaves (Webster and Benfield 1986). Species-specific breakdown rates may vary with stream, location in the stream, time of year, activity of microbes, presence of shredders, and other stream-specific factors (Webster and Benfield 1986).

Overall, leaf-litter decomposition is thus an integrative, ecosystem-level process because it links terrestrially derived matter and energy sources to various elements of stream ecosystems (i.e., microbial activity, invertebrates, and physical and chemical features of the stream).

Experimental design and factors to test

The overall process of measuring leaf-litter decomposition rates involves placing pre-weighed "leaf packs" in a stream, periodically sampling those leaf packs over time, and estimating the rate at which the packs lose mass in the stream. Specifically, you will construct bags for the incubation (mesh size appropriate to in/exclude macroinvertebrates). Then you will weigh dried leaves and packed known quantities into the bags. These bags will then be anchored at a specific location in the stream. Over the course of the study, bags will be retrieved regularly, cleaned of debris and invertebrates, dried, combusted and weighed. Auxiliary parameter, such as stream water temperature, dissolved oxygen concentration and electrical conductivity will be measured at each sampling occasion. Decomposition rates (-k) are computed using an exponential decay model that assumes the rate of mass loss from the packs is a constant fraction of the amount of material remaining. Operationally, -k is the negative slope of the line produced by a linear regression of the natural log of percent leaf mass remaining plotted against time.

Protocol for leaf-litter decomposition experiments

1. Construct mesh bags from netting or similar material such as poultry fencing, hardware cloth, or large mesh plastic screen (Figure 5). Construct enough mesh bags to sample 3 replicates per treatment for 4 time points.

2. Select leaf litter material from previously air-dried leaves. Weigh out 5 to 10 g (± 0.25 g) portions of leaves on an analytical balance, and fashion them into leaf packs. Use approximately the same mass of leaves for each pack. Record the initial dry mass $(DM)_{t=0}$ on the data sheet. Select a method to label the leaf packs/bags. Color coded plastic "embossing" tape stapled to the bags works well, as do strips of "flagging tape" tied to the bags.

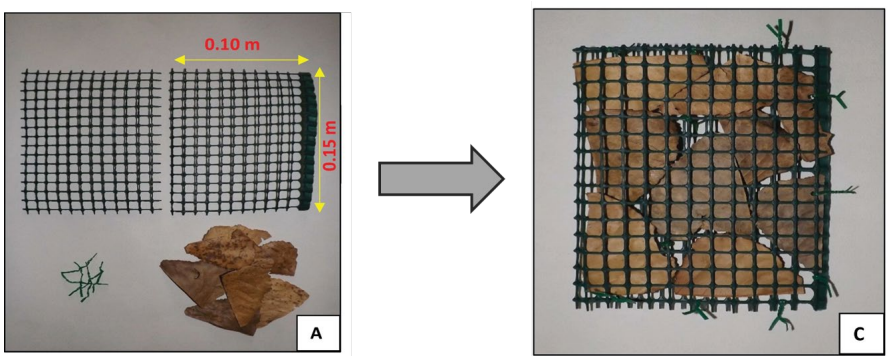

Figure 5 Bags for leave litter degradation experiments

3. Transport all the bags to the study site, handling carefully to avoid unnecessary breakage.

4. Packs must be secured in the study site. Use steel rods driven into the streambed to anchor the leaf packs.

5. At every sampling occasion, remove the appropriate number of leaf packs and place each pack into individual Zip bag. Include an internal label (pencil or permanent marker on waterproof paper) identifying the sample with all pertinent information (e.g., retrieval date, site). Write the same information on the outside of the Zip bag, using a permanent marker. Return the samples to the laboratory.

6. Apart from retrieving the leave litter bags, use the hand-held field sensors to measure the basic physical and chemical properties of the stream (water temperature, conductivity and dissolved oxygen). Record the results of the field measurements. Similarly, sample 3 cobbles of approximately 7 cm diameter for further Chlorophyll-a analysis in the laboratory. Insert the cobbles into additional pre-labelled Zip bags. These samples are stored frozen in the lab and processed all together at the end of the experiment (see below).

7. In the laboratory, remove the leaf material from the bags and gently rinse the leaves of silt and debris. Because the decomposition model represents loss from the original mass, ignore small leaf fragments that may have been lost from the original mass but retained by the bag.

8. Place the cleaned leaves into the pans (Figure 5) and dry in a hot air oven at 45-50 °C. It is very important to label the pans by scratching the metal surfaces with a metal pointer (and impressing a code using a metal probe). After drying, weigh leaf material and record the dry mass $(DM)_{t=f}$ on the data sheet.

9. Regress the natural log (ln) of mean dry mass (y-axis) on days of exposure (x-axis). The negative slope of the regression line is equal to the decomposition rate (-k).

Figure 6 Bags and pans containing the leaves for drying and ashing. Make sure to label the pans by indenting

6. Terrestrial Ecosystem: Plant performance in contrasting environments

Why do we need experiments with plants?

As sedentary and in some cases quite long-lived organisms, plants need to adjust to their environment in effective ways to ensure their survival and reproduction. Plant performance can be limited by a wide variety of environmental factors, such as water and light availability, air temperature, soil nutrient supply, but also the presence of environmental stressors like salt, heavy metals, etc. (Schulze et al. 2005). In particular, with rising global air temperatures under climate change, heat and drought stress are becoming increasingly frequent and severe (Dai 2013, Spinoni *et al.* 2018), with devastating impacts on terrestrial ecosystems (Allen *et al.* 2015). Plant species differ quite heavily in their responses to a changing environment. By subjecting plants to contrasting situations in a controlled experiment, we are able to gain insight on how plants may deal with situations that we don't yet observe in nature, but that might become common with changing climatic conditions. While we can study plants *in situ* and assess their responses to current climatic conditions, experiments allow to extend the range of environmental conditions and help to create scenarios about the future of the studied species and ecosystems (Beier et al. 2012).

Objective

The goal of this project is to give the students practical knowledge in plant ecology by applying simple plant physiological measurements in an experimental setting. You will design your own experiment by choosing one of the following environmental factors to test on an ivy plant (*Hedera helix*): drought (low *vs.* high water supply) or temperature (heating *vs.* ambient condition). For the treatments, different time intervals can be chosen, as for example continuous or intermitted (impact and recovery). You will investigate the growth and performance of the selected plant in the chosen experimental condition with repeated physiological measurements and at the same time monitor soil and atmospheric conditions.

Measurements

Plant performance can be measured with a wide variety of methods, depending on the focus of the study. As plant performance fundamentally relies on the assimilated carbon provided by photosynthesis, we will focus on measurements around this process (but we will not measure photosynthesis directly, because it is very time consuming). The measurements will thus include (1) stomatal conductance, estimating plant transpiration and therefore water loss, (2) chlorophyll content and chlorophyll fluorescence, as indicators of the capacity and efficiency of the photosynthetic process, (3) growth: counting newly formed leaves and shoot elongation, (4) plant total leaf area, indicating total surface available for photosynthesis and (5) shoot vs. root biomass allocation as indication of the resources invested into above- or below-ground organs. Environmental measurements revolve around the measurements of plants performance and thus include most limiting factors for plant growth, such as (1) soil water availability, (2) air temperature and (3) light availability.

Study site

The study site is on the EPFL campus in a grassland south of the LESO building (see map Figure 2). Different structures are built to set the environmental conditions.

- Drought: a roof that intercepts rainfall, reducing water availability
- Temperature: a deep plastic roof that increases temperature with light radiation, heating up the plants underneath it
- Control: a designed site to place your control plants under optimal conditions

Experimental design and statistical analysis

You will test the effect of the environmental factors by comparing the growth and performance of the plants in the two selected conditions (for example: drought condition *vs.* wet condition). You will test the treatment effects using a T-test with the statistics program R, which will be introduced during the course. Three ivy plant per pots (pseudo-replicates) in three pots (replicates) will be planted for each conditions, making a total of 6 pots. Only the pots (i.e., the replicates) will have to be considered for the statistical analyses, and the way to deal with this will also be introduced during the course. The pseudo-replicates (i.e., the three plants per pot) are used to ensure the survival of at least one plant during the whole experiment, on which to perform the measurements.

Protocol

1. Experiment set-up:

- Choose and describe the experimental design with a sketch of the sites, pots and plants.
- Set up the experiment: Label the pots you will use during your experiment with an indication of your group number and the treatment you will apply. Then pot your individual plants in each pot and label the plants you will measure with a ribbon (to ensure continuity in your measurements). Measure the shoot length and count the number of leaves of all selected plants to assess initial conditions.

2. Field measurements 1-3:

A) Soil conditions

- Measure soil temperature with a digital temperature meter at 10–15 cm soil depth at the centre of the pot.
- Measure soil humidity with a TDR (FieldScout) at 10–15 cm soil depth. Repeat the measure three times at different places to account for variability.

B) Plant measurements

Be careful to select the same plant for the measurements at all sampling dates, because individuals might differ in their properties (i.e., always measure the one you marked with a ribbon the first day of field measurements).

- Stomatal conductance with a Porometer
- Chlorophyll content with a Chlorophyllmeter
- Chlorophyll fluorescence with a FluoroPen
- Number of new leaves and elongation of the shoot (growth)

Please, the material is expensive so handle it very carefully!!

3. Field measurements 4:

A) Soil measurements: same as Measurements 1-3

B) Plant measurements: same as Measurements 1-3

C) Additional measurements:

- Average leaf area: estimate the total leaf area of the plant you performed your measurements on (calculating the leaf area of all leaves on the shoot). You will take pictures of all the leaves for each selected plant and measure the leaf area using the software ImageJ, following a protocol that will be provided on Moodle.
- Above- vs. below-ground biomass: calculated on dried leaves + shoot biomass and dried root biomass. The different parts will be collected and cleaned during the last measurement and dried in the oven for two days. Their weight will need to be measured individually by every group the next week during an individual session in the lab.

4. Environmental measurements

To assess the environmental conditions experienced by your plants during the experiment, we installed small meteorological stations in the grassland and under the shelter that record continuously:

- Air relative humidity (RH, %) at 50 cm height from the ground
- Air temperature (Tair, °C) at 50 cm height from the ground
- Photosynthetic active radiation (PAR, W/m^2) at 150 cm height from the ground (only in the grassland)
- Rainfall (mm) (only in the grassland)

The corresponding data will be provided to you at the end of the measuring period, so that this information can be used to interpret your results and included in your report.

References

Beier C, Beierkuhnlein C, Wohlgemuth T, et al (2012) Precipitation manipulation experiments - challenges and recommendations for the future. Ecol Lett 15:899–911. https://doi.org/10.1111/j.1461-0248.2012.01793.x

Schulze E-D, Beck E, Müller-Hohenstein K (2005) Plant Ecology. Springer