BugNet Evolution-Protocol

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Aim

The experimental part of BugNet is using invertebrate herbivore and fungal exclusion experiments to quantify plant community and ecosystem responses to insects, mollusks and fungal pathogens in a wide range of herbaceous-dominated ecosystems. The BugNet exclusion experiments will also allow to test whether and how rapidly plants respond evolutionarily to an experimental pest suppression. It can be assumed that by excluding or decreasing impact of insect, mollusks or fungi, plants will reduce investment into defense but will increase allocation to growth and reproduction. Using the suppression of pest groups alone and in combinations provides rare opportunity to explore how multiple pest groups drive evolution of plant defense. By collecting leaf and seed material in the beginning we will be able to address evolutionary questions at some point in the future.

Selection of species

We should identify the most dominant species of each site, ideally each species should occur in all the plots in sufficient numbers. Of those species, select 3-5 species (depending on your time), and try to select species from different plant families. We try to focus on the plant families **Poaceae**, **Asteraceae, Fabaceae and Cyperaceae**, and if you have dominant species from those families these should have priority. However, other families are also fine if your site does not contain species that occur in all plots in sufficient numbers from those families. Avoid sampling long lived shrubs. The focus on a few target plant families ensures that we have some replication per plant family.

Sampling per species

1) Sampling leaves

For each species, please sample leaves from 5 individuals per plot (ideally from different locations within the 2.5 x 2.5 m core subplot, but try to avoid the 25 cm buffer zone).

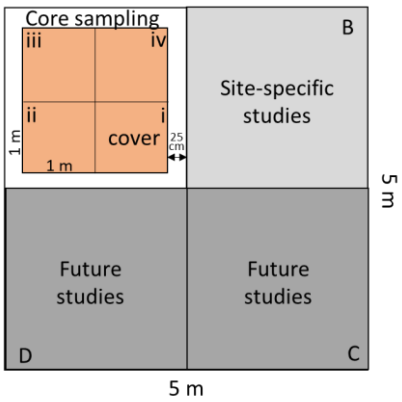

Fig. 1. For each selected species, leaves from five individuals should be collected in the core sampling area excluding the 25 cm buffer zone (red). The individuals should be evenly spread out throughout the plot. Leaves should be placed in tea or coffee filters for storage.

Per individual, collect at least 3 leaves (at least 0.5 g fresh weight), for small leaves rather more (we need 25 mg and 100 mg dry leaf material for the analysis of leaf metabolites and genetic analysis, respectively). Select young, visibly healthy, green leaves. Put the leaves separated per individual in labelled tea or coffee filters (labelled with Year, Plot, Species, Individual number (from 1-5)).

If leaves are very moist, air-dry them for a little bit and then put the tea bags or coffee filters into zip-lock bags filled with ca. 30-50g of silica gel (the equivalent of an espresso cup). We can add the five individuals per species and plot together in one zip-lock bag. You will ideally have samples of 24 x 5 individuals per species in the end (24 zip-lock bags per species).

Please also make one herbaria specimen voucher for each plant species (plant can be from outside the plot). If you are not sure with identification of the species, let us know. Collect the material at peak growing season.

Fig. 2. Leaves should be placed in tea or coffee filters for storage.

Please send us the dry leaves in silica-gel and the herbaria specimen voucher, ideally in the same package when you also send us your biomass samples. We will archive them in a safe place in Davos. Please wrote Anne Kempel an email before sending (anne.kempel@slf.ch). She will send you a permit that you should place inside the package. Please send the package to:

Anne Kempel
WSL Institute for Snow and Avalanche Research SLF
Flüelastrasse 11
7260 Davos, Switzerland

2) Sampling seeds

We also want to store seeds for potential future experiments. Ideally, we will **harvest seeds from the same five individuals** that were used to collect leaves. Sometimes it will not be feasible to collect both leaves and seeds at the same time. In this case, plants should be marked after collecting leaves to return to the same individuals to collect the seeds. If it is not feasible to return to the same individuals, it is also ok to collect seeds from five different individuals. Put the seeds separated per individual in labelled paper bag (Year, Plot, Species, Individual Number (1-5)) and keep in dry and well-aerated place. Number of available seeds will vary across the species. To secure a sufficient number of seeds for future experiments, we will also do **bulk sampling of seeds.** This means that we will collect seeds from several individuals per plot to obtain at least 100 seeds per species and plot, and place them together in one labelled bag per plot (24 paper bags per species and plot). However, less than 10% of seeds in the plot should be harvested. The dry seed samples should also be sent to Anne Kempel for long term storage (see address above).

Timing – when should I sample and how often?

Start of sampling. Every site can start sampling leaves and seeds in 2023, even if you have already applied the treatments the years before. Sample at peak biomass and when seeds are ripe. The treatments should be applied at the beginning of the growing period.

Repeated sampling. We will repeat the leaf and seed sampling **every 3 years** to be able to track changes in metabolites and other traits over time. However, it can also be performed on an annual basis if capacity allows. In this case never sample more than 10% of the seeds per plot.

Analyses & experiments

An exact design of future analyses & experiments is not clear yet. However, we plan to use leaves for the analysis of metabolites. Leaves will also be used for genetic (genomic) analyses to differentiate between treatment-induced changes versus nonadaptive evolution such as genetic drift. Seeds will be used for common garden experiment to analyze an anticipated divergence between pesticide-treated and not-treated plots in plant defense traits and investment to growth.