The "Bug-Network" – Experimental part

A global assessment of the impact of invertebrate herbivores and pathogenic fungi on plant communities and ecosystems

Focal research questions

1. Do insect herbivores, molluscs and fungal pathogens differ in their impact on plant communities? And do they interact with each other?
2. When do invertebrate herbivores and fungal pathogens have the strongest effects on plant communities (productivity, community composition, diversity and functioning)?

BugNet Goals

Global research networks can rigorously test for general patterns and mechanisms and several, such as the NutNet or Drought-Net, have led to important advances. The goal of BugNet is to survey consumer and plant communities across sites and set-up identical insect herbivore, mollusc and fungal exclusion experiments in many parts of the world.

BugNet aims to implement a cross-site study requiring minimal investment of time and resources by each investigator. Firstly, we will conduct a comparative study to investigate how the functional composition of invertebrate communities changes along abiotic and biotic gradients. Secondly, we will initiate an experimental study to quantify plant community and ecosystem responses to invertebrate herbivores and fungal pathogens in a wide range of herbaceous-dominated ecosystems, such as desert grasslands to arctic tundra, but also heathlands or Mediterranean shrublands.

Protocol

1.1. Selection of sites

The site should be relatively homogeneous, dominated by herbaceous or shrub vegetation. Natural disturbances, such as fire or browsing by vertebrates, do not need to be excluded from the site, but a record of the disturbance regime, and ideally a quantification of vertebrate herbivory, is required. It is preferable that the site is not heavily grazed by livestock. Grazed sites can be included if the plots are fenced, though.

During the experiment, the sites should be managed as it is common in the area, i.e. if the grasslands are mown once or twice a year, then the experimental site should also be mown. In this case make sure to plan your measurements to take place at peak plant biomass.

1.2. Setting up the experiment

The experiment will be a randomized block design with three blocks, 8 treatments, and three replicates per treatment (N = 24 total experimental plots, Fig. 1). Each experimental plot will be 5 x 5 m in size, separated from the other plots by a 1m walkway. Each 25m^2 plot will be subdivided into four 2.5 x 2.5 m subplots (A, B, C, D), with one dedicated to the core sampling, one to additional site-specific studies and two for future network-level research (e.g. exclusion of oomycetes, warming treatment, …). The position of the treatments in the three blocks should be randomly assigned, and also the subplots should be randomly assigned to the different uses. The subplot dedicated to the core sampling will further be divided into four 1m x 1m small plots (i, ii, iii, iv), with the one located closest to the centre designated for the assessment of species composition (cover, i). The other three small plots will be designated for destructive sampling such as the assessment plant biomass, or herbivore and pathogen damage (see Fig. 1).

To quantify the impact of different consumer groups, they will be excluded (reduced) using biocides. Treatments will involve the removal of consumer groups alone, i.e. insects, mollusc, and fungi, in all possible two-way combinations, all consumer groups together and a control, giving a total of 8 treatments (Fig. 1).

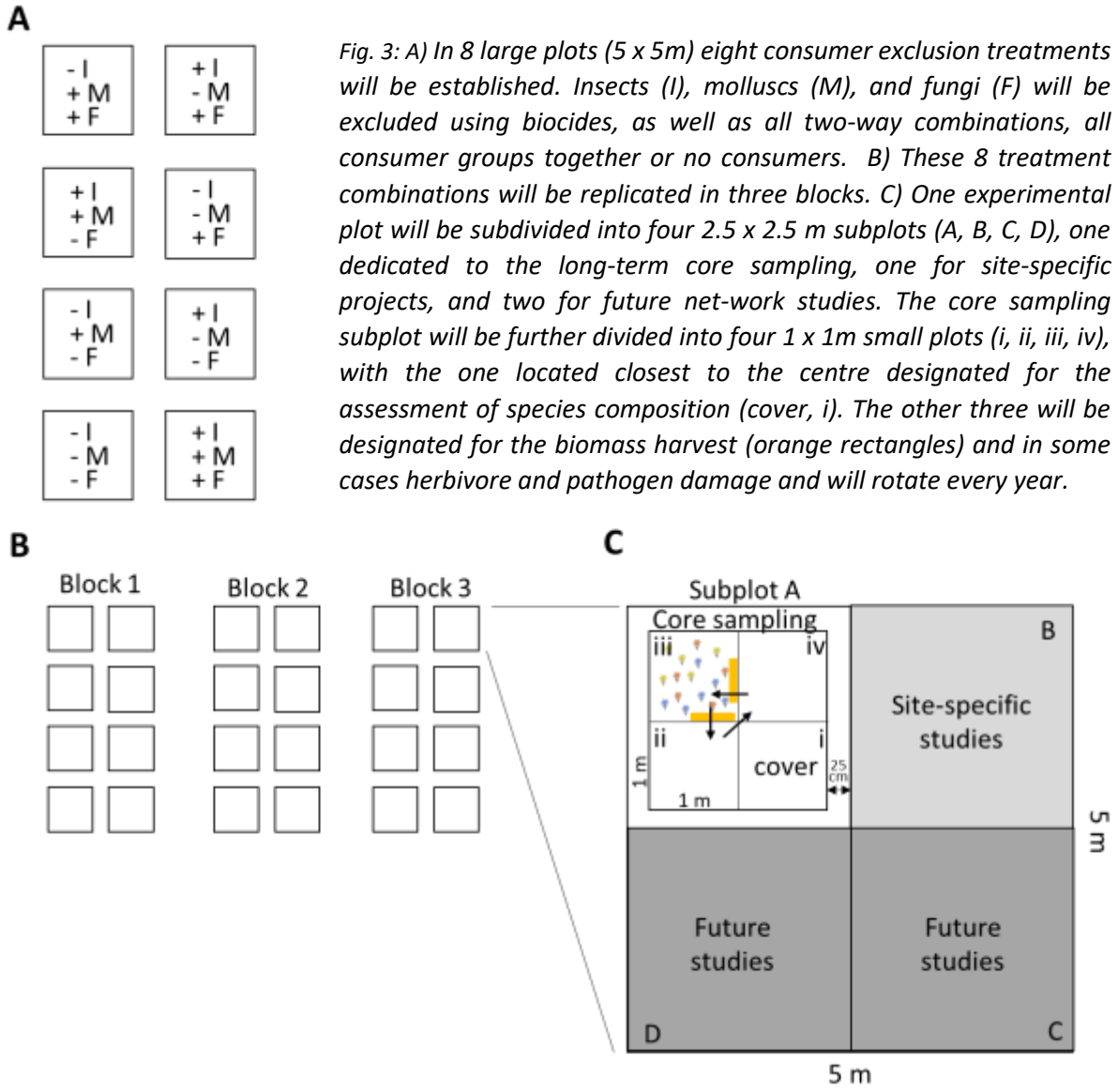


Fig. 3: A) In 8 large plots (5 x 5m) eight consumer exclusion treatments will be established. Insects (I), molluscs (M), and fungi (F) will be excluded using biocides, as well as all two-way combinations, all consumer groups together or no consumers. B) These 8 treatment combinations will be replicated in three blocks. C) One experimental plot will be subdivided into four 2.5 x 2.5 m subplots (A, B, C, D), one dedicated to the long-term core sampling, one for site-specific projects, and two for future net-work studies. The core sampling subplot will be further divided into four 1 x 1m small plots (i, ii, iii, iv), with the one located closest to the centre designated for the assessment of species composition (cover, i). The other three will be designated for the biomass harvest (orange rectangles) and in some cases herbivore and pathogen damage and will rotate every year.

1.3. Treatment applications

To control insect herbivores, we will use Lambda-Cyhalotrhin (e.g. Karate Zeon, Syngenta), which is a broad spectrum, non-systemic insecticide frequently used in herbivore exclusion studies and with few non-target effects. It will be sprayed four times during the growing season. To control foliar fungi a combination of azoxystrobin and difenoconazole (azoxystrobin inhibits fungal mitochondrial respiration, Difenoconazole interrupts the synthesis of ergosterol, a fungal cell membrane component, e.g. a mix of Score Profi and Ortiva, Syngenta), will be spayed four times during the growing season. To control molluscs molluscicide pellets based on ferric phosphate (e.g. Limax Ferro, Syngenta) will be applied four times during the growing season. It might be that in some countries some biocides are not approved. If this is the case, please contact us and we will discuss alternative products that can be used. Biocides may not wipe out infestation, but they do significantly reduce

enemy attack on plants and are so far the only experimental approach to assess the importance of invertebrate herbivores and pathogens in natural plant communities.

1.4. Measurements per site – Baseline data (prior to treatment application)

To characterize the different sites around the globe, several measurements of soil conditions, and invertebrate communities and plant traits will be taken. This allows us to link consumer impact to several drivers (latitude, altitude, soil fertility, above- and belowground consumer and predator abundance and characteristics, diversity, and biomass), and to shed light at the context dependency of biotic interactions.

If you want your experimental site to be part of the comparative study and the manuscripts involved, the baseline measures are the same as those described in the "comparative part" and are described under 1.4.1. Thus, collaborators doing the experimental part and assessing all the required data under 1.4.1 automatically participate in the comparative part.

If you don't want to do the extra work, and only want to participate in the experimental study, the baseline data you need to assess is much less work and you can go directly to 1.4.2.

1.4.1. Measurements if you want your experimental site to be part of the comparative part

Sampling of the aboveground invertebrate community

To test whether there are large scale patterns in invertebrate community characteristics, the invertebrate community of a site will be characterized using suction sampling. It is preferable to do this at the very beginning of your sampling, as invertebrates may move away when you are working on the plots.

In seven randomly chosen plots (out of the 24 plots), you should cover an area of 0.16 m^2 of vegetation in the middle of the plot dedicated for site specific studies with a cylindrical, fine-meshed gauze-cage of 45 cm diameter (Ikea laundry basket, see detailed protocol) to prevent insects from escaping. You will sample invertebrates within the cage following a standardized protocol with a leaf blower set to suction mode (Stihl SH86), equipped with a fine gauze-bag inserted into the suction tube. Transfer the samples to plastic containers filled with 70% ethanol. If possible, sort the invertebrates into major groups (orders, see detailed protocol), count them and classify them as herbivores or predators if possible (this will be difficult for several groups such as Heteroptera, Diptera and Coleoptera), and send them to the project coordinators for further measurements. If you will not be able to sort and classify the samples, this will be done by the project coordinators. You can find detailed protocols on how to sample invertebrates, as well as ideas on where to find leaf blowers to borrow here.

https://youtu.be/2h3R-QQsnqA

Plant species composition

As baseline plot measures, estimate the percent plant cover per plant species in each of the 24 plots, in the subplot dedicated to the core sampling, in the small plot located closest to the centre (see Fig. 1, cover). Cover for each plant species rooted within the plot will be estimated to the nearest 1% (up to 20% cover) and the nearest 5% for cover 20-100%. Assign 0.5% to very rare species with less than 1% cover. Estimate also the percent cover for woody over storey, bryophytes, litter, bare soil, and rocks if present. Total cover will typically exceed 100% because species cover is estimated independently for each species (see cover datasheet). Take these measures before the application of the treatments. This means that data from the experimental field sites can be used in several analyses immediately.

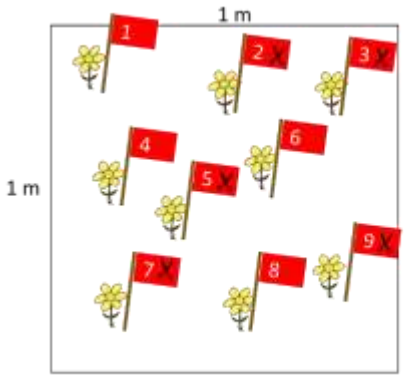
To reduce bias in cover assessment, it is helpful to train yourself by placing differently sized pieces of paper on the plot: e.g. 10cm x 30cm = 3 %, 10cm x 10cm = 1 %, 3.1cm x 3.2cm = 0.1%, 31cm x 32cm = 10 % …

Herbivore damage and fungal infection

As a baseline measure of herbivore damage and pathogen infection per site, you will score damage and infection in ten randomly chosen plots, similar to the comparative part. Assess herbivore and pathogen damage species-wise. If you work in systems that are regularly mown, you can assess the damage in the small plot dedicated for the cover estimation (small plot i). If you work in very unproductive systems and removing individuals for the assessment of damage might have strong influences on the vegetation (as regrowth is very slow), then please assess damage in one of the small plots dedicated for destructive sampling (small plots ii, iii or iv, Fig. 1). Alternatively, you can assess damage in the cover small plot i without removing plants.

Selection of species: Start with the species having the highest cover, followed by the species with the second highest cover, and so on, until the cover-sum of the species reaches 80% (relative cover excluding bare ground, rocks). However, do not assess damage on more than five plant species per plot. E.g. if Species A has a cover of 50%, Species B a cover of 20%, Species C a cover of 10% and Species D a cover of 8% assess damage on A, B and C. If Species A has already a cover of 90%, then only assess cover on species A. If Species A, B, C, D, and E have each a cover of 15% and species F, G, H, I… a cover of 5% then only assess damage on the five most abundant species (A,B,C,D and E) although the sum of their cover does not add up to 80%.

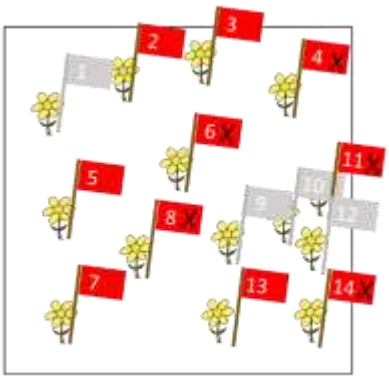
Selection of individuals per species: If your species has less than 10 individuals, select all individuals. If your species has between 10 and 20 individuals per plot, mark all individuals in the $1m^2$ with grill sticks numbered from 1-20. Use a random number generator (or quickly select 10 numbers in your head) to select 10 individuals (it is important that the individuals are selected randomly, and that there is no bias towards particularly damaged or undamaged ones). If your species has more than 20 individuals, divide your $1m^2$ plot into four quadrants, and estimate the proportion of individuals in each of the four quadrants. Randomly select individuals per quadrant in proportion to their numbers of individuals, e.g., if quadrant 1 contains 80% of all individuals, and quadrant 3 20%, then randomly select eight individuals of quadrant 1 and two of quadrant 3. Particularly if the distribution of your species is very patchy (e.g. one large patch with many individuals, and 3 isolated individuals) this methods prevents that you will select isolated individuals with a higher likelihood (see Fig. 2).



Random numbers:
7
20
10
2
18
17
9
15
16
14
3
5
12
8
6
13
4
19
1
11

Species has 1-10 individuals

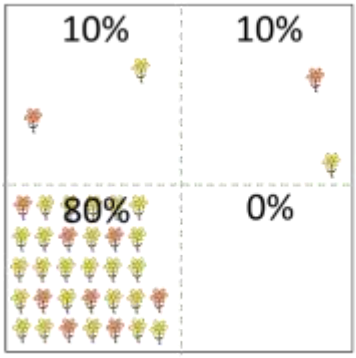
If a species has ten or less than ten individuals assess presence/absence of damage signs on all of them (incidence, bold). To select five individuals for the assessment of % damage, mark all individuals with your numbered grill-sticks, and cut the individuals with the five Random-numbers that appear first (red numbers, scissors).



Random numbers:
18
17
14
4
20
11
6
8
13
5
3
7
15
2
9
19
1
10
16
12

Species has between 11-20 individuals

If a species has between 11 and 20 individuals, mark all individuals with your numbered grill-sticks, and select the individuals with the 10 Random-numbers that appear first (bold). Assess the presence/absence of damage signs on all of them. To select five individuals for the assessment of % damage, cut the individuals with the five Random-numbers that appear first (red numbers, scissors).



Species has > 21 individuals

If a species has > 20 individuals, divide the 1m² in four quadrats and estimate the proportion of individuals in each quadrat. Randomly select individuals per quadrat in proportion to their numbers of individuals, i.e. randomly select 8 individuals in the quadrat that contains 80% of all individuals, and each one individual in the quadrats that contain 10% of all individuals. Assess the presence/absence of damage signs on all ten individuals. To select five individuals for the assessment of % damage, mark them all with your numbered grill-sticks and proceed as above.

Fig. 2: *Selection of individuals per plant species. For each selected plant species, select 10 individuals on which you will assess the presence/absence of damage signs of the different damage categories (incidence). Of these 10 individuals you will select five individuals which you will cut at ground level (respectively a subset of them), bag, and bring to the lab for the assessment of % leaf damage and the measurements of plant traits.*

For more guidance – this youtube video gives a tutorial on how to select species and individuals for damage incidence and damage % measurements.

https://youtu.be/em7nRyWr-mE

Measurements: On each of the selected individuals per species (max. of 10), record the presence or absence (0,1) of damage signs by chewing, mining, galling and sucking/rasping herbivores, and

pathogen disease symptoms of the categories downy mildews, powdery mildews, rusts and leaf spots (see damage gallery). This will give us an estimation of *damage incidence*.

In addition, randomly pick five of the ten individuals (select them using the grill sticks with the first five random numbers from your random-number list), and measure the maximal **height** (stretch out if necessary, see section on plant traits below). Then, cut them at ground level. If your individual is very large, or builds large tussocks as is often the case for grasses, then take a subsample from the middle of the individual which contains at least five leaves. If you selected only one plant species per plot because this species had a relative cover of 80% or more, then cut all 10 individuals. On these individuals you will estimate the **leaf area (%)** that has been **damaged**. You can either do this directly in the field, or you can bag the plants in labelled plastic bags, place them in a cooler, bring them to the lab, and do the % damage assessment there. In any case, visually survey five *random*, mature, and non-senescing leaves (or leaflets if your leaves are very small) per individual for damage and disease symptoms. For easier inspection you can use hand lenses to better assign damage types. On each of the five leaves estimate the leaf area (%) that has been removed by chewing herbivores, mining, galling and sucking/rasping herbivores, and the leaf area that is covered by pathogenic disease symptoms of the categories downy mildews, powdery mildews, rusts and leaf spots. Some plant individuals will have fewer than 5 leaves, and for these all leaves should be surveyed (but leave out senescent leaves). Note that in some cases, damage is present on only the underside of leaves, so remember to check both sides of the leaf for damage. You can check out our detailed protocol on how to assess damage and also our photo gallery of the most common damage types. We also highly recommend that you train your damage assessment with the ZAX Herbivory Trainer app by Zoe Xirocostas and Angela Moles!

With this method you assess damage on a minimum of 10 individuals (if one species had a cover of 80% or more) or on up to 25 individuals (if you selected 5 plant species with each 5 individuals) per plot. Five individuals per selected species and plot will be used to measure several plant leaf traits. Your bagged plants (if you do the assessment of % damage in the lab) can be used for this, see below.

UPDATE: The damage assessment is very laborious, and we realized that we slightly underestimated the time needed for this task. We therefore offer the possibility to assess the % leaf area damaged in only five instead of the ten plots. Incidence and height should be measured in all ten plots.

Shrublands: If you work in shrublands, you may not find 10 individuals of a species in your $1m^2$ plot but very likely will have only one or two individuals. You may also have spreading shrubs where it can be difficult to distinguish individuals. In this case, randomly pick 50 leaves from 10 different branches per shrub species throughout the plot (5 leaves per branch). The five leaves per branch should always originate from the same leaf position, i.e. from the tip of each branch, count the first ten leaves and start sampling from there (leaf 11 to 15). Make sure that the 10 branches are from either as many shrub individuals as possible, or else take branches from different positions and orientations within the shrub patch. Assess how many of those 50 leaves are damaged. This allows us to assess the proportion of leaves that are damaged by the different damage categories per shrub individual (incidence). In addition, assess the % leaf area damaged on at least 25 randomly chosen leaves per shrub species.

If your shrub species is leafless and instead has photosynthetic stems (e.g. *Retama ssp*.), instead of picking leaves, randomly cut 5 cm pieces from 10 different branches throughout the plot. On these,

assess how many of those pieces show damage signs of the different damage categories (incidence). In addition, on five of those branches, assess the % photosynthetic area that is damaged.
You can assess % damage either in the field or collect the leaves to assess damage in the lab. In any case you should collect a few leaves to measure several plant traits (SLA and LDMC), except on species that have photosynthetic stems where SLA and LDMC cannot be measured. These leaves for plant trait measurements should ideally not contain any damage symptoms.

UPDATE: The damage assessment is very laborious, and we realized that we slightly underestimated the time needed for this task. We therefore offer the possibility to assess the % leaf area damaged in only five instead of the ten plots. Incidence and height should be measured in all ten plots.

Plant traits

At each site**,** several plant traits - plant height, specific leaf area (SLA) and leaf dry matter content (LDMC) - will be measured to characterize the plant communities. These traits are closely associated to two major axis of plant functional variation, the size of plants and their parts, and the resource economics spectrum (Wright et al. 2004, Díaz et al. 2016). You will measure the traits according to protocols in Garnier et al. 2001. The traits will be measured on the same species that you have selected for the assessment of herbivore and pathogen damage. The height will be directly measured in the field, on five individuals per selected species. Use the same five individuals that you have selected for the % damage assessment. SLA and LDMC will be measured on three individuals (or subsets of the individuals) per selected species. For this, pick three random individuals per selected species (these can be the same individuals that you used to assess the % damage), bag them in a labelled plastic bags and place them in a cooler. If possible, your individuals have > 5 leaves without any damage symptoms, as ideally the leaf traits are measured on undamaged leaves (see detailed protocol on how to measure SLA and LDMC).

In addition to this, height, SLA and LDMC of all species at a site should be measured. This is important to test whether the response of plants to enemy exclusions follow patterns predicted by defense-deployment strategies (e.g. growth defense-tradeoff). For each plant species present at a site, five individuals per site should be randomly sampled, and their height, SLA and LDMC assessed. As you will already have measured traits on most of the species from a site within the 10 plots, you only need to measure plant traits of the species that you haven't measured yet. These measurements can also be done at a later stage but should be completed after the fourth year.

Aboveground biomass

As baseline plot measures of plot productivity in each of the 24 plots, in one of the small plot dedicated for destructive sampling (ii, iii or iv), clip the aboveground plant material to 2 cm above ground level, in two 10cm x 50cm strips (orange rectangles, Fig. 1). Collect the total aboveground biomass, dry it for 3 days at 70 °C and weigh it. Send a subsample of the dry biomass samples to the project coordinators. If you can grind the biomass to powder, that would be ideal, but if not you can cut the biomass sample in pieces and send us a well-mixed subsample. Please send us ca. 20 g of dry weight per plot (e.g. in a zip-block plastic bag or a jar, see labelling protocol). We will use the samples to measure several leaf characteristics (leaf N and P, fibre content etc.) and to identify the phyllosphere microbiome. Take these measures before the application of the treatments. This means that data from the experimental field sites can be used in several analyses immediately.

If you work in shrublands the biomass of the shrub species will be estimated using allometric equations. For each shrub species, measure the height and canopy diameter of 20 individuals of contrasting sizes outside of your experimental plots. Clip, dry and weigh them. If your shrubs form big

patches and individuals are difficult to isolate, just take 20 “sampling units” with known height and canopy diameter, and collect the biomass of this sampling unit instead. To be able to obtain a measure of shrub green vs brown biomass, please separate the leaves from the woody biomass. For some species this might work better once the leaves are dry. Now measure the height and canopy diameter of all shrubs in the subplot dedicated to the core sampling, in the small plot in which you also assess the plant cover (Fig. 1, cover).

Soil samples

Soil cores will be collected to assess a range of soil characteristics. In each of the ten plots, collect two soil cores (soil corer 2.5 x 10 cm) and homogenize the soil into a single sample per site. Please sieve the soil through a 2 mm mesh. Soils should be air-dried and send to the project coordinators (see labelling and mailing protocol sheet for more details). There, total organic C, total N and P stocks, as well as mineral N (ammonium, nitrate) and P will be measured and will give information on soil characteristics at the site level.

In addition, we would like to obtain information of the soil microbial community at the plot level. The soil microbial community is likely to change in response to the pesticide treatments. To be able to analyse this change at some point in the future, it is necessary to have information on pre-treatment conditions. We therefore encourage you to store soil samples **per plot** prior to the application of the treatments in a freezer:

Please take **five soil** cores (e.g. 2.5 x 10 cm) from random locations per plot with a soil corer, and homogenize the soil into a single sample per plot. Make sure to carefully clean your soil corer between plots. We ask you to store **50g** of soil per plot in a labelled zip block bag or any other container at -20°C, and 5g of soil per plot at -80°C (if you have access to such a freezer). Don’t forget to label your bags well, as they might sit in the freezer for a few years before we analyse the microbial community at some point in the future.

1.4.2. Measurements – experimental part only

If you only want to participate in the experimental study, you only need to assess baseline plant species composition and biomass per plot. Additionally, we ask you to collect soil and to assess several plant traits of all plant species in your site:

Plant species composition

As baseline plot measures, estimate the percent plant cover per plant species in each of the 24 plots, in the subplot dedicated to the core sampling, in the small plot located closest to the centre (see Fig. 1, cover). Cover for each plant species rooted within the plot will be estimated to the nearest 1% (up to 20% cover) and the nearest 5% for cover 20-100%. Assign 0.5% to very rare species with less than 1% cover. Estimate also the percent cover for woody over storey, bryophytes, litter, bare soil, and rocks if present. Total cover will typically exceed 100% because species cover is estimated independently for each species (see cover datasheet). Take these measures before the application of the treatments. This means that data from the experimental field sites can be used in several analyses immediately.

To reduce bias in cover assessment, it is helpful to train yourself by placing differently sized pieces of paper on the plot: e.g. 10cm x 30cm = 3 %, 10cm x 10cm = 1 %, 3.1cm x 3.2cm = 0.1%, 31cm x 32cm = 10 % …

Aboveground biomass

As baseline plot measures of plot productivity in each of the 24 plots, in one of the small plot dedicated for destructive sampling (ii, iii or iv), clip the aboveground plant material to 2 cm above ground level, in two 10cm x 50cm strips (orange rectangles, Fig. 1). Collect the total aboveground biomass, dry it for 3 days at 70 °C and weigh it. Send a subsample of the dry biomass samples to the project coordinators. If you can grind the biomass to powder, that would be ideal, but if not you can cut the biomass sample in pieces and send us a well-mixed subsample. Please send us ca. 20 g of dry weight per plot (e.g. in a zip-block plastic bag or a jar, see labelling protocol). We will use the samples to measure several leaf characteristics (leaf N and P, fibre content etc.) and to identify the phyllosphere microbiome. Take these measures before the application of the treatments. This means that data from the experimental field sites can be used in several analyses immediately.

If you work in shrublands the biomass of the shrub species will be estimated using allometric equations. For each shrub species, measure the height and canopy diameter of 20 individuals of contrasting sizes outside of your experimental plots. Clip, dry and weigh them. If your shrubs form big patches and individuals are difficult to isolate, just take 20 "sampling units" with known height and canopy diameter, and collect the biomass of this sampling unit instead. To be able to obtain a measure of shrub green vs brown biomass, please separate the leaves from the woody biomass. For some species this might work better once the leaves are dry. Now measure the height and canopy diameter of all shrubs in the subplot dedicated to the core sampling, in the small plot in which you also assess the plant cover (Fig. 1, cover).

Plant traits

At each site**,** several plant traits - height, SLA and LDMC of all species should be measured. This is important to test whether the response of plants to enemy exclusions follow patterns predicted by defense-deployment strategies (e.g. growth defense-tradeoff). For each plant species present at a site, five individuals per site should be randomly sampled, and their height, SLA and LDMC assessed. You will measure the traits according to protocols in Garnier et al. 2001. If possible, your individuals have > 5 leaves without any damage symptoms, as ideally the leaf traits are measured on undamaged leaves (see detailed protocol on how to measure SLA and LDMC). These measurements can also be done at a later stage but should be completed after the fourth year.

Soil samples

Soil cores will be collected to assess a range of soil characteristics. In each of ten randomly chosen plots, collect two soil cores (soil corer 2.5 x 10 cm) and homogenize the soil into a single sample per site. Please sieve the soil through a 2 mm mesh. Soils should be air-dried and send to the project coordinators (see labelling and mailing protocol sheet for more details). There, total organic C, total N and P stocks, as well as mineral N (ammonium, nitrate) and P will be measured and will give information on soil characteristics at the site level.

In addition, we would like to obtain information of the soil microbial community at the plot level. The soil microbial community is likely to change in response to the pesticide treatments. To be able to analyse this change at some point in the future, it is necessary to have information on pre-treatment conditions. We therefore encourage you to store soil samples **per plot** prior to the application of the treatments in a freezer:

Please take **five soil** cores (e.g. 2.5 x 10 cm) from random locations per plot with a soil corer, and homogenize the soil into a single sample per plot. Make sure to carefully clean your soil corer between plots. We ask you to store **50g** of soil per plot in a labelled zip block bag or any other container at -

20°C, and 5g of soil per plot at -80°C (if you have access to such a freezer). Don't forget to label your bags well, as they might sit in the freezer for a few years before we analyse the microbial community at some point in the future.

1.5. Annual measurements per plot

Plant species composition

Once annually, estimate the percent plant cover per plant species in the subplot dedicated to the core sampling, in the small plot located closest to the centre (see Fig. 1, i, cover). Cover measures follow the same protocol as for the baseline data. In systems in which species composition shifts strongly within the year or which have a two-times mowing regime, we recommend that species composition is assessed twice, once in spring/early summer and once in late summer. This allows us to account for differences in phenology and to capture the maximum cover of each species.

Herbivore damage and fungal infection

If you work in a system that is regularly mown, measure the herbivore damage and fungal infection per plot in the small plot dedicated for the plant cover (small plot i), using the same method as described for the baseline data. If your system is very unproductive and removing individuals for damage assessment would have strong influences on the vegetation, then measure the herbivore damage and fungal infection in one of the other small plots dedicated for destructive sampling (ii, iii, iv), or alternatively, assess damage in the cover small plot without removing individuals. Damage does not need to be assessed every year and we will communicate the years in which the damage should be assessed.

Above- and belowground biomass

To quantify consumer impact on productivity (top-down control), clip the aboveground plant material to 2 cm above ground level, in two 10cm x 50 cm strips of each core sampling subplot, in one of the small plots that are used for destructive sampling (ii, iii, iv). Each year, the biomass harvest should be done in a different small plot (see Fig. 1).

If you work in very unproductive systems which are not mown, and in which the removal of biomass might still be visible after three years, then try to not overlap the stripes for biomass harvest for as long as possible (see example of harvest positions, Fig. 3).

Fig. 3: In very unproductive systems, in which plants grow very slowly and the removal of biomass might still be visible after a few years try to not overlap the stripes for biomass harvests for as much time as possible.

Collect the total aboveground biomass, dry and weigh it (the two subsamples per plot can be combined). Sampling should be done at peak biomass production (the timing of peak biomass will vary between sites and will be defined by local researchers for their system). If your site has a two-times mowing regime, biomass should be collected twice per year to better estimate site productivity

(before the cuts). Send the dry biomass samples to the project coordinators. We will then measure several leaf characteristics (leaf N and P, fibre content etc.). Please send us ca. 20 g of dry weight *per plot* (e.g. in a zip-block plastic bag or a jar, see labelling protocol).

Root biomass will be measured as standing root biomass in year 3 of the experiment: take a soil core of 5cm diameter, 30 cm deep, and sort to separate roots.

If you work in shrublands the biomass of the shrub species will be estimated using the allometric equations that you have developed for the baseline measures. Measure the height and canopy diameter for all shrubs in the subplot dedicated to the core sampling, in the small plot in which you also assess the plant cover (Fig. 1, cover, i).

1.6. Optional measurements per site

There will be the possibility to propose additional measurements. We will also hold several workshops and discussion groups where we can develop ideas together.

Time estimation

TASK	Estimated time per plot in min	Estimated time all plots in min
Baseline measurements Field:		
insect suction in seven plots	4 min	28
Cover in 24 plots	Ca. 20 min	480
Damage incidence in 10 plots	Ca. 16 min	160
Height in 10 plots	3 min	30
Plant biomass in 24 plots	5 min	120
Soil cores in 10 plots	5 min	50
Plant traits of all species at the site		30 min
Lab:		
Leaf damage (max of 25 ind per plot)	35	350
Leaf area (max. of 25 ind per plot)	35	350
Leaf area of additional species		60
Baseline measurements	**TOTAL**	**=1658 min (27.5 hours = 3.5 days)**
Annual measurements Field:		
Cover in 24 plots	20 min	480
Cover in 24 plots (damage small plot)	20 min	480
Damage in 24 plots	16 min	384
Biomass in 24 plots	5 min	120
Lab:		
Leaf damage (max of 25 ind per plot)	35 min	840
Annual measurements	**Total**	**2304 min (38.5 hours = ca. 4.8 days)**
Optional:		
Insect sorting		

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