The “Bug-Network” (BugNet)

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

Background

A wide range of organisms attack plants and they have strong impacts on primary production, plant community assembly and coexistence, and ecosystem processes such as nutrient cycling (Weisser and Siemann 2004, Duffy et al. 2007, Borer et al. 2014, Terborgh 2015). While a few studies have shown big impacts of invertebrates and fungal pathogens, we know little about how generally important they are, and how their functional composition varies across a range of different ecosystems.

A lot of theory predicts that consumer impact varies across environmental gradients (van Euler et al. 2014). Impact is likely to depend on abiotic conditions at large spatial scales such as climate (latitude, altitude) and plant productivity, but also on abiotic and biotic drivers operating at smaller spatial scales, such as plant diversity and soil fertility (bottom-up) and predator abundance (top-down). Our understanding of how consumer communities and their impact varies across environmental gradients is surprisingly limited (Moles and Ollerton 2016, Jia et al. 2018). Existing studies differ substantially in methodology, making generalities across large scales difficult, which calls for comparative approaches that implement standardised protocols across sites. This is particularly important if we are to understand how global change drivers, such as climate and land use change, will alter consumer communities and their functioning.

A first step in understanding how consumer impact varies along environmental gradients is to determine how consumer communities vary. In particular, it is important to understand how variation in abiotic conditions (principally climate) directly impacts consumer communities and how environmental variation alters plant communities (productivity, functional composition) and thereby indirectly affects consumers. The second step is to understand how consumer impact changes along environmental gradients. To robustly assess impact, exclusion experiments are necessary. Only a few studies have explored the impact of certain consumer groups, such as belowground invertebrates and fungal pathogens (Borer et al. 2015), and we know little about whether the impact of different consumers varies along environmental gradients (Seabloom et al. 2018). Different groups of consumers may also interact (Allan and Crawley 2011) and factorial exclusion are needed to test for these. In general, we could expect compensatory interactions between consumers attacking different, competing, plant species and additive interactions between consumers attacking the same plant species.

A powerful tool to quantify the variation in plant consumer communities and their impact are globally coordinated experiments, using standardized measurements and replicated experiments across ecological gradients. The “Bug-Network” will be such a project and aims to explore the context dependency of biotic interactions within a coordinated research network comprised of many grassland- and shrubland sites worldwide.

Focal research questions

1. How does the functional composition of invertebrate communities vary with abiotic and biotic drivers?
2. How important are direct effects of climate vs. indirect effects of changes in the plant community?
3. When do invertebrate herbivores and fungal pathogens have the strongest effects on plant productivity, plant community composition and diversity (large and small spatial scales, abiotic and biotic drivers, e.g. soil fertility, climate, plant diversity, predator abundance, enemy diversity)?
4. Do above- and belowground invertebrates and fungal pathogens differ in their impact on plant communities? And do they interact with each other?

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 above- and belowground invertebrate herbivore 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 above and belowground 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.

Get involved

There are two ways that you can get involved in BugNet. 1) by participating in a cross-site survey by collecting data on plant- and invertebrate functional community characteristics in your study region ("**comparative study**") and 2) By establishing exclusion experiments in addition, in your herbaceous-dominated study system and committing to be involved in the network for the next five years or longer ("**experimental study**").

Preliminary protocol

1. Comparative study (Europe focussed)

In this study we will investigate variation in invertebrate and plant community characteristics along environmental gradients (e.g. climate, soil fertility). If the implementation of an experiment in your area requires too much of a commitment it is possible to only participate in the observational part. If you are able to set up the experiment (see below) the baseline data that you collect can be used for the comparative study. The comparative study will focus initially on a climatic gradient within Europe but we hope to expand the analysis to a global comparison later.

1.1. Selection of sites

The plant and invertebrate communities in at least two herbaceous communities should be assessed. If you participate in the experimental study, then only the baseline data of one site is required. The climatic conditions should be similar between the two plant communities but they should differ in their plant functional composition, for instance due to variation in soil conditions, to disentangle the direct (climate) and indirect (plant characteristics) drivers of changes in the invertebrate community. Each site should be relatively homogeneous, dominated by herbaceous or shrub vegetation, and representative of a particular ecosystem (e.g. shortgrass steppe, tallgrass prairie, heathland). 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 grazed by livestock. The sites should be visited at peak biomass production (the timing of peak biomass will vary between sites and will be defined by local researchers for their system).

1.2. Measurements per site

Soil samples

Soil cores will be collected to assess a range of soil characteristics. In 10 randomly chosen locations per site, soil cores (soil corer 2.5 x 10 cm) should be taken and homogenized into a single sample per site. Soils should be air-dried and send to the project coordinators. There, total organic C, total N and P stocks, as well as mineral N (ammonium, nitrate) and P will be measured.

Plant species composition

The percent plant cover per plant species will be estimated in ten 1m x 1m plots. Plots should be randomly distributed across the site and should be at least 3m apart from each other. 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%. Percent cover should be also estimated for woody over storey, litter, bare soil, disturbance (animal digging), and

rocks if present. Total cover will typically exceed 100% because species cover is estimated independently for each species. We will offer a training tool to reduce bias in cover assessment.

Aboveground biomass

To quantify site productivity, in each of the ten **1m x 1m plots** aboveground plant material will be clipped to 5cm above ground level, in two 20cm x 50cm strips, and total aboveground biomass will be collected, dried and weighed. Dry biomass samples should be sent to the project coordinators and will be used to measure several leaf characteristics (leaf N and P, fibre content etc.).

Herbivore damage and fungal infection

To assess herbivore damage and pathogen infection per site herbivory will be assessed on 20 plants in each of the ten 1m x 1m plots (200 plants per site). To select plants per plot, randomly place a stick in each of the 20 grids indicated in the plot scheme (green dots, Fig. 1) and pick the nearest rooted individual to the stick. The plants will be also used to assess plant traits (see below). Assess herbivore damage, pathogen infection, and plant species identity of each plant. Damage and infection caused by different feeding guilds or pathogen groups should be recorded separately. We will provide clear instructions on how to estimate damage and infection, and will provide a training set of digital leaves to estimate and improve precision and bias.

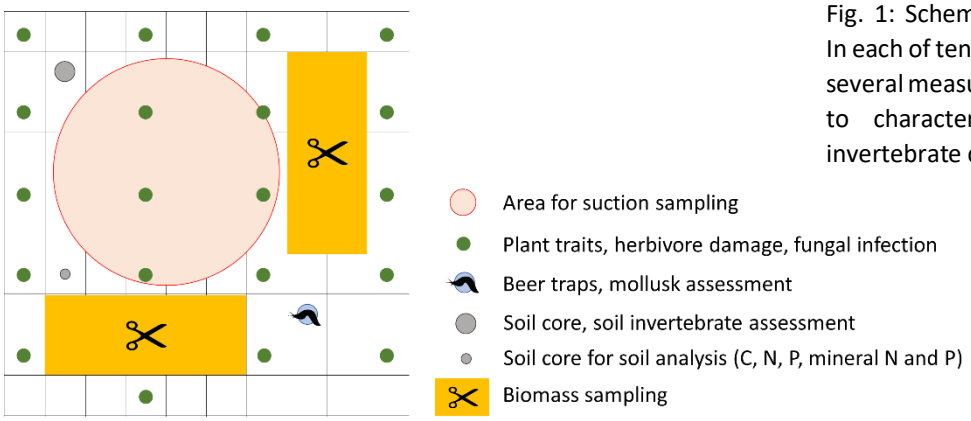

Fig. 1: Schematic sampling design. In each of ten 1m x 1m plots per site several measurements will be taken to characterize the plant and invertebrate community.

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 axes of plant functional variation, i.e. the resource economics and size spectra (Wright et al. 2004, Díaz et al. 2016). Traits will be measured according to protocols in Garnier et al. 2001. To reduce the work load community weighted mean trait values will be calculated on the same 20 randomly chosen individuals per plot that were collected to assess herbivore damage and fungal infection (similar to a taxon free approach, Lavorel et al. 2008). On these, height, SLA and LDMC will be measured (we will provide protocols on how to measure SLA and LDMC).

Sampling of the aboveground invertebrate community

To assess whether impact is related to consumer or predator characteristics (consumer or predator abundance, diversity, biomass, functional composition), and to test whether there are large scale patterns in invertebrate community characteristics, the invertebrate community of a site will be characterized using suction sampling. In 5 of the ten $1m^2$ plots, an area of $0.25m^2$ of vegetation will be covered with a cylindrical, fine-meshed gauze-cage of 56 cm diameter (laundry basket) to prevent insects from escaping. Invertebrates within the cage will be sampled following a standardized protocol with a leaf blower set to suction mode (Stihl SH86), equipped with a gauze-bag inserted into the suction tube. Samples will be transferred to plastic containers filled with 70% ethanol. If possible, invertebrates will be sorted into major groups (orders), counted and classified as herbivores or predators, and will be send to the project coordinators for further measurements. If sorting and classification is not possible, this will be done by the project coordinators. We will provide detailed protocols on how to sample invertebrates.

1.3. Optional measurements per site

Sampling of molluscs and belowground invertebrates

Mollusc abundance will be assessed using 5 randomly placed pit-fall traps baited with beer (Heineken or Carlsberg). Molluscs will be counted, dried and weighed. To characterize belowground invertebrates, 10 randomly placed soil cores should be taken and invertebrates should be extracted using Tullgren Funnels and stored in ethanol. We will provide detailed information on how to easily build Tullgreen funnels for the collection of the living soil organism. Ideally, soil invertebrates will be sorted into major groups, counted and classified as detritivores, herbivores or predators and samples will be send to the project coordinator for further size-related measurements. If sorting is not possible, this will be done by the project coordinators.

Pollinator assessment using pan traps

To characterise the pollinator community at a site, coloured pan trapping is considered a simple, efficient method. We will provide a standard protocol to collect flying insects at a site.

Bird predation rate assessment using plasticine caterpillars

Fake caterpillars using plasticine is a standard method to assess predation rates by birds, rodents, or arthropods. We will provide a standard protocol for the assessment of predation rate using plasticine caterpillars.

2. Experimental study

2.1. Selection of sites

The same conditions as for the comparative part, however, only one site is needed for the experiment. If you can do more that is of course welcome!

2.2. Setting up the experiment

The experiment will be a randomized block design with three blocks, 17 treatments, and three replicates per treatment (N = 51 total experimental plots, Fig. 2). Each experimental plot will be 4 x 4 m in size, separated from the other plots by a 1m walkway. Each 16m^2 plot will be subdivided into two 2x4 m subplots, with one dedicated to the core sampling, and one to additional site-specific studies or future network-level research (e.g. N-addition).

To quantify the impact of different consumer groups above and belowground, they will be excluded (reduced) using biocides. Treatments will involve the removal of consumer groups alone, i.e. aboveground insects, belowground insects, molluscs, aboveground fungi, belowground fungi (5 main factors), in all possible two-way interactions (10 two-way combinations), all consumer groups together and a control, giving a total of 17 treatments (Fig. 2).

2.3. Treatment applications

To control aboveground herbivores, we will use Dimethoate (e.g. Perfekthion, BASF, Ludwigshafen, Germany), which is a broad spectrum, quasisystemic foliar insecticide frequently used in herbivore exclusion studies and with few non-target effects (Stein et al. 2010, Allan and Crawley 2011). It will be sprayed every two weeks during the growing season. To control belowground herbivores we will use Chlorpyrifos, a contact soil pesticide without systemic effects (Stein et al. 2010). To control aboveground foliar fungi a combination of azoxystrobin and propiconazole (azoxystrobin inhibits fungal mitochondrial respiration, propiconazole inhibits fungal demethylation, e.g. Quilt, Syngenta), will be spayed biweekly during the growing season. Alternatively, Difenoconazol alternated with Picoxystrobin could be used depending on local laws. To control belowground fungi a soil drench fungicide containing mefenoxam (inhibits ribosomal RNA synthesis in soil-living fungi) will be used, e.g. Ridomil Gold (Syngenta Crop Protection). Alternatively, Dimethomorph alternating with Epixiconazol, Fenpropiorph and Pyraclostrobin could be used. To control molluscs molluscicide pellets (metaldehyde) will be applied every two or four weeks. All biocides have been used in exclusion studies and have been shown to have no non-target effects (Allan et al. 2010, Allan and Crawley 2011, Borer et al. 2015, Seabloom et al. 2017). It might be that in some countries some biocides are not approved. If this is the case please contact us and we will find 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.

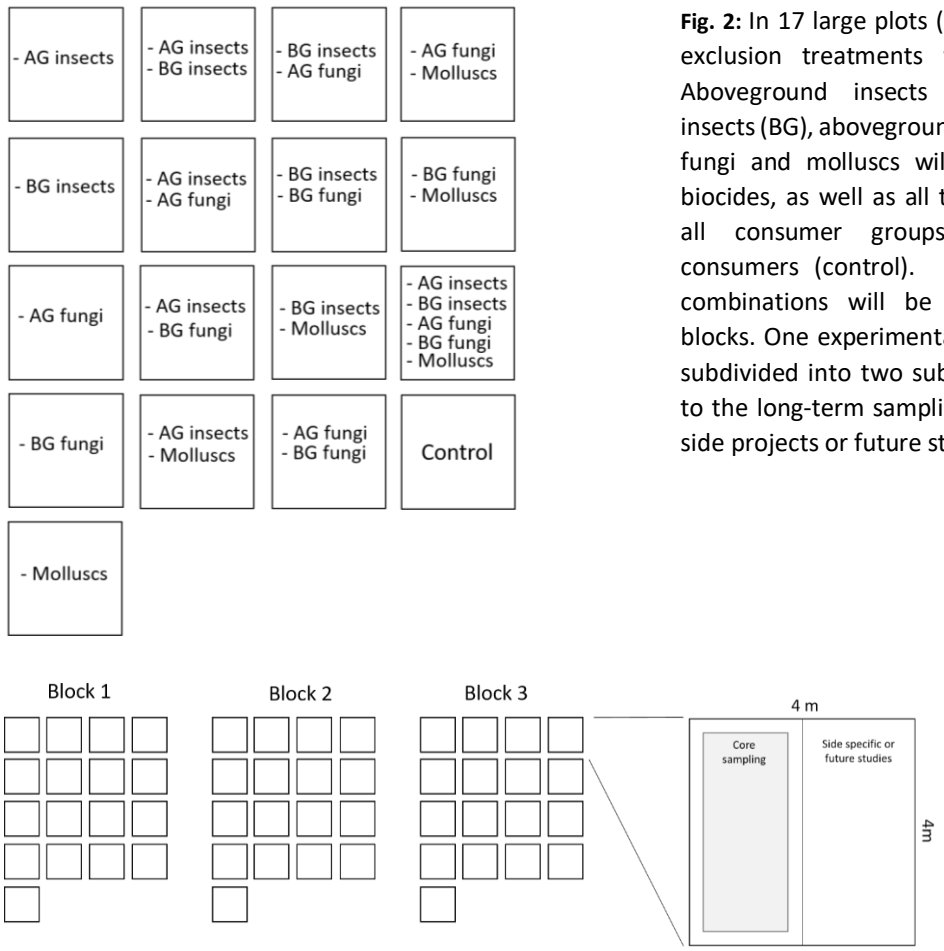

Fig. 2: In 17 large plots (4 x 4m) 17 consumer exclusion treatments will be established. Aboveground insects (AG), belowground insects (BG), aboveground fungi, belowground fungi and molluscs will be excluded using biocides, as well as all two-way interactions, all consumer groups together or no consumers (control). These 17 treatment combinations will be replicated in three blocks. One experimental plot (4x4m) will be subdivided into two subplots, one dedicated to the long-term sampling, the other one for side projects or future studies.

2.4. Measurements per site – Baseline data (see comparative part)

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. The baseline measures are the same as those described in the "comparative part". This means that data from the experimental field sites can be used in several analyses immediately.

In addition to the measurements described above, plant traits of all plant 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, five individuals per site should be randomly sampled, and their height, SLA and LDMC assessed. Samples should be sent to the project coordinators, where leaf nutrients will be measured.

2.5. Annual measurements per plot

Plant species composition

Before the start of the experiment, and once annually, the percent plant cover per plant species will be estimated in the subplot dedicated to the core sampling. Cover measures follow the same protocol as for the baseline data.

Above- and belowground biomass

To quantify consumer impact on productivity (top-down control), aboveground plant material will be clipped to 5 cm above ground level, in two 10cm x 50 cm strips of each core sampling subplot, and total aboveground biomass will be collected, dried and weighed. Sampling will 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). Root biomass will be measured as standing root biomass in year 3 of the experiment: a soil core of 5cm diameter, 30 cm deep, will be taken and sorted to separate roots.

Dry biomass samples should be sent to the project coordinators in the first and third year of the project, to measure several leaf characteristics (leaf N and P, fiber content etc).

Herbivore damage and fungal infection

Herbivore damage and fungal infection per plot will be measured the same way as described for the comparative part.

3. Authorship Guidelines

Researchers will be included as co-authors on BugNet manuscripts if they do the following:

1) To become a co-author of BugNet manuscripts related to the **observational study**, contribution of data from at least two sites is required. This entitles the co-authorship for two scientists (e.g., 2 PIs, or 1 PI + 1 student).

2) To become a co-author of BugNet manuscripts related to the **experimental study**, contribution of data from one site for the duration of at least 4 years is required. This entitles the authorship for three scientists. If you run an experimental site in your area, you will also become a co-author on manuscripts related to the observational study.

Co-authors should also contribute intellectually to either the development of research questions, data analysis, identification of invertebrates, writing and editing.

References

Allan, E., and M. J. Crawley. 2011. Contrasting effects of insect and molluscan herbivores on plant diversity in a long-term field experiment. Ecology Letters 14:1246–1253.

Allan, E., J. van Ruijven, and M. J. Crawley. 2010. Foliar fungal pathogens and grassland biodiversity. Ecology 91:2572–2582.

Borer, E. T., E. M. Lind, E. J. Ogdahl, E. W. Seabloom, D. Tilman, R. A. Montgomery, and L. L. Kinkel. 2015. Food-web composition and plant diversity control foliar nutrient content and stoichiometry. Journal of Ecology 103:1432–1441.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig, Y. M. Buckley, M. Cadotte, C. Chu, E. E. Cleland, M. J. Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Du, J. Firn, Y. Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. A. Klein, J. M. H. Knops, K. J. La Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock, J. Pascual, S. M. Prober, D. A. Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517.

Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet'ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. Nature 529:167–171.

Duffy, J. E., B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thébault, and M. Loreau. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. Ecology Letters 10:522–538.

Garnier, E., B. Shipley, C. Roumet, and G. Laurent. 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. Functional Ecology 15:688–695.

Jia, S., X. Wang, Z. Yuan, F. Lin, J. Ye, Z. Hao, and M. S. Luskin. 2018. Global signal of top-down control of terrestrial plant communities by herbivores. Proceedings of the National Academy of Sciences 115:6237.

Lavorel, S., K. Grigulis, S. McIntyre, N. S. G. Williams, D. Garden, J. Dorrough, S. Berman, F. Quétier, A. Thébault, and A. Bonis. 2008. Assessing functional diversity in the field – methodology matters! Functional Ecology 22:134–147.

Moles, A. T., and J. Ollerton. 2016. Is the notion that species interactions are stronger and more specialized in the tropics a zombie idea? Biotropica 48:141–145.

Seabloom, E. W., E. T. Borer, and L. L. Kinkel. 2018. No evidence for trade-offs in plant responses to consumer food web manipulations. Ecology 99:1953–1963.
Seabloom, E. W., L. Kinkel, E. T. Borer, Y. Hautier, R. A. Montgomery, and D. Tilman. 2017. Food webs obscure the strength of plant diversity effects on primary productivity. Ecology Letters 20:505–512.
Stein, C., S. B. Unsicker, A. Kahmen, M. Wagner, V. Audorff, H. Auge, D. Prati, and W. W. Weisser. 2010. Impact of invertebrate herbivory in grasslands depends on plant species diversity. Ecology 91:1639–1650.
Terborgh, J. W. 2015. Toward a trophic theory of species diversity. Proceedings of the National Academy of Sciences 112:11415.
Weisser, W. W., and E. Siemann. 2004. Insects and Ecosystem Function. Springer-Verlag, Berlin.
Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M.-L. Navas, Ü. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821–827.