The Bayreuth Phytometer – A common metric for community ecology

Peter Wilfahrt, Bernd Berauer, Andreas von Hessberg, Anke Jentsch

Disturbance Ecology, BayCEER, University of Bayreuth, 95440 Bayreuth, Germany

Introduction:

Scientific rationale:

Disentangling the various environmental factors driving emergent plant community properties such as productivity is a central goal in community ecology. Here, we propose a new phytometer experiment, which is intended to accompany on-going studies that manipulate environmental drivers of plant communities either through experimental manipulation or along natural gradients such as elevational ranges, countries and continents. We aim to develop the phytometer as a living reference system to be used as a tool for community ecology to improve ecological theory and prediction.

In order for a standardized phytometer to be a useful tool for community ecologists, several conditions should be met. First, a phytometer for community ecology must itself be a community of interacting species. Community effects can shape species-level outcomes in response to climate change, effects that would otherwise be missed by single species phytometers. Second, because of unique interactions between species, climate, and soil, a standardized phytometer gains efficiency when paired with a standardized substrate, which can then be compared to local soils to disentangle of climatic and edaphic conditions. Thus, an ideal phytometer is composed of a standardized plant mixture grown in

standardized substrate. Our phytometer allows for the quantification of climatic and edaphic effects on plant growth independently from one another.

We introduce a phytometer consisting of a three-species mixture representing common European weeds, which are naturalized but non-invasive on six continents, combined with an inert standardized substrate mixed with a standard amount of fertilizer. Our goal is to provide researchers a common response variable that is independent of local soil resource pools and regional species pools. By providing such a common metric, emergent community properties such as productivity can be gauged relative to other sites that have implemented the same study. The protocol is streamlined for relative ease, and undemanding in terms of effort required.

Guidelines for participation:

The phytometer as a tool is intended to accompany experimental or observation studies on herbaceous plant communities that operate across gradients with turnover in soils and species. This could mean distances between phytometer sites of 5 meters at an experimental site, or across countries and continents for large-scale gradient studies. We are also interested in single site phytometer installations to build a larger phytometer database in order to establish a geographic network of phytometer performance. Our phytometers are designed for herbaceous, grassland systems so should be placed in an area of full sunlight. The phytometer initiative is intended to accompany, not replace, on-going studies that manipulate environmental drivers of plant communities either through experimental manipulation or along natural gradients.

We have designed the phytometers to be low cost and low effort. While we are able to provide the basic supplies for phytometers, there is some cost required by local sites in order to obtain additional environmental data. This is detailed in the 'Materials required' section, but we anticipate the cost being ~€550 per site plus shipping costs, with additional sites per group being ~€350. Labor requirements for each site consist of growing phytometer species from seeds, filling phytometer pots with soil and transplanting species into them, and two harvest dates separated by one year. Phytometers should be planted so that the first 50-days of growth coincide with peak growing conditions of the site, as determined by local investigator expertise. Note that there are 66 days of greenhouse growth required prior to this 50-day period. Participation requires that sites have access to local climate information, with daily resolution for minimum and maximum temperature, and daily precipitation. This document provides a short overview of the timeline of events, and a detail section describing a step-by-step procedure.

Abbreviated protocol (follow line numbers for more details):

1.1) Receive package from Bayreuth with all materials needed for growing and planting the phytometers.
1.2) Order additional materials – loggers, PRS probes, vermiculite

2.1) Begin germinating seeds in potting soil in trays in greenhouse; keep soil moist at all times.
2.2) Transfer individuals to quickpots; 1 per cell.
2.3) After three weeks, move quickpots outside to harden off. Keep soil moist at all times.

3.1) Fill vermiculite phytometer pots and water.
3.2) Retrieve local soil from site and fill pots to the rim. Insert in-growth cores into all pots.
3.3) Dry teabags in 70°C oven for 48 hours. Weigh.
3.4) Refill vermiculite pots to rim. Mix in Osmocote fertilizer evenly across pot in the top 1-3cm.
3.5) Transplant seedlings to phytometer pots using the planting scheme, planting disc, and stamping tool.
3.6) Weigh non-planted individuals to determine average starting biomass.
3.7) Attach labels to phytometer pots.
3.8) Install add-ons: teabags, in-growth cores, and TidbiT dataloggers.
3.9) Begin 10 day watering period; 1L per pot per day
3.10-3.12) Move pots to field site, dig them in up to the rim in checkerboard fashion.
3.13) Insert PRS probes into local and standard soil.

4.1-4.2) Measure maximum vegetative height of all living individuals. Record mortality and number flowering/seeding individuals in each pot.
4.3) Harvest aboveground biomass in pots. Individuals should be clipped 3cm above ground. Dry biomass at
4.4) Remove root in-growth cores, extract roots from soil, replace cores with root free soil/substrate.
4.5) Remove PRS probe and ship.
4.6) Dig up dataloggers, download data, and rebury.
4.7) Weed out non-phytometer species

5.1) Weed phytometer of any non-phytometer species in spring of next year.
5.2) One year after first harvest, repeat steps from first harvest, except for PRS probes.
5.3) Remove teabags, dry in 70C oven for 48 hours and weigh.

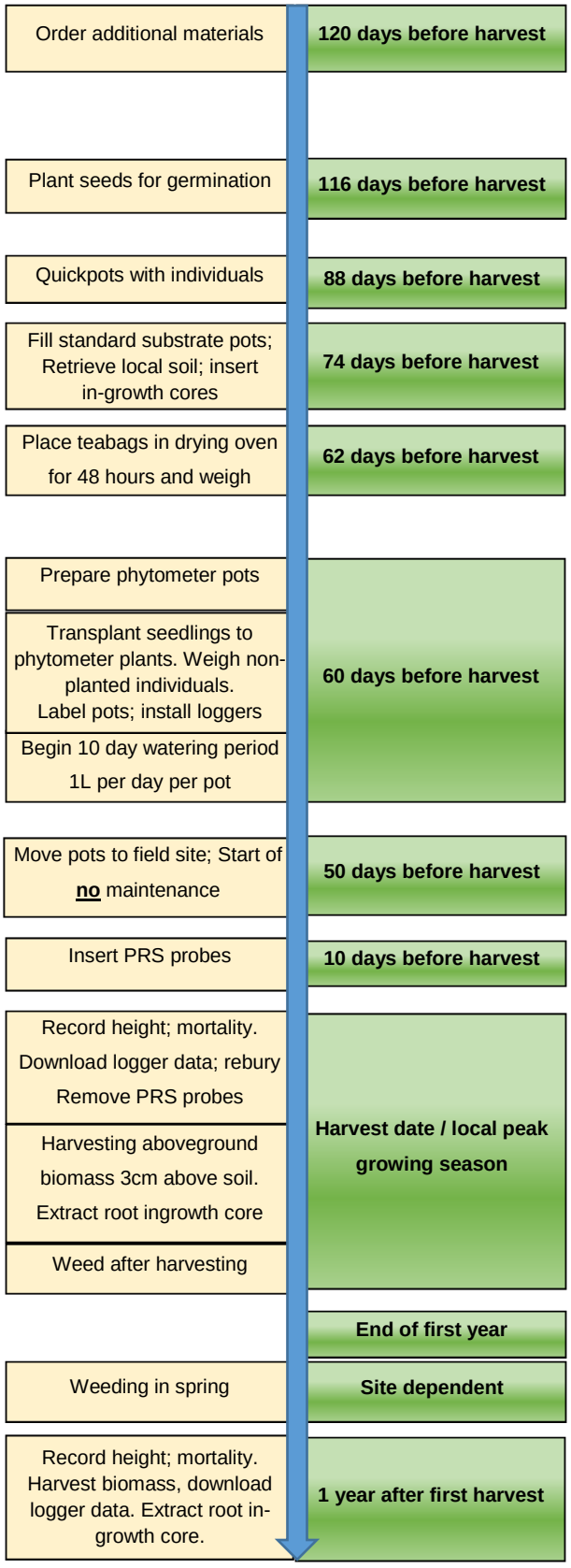

Figure 1: Workflow for key steps. Dates are referenced to the first harvest event, which should closely coincide with peak growing seasons on a site-by-site basis

1. Materials required*:*

In the following list of required materials, some line items are labeled as 'per site'. A site may be each observational area along an ecological gradient, or each treatment within an already existing experiment.

1.1 *Provided by Bayreuth team*:

1.1.1) Seeds of three target species.

1.1.2) 3 trays for germination and 3 Quickpots for early growth (separate by species)

1.1.3) Greenhouse potting soil for germination and Quickpot growth

1.1.4) 10 black plastic pots (30 cm inner diameter, 23 cm height) per site

1.1.5) Planting scheme

1.1.6) Planting disc (Allows planting at uniform density and size).

1.1.7) Stamping tool (Ensures plant plugs are inserted at uniform depth).

1.1.8) OSMOCOTE Exact Standard 12-14M" slow-release fertilizer. Composition: NPK(MgO): 15+9+11+2; Amount: 4 g / standardized substrate pot.

1.1.9) Rooibos and green teabags for decomposition rates, 10 of each per site.

1.1.10) Plastic mesh ingrowth cores for belowground biomass. 10 per site.

1.1.11) Plastic cylinder for installing in-growth cores.

1.1.12) 100 liter bag of Vermiculite per site (Vermiculite G: K1 - 0-2mm grain size).

1.2 Items that must be ordered by site investigator. Links are provided to the company, which can link you to your nearest distributor:

1.2.1) TidbiT dataloggers. Two required per site. Ordered at:

- http://www.onsetcomp.com/products/data-loggers/utbi-001

1.2.2) TidbiT dataloggers require a device to download data and computer software. This is a one-time cost for your group (i.e. *not* per site). Note: The software is used for all HOBO logger products, so please check if your lab group already has this.

- http://www.onsetcomp.com/products/communications/base-u-4
- http://www.onsetcomp.com/products/software/bhw-pro-dld (also available as CD)

1.2.3) Two pairs of PRS probes per site. PRS probes can be ordered here:

- https://www.westernag.ca/innovations/customer/order

1.2.4) Paper bags (for harvested plant mass).

1.2.5) Access to 60˚C drying oven.

Approximate cost: €550 per site + Shipping costs; €350 for additional sites.

2. *Planting and greenhouse management*:

Figure 2: Material for steps 2.1-2.3

2.1 Sow seeds of each species in separate trays with the provided potting soil. Each species should have its own tray, with about 10g seeds per tray (Approximately double the number of individuals needed to account for germination rates). This should be done 116 days prior to the planned date of harvesting the phytometers. After sowing, lightly press the seeds into the potting soil and cover them with a thin layer of soil to prevent desiccation. Trays and quickpots should be watered daily such that the soil never dries out. However, the soil should never be oversaturated for more than an hour (i.e. do not over water). During the germination period, the temperature of the greenhouse should be between 18°C and 20°C. If a greenhouse is unavailable, this step can be done in a normal room that is temperature maintained and has natural light, though this should only be done as a last resort.

2.2 After 4 weeks of germination, transplant the individuals into the quickpots with one individual per cell. Fill the quickpots with the potting soil sent to you, with a small hole lightly impressed at the center of each cell. Carefully remove individuals from the germination tray and lightly press them into each quickpot cell; make sure that the roots are completely covered by soil at the end.

2.3 After three weeks of growth in the quickpots, move them outside of the greenhouse (or into a cold or temperate greenhouse if there is the risk of frost) in order to harden off. After 1 week of hardening off, transplant the individuals into the phytometer pots.

Figure 3: Dactylis glomerata in Quickpots prior to transferring to phytometers.

3. *Pot installation*:

Figure 4a-d: Phytometer pot production.

3.1 The root in-growth cores must be inserted during the following two steps. When filling pots, place the plastic cylinder provided by the Bayreuth team in place 'C' (Figure 5). You can use the planting disc to guide its placement when filling. After all soil/vermiculite is placed, water it thoroughly so the soil stays in place, remove the cylinder and line the hole with the plastic mesh. For standard substrate pots, this mesh should be filled with vermiculite. For local soil, this should be filled with root-free soil. This means you must sieve a small amount of local soil free of roots (approximately 1 liter per site). The top of the in-growth core should be even with the soil when finished.

3.2 Two weeks prior to planting the phytometers (74 days before harvest), fill the five standard pots provided by the Bayreuth team with vermiculite substrate and soak them with water. The vermiculite naturally settles and this minimizes this effect. The pots have holes in the bottom to allow water drainage, but place a paper towel at the bottom of the pot to prevent Vermiculite from spilling out prior to planting.

3.3 Transport local soil from your site to the greenhouse (~80L per site). Ideally, soil is coming from 5 - 30 cm below the surface, which is thoroughly mixed prior to potting. This soil should not include any green plant material or leaf litter. Fill soil up to rim. If it is not possible to transport soil to the greenhouse, pots can be prepared on-site but please make note of it. However, this requires daily visits to the field for ten days post-planting to water in the plants.

3.4 Label the teabags (follow labeling from step 3.6). At least two days prior to planting (62 day before harvest), place teabags in a drying oven at 70°C for 48 hours. Remove, weigh the teabags to the nearest milligram and record this weight. Removal and weighing should be done shortly before planting.

3.5 Refill vermiculite to the rim in standard pots. Add 4g of Osmocote provided by the Bayreuth team to the top of the Vermiculite, and gently mixed in so that it is still within the top 1 - 3 cm of the pot. Water the vermiculite and local soil as this makes planting easier.

3.6 At this step, take care that you do not plant non-target species, as seed mixes often contain a low rate of contamination from other species. Place the planting disc over the top of each pot

(Fig.4b), align the small notch in the disc with the hole drilled into the rim of the pot, and press holes into the soil using the “stamp”. The notch aligns with the ‘top’ of the planting scheme. Place all holes prior to planting to keep the distance between individuals consistent. Once 18 holes are created, remove the disk, carefully remove healthy individuals from plugs and press them into the holes, making sure that no roots are exposed above the surface. While only healthy individuals should be used, avoid taking the biggest individuals first. Rather select in a ‘typewriter’ fashion (left to right, top to bottom). Cover the potting soil from the plug with the soil / substrate from the pot and gently pressed around the stem of the plant to minimize desiccation (Fig.4c).

3.7 Measure the natural maximum vegetative height of 25 random, but healthy, individuals of each species that remain in the quickpots (i.e. not planted). Then, clip these individuals at 3cm above the soil, place them in paper bags in groups of five, and dry them at 60˚C for at least 48 hours, and weigh them to the nearest milligram. This provides an average starting mass for species by site.

3.8 Affix the provided labels to the small hole drilled in the pot rims with the following labeling scheme [Fig.4d]: Site code - Soil type code - pot number. Site codes are two digit codes assigned to you by the Bayreuth group; soil type is either LS for local soil or SS for standard substrate, and pot number is 01-05. As an example, the third replicate of local soil in Bayreuth would be labeled ‘BT-LS-03’. These labels must be identical to those entered when reporting data. Pots labeled -03 should be those with the data loggers.

3.9 Dig a small hole for both teabags in all ten pots at 5cm depth for measuring decomposition rates, insert teabag, and cover. Finally, data loggers should be placed at 5cm depth in one pot each for local and standard soil (see Fig 5 for placement of all items). **Make sure to activate loggers before planting!** Tie a piece of sturdy string to the data loggers and affix them to the hole drilled for the label in the rim of the pot. Care must be taken at this stage not to injure the plants.

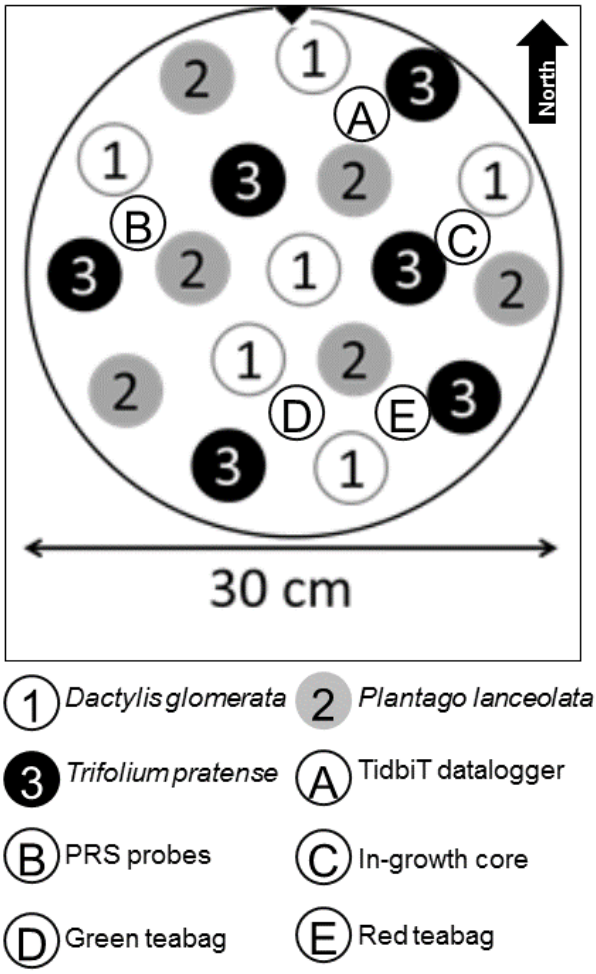

Figure 5: Planting scheme and pot location for add-ons. Planting disc creates holes only for plants

3.10 After planting, water in pots for 10 days at a rate of 1 liter per pot per day.

3.11 Transport pots to the site, taking care that they stay upright. Settling of the soil and substrate may occur during transportation. Pots with local and standardized soil should be placed in a checkerboard fashion, 5x2, as space allows [Fig.6]. Align the pots so the hole on the rim with the label faces north. Once all pots are placed in the ground, fill in the soil surrounding the pots and compress to ensure insulation. Extreme care should be taken to prevent local soil from getting into the standardized Vermiculite substrate phytometers at this step. We suggest inverting an empty pot over the standardized Vermiculite substrate pots to accomplish this. Please photograph each site such that the arrangement of all ten pots is visible and send to the Bayreuth Group.

3.12 If alternate pot arrangements are required to fit the available space, this should be diagrammed and sent to the Bayreuth Group. In the event that separate blocks are required due to space constraints, standardized substrate and local soil phytometers should be present in each block.

3.13 This is the start of the 50-day growth period for the phytometers. No maintenance of the phytometers occurs at this stage.

3.14 PRS probes should be inserted 10 days prior to harvest (see Figure 5). Samples consist of four pairs (anion/cation probes). One pair should be inserted into phytometer pots 01-04 for each soil type. Insert probes vertically, XX cm apart from one another, facing XX direction.

Figure 6: Preferred arrangement of pots in checkerboard fashion (left).
Alternate arrangement showing one block necessary to fit in footprint of experiment (right)

4. Harvesting

4.1 Prior to harvesting, record the highest, naturally standing vegetative height of each individual to the nearest millimeter.

4.2 Record number of individuals of each species in each pot that are A) still alive and B) have reproductive biomass.

4.3 To harvest biomass, pull each individual erect and then clip 3 cm above the soil. Place the biomass of the same species within each pot in one paper bag that can be placed in a drying oven. Thus, each pot should have three bags, and each site should have 30 bags. Reproductive biomass should be included. Dry bags in a drying oven at 60˚C for at least 48 hours, and then weigh to the nearest milligram, or most accurate mass available. Once weighed, retain biomass in case of future leaf chemistry analysis. Enter height, survivorship, and biomass data into the provided data sheet and sent to Peter Wilfahrt (peter.wilfahrt@uni-bayreuth.de).

4.4 Carefully remove in-growth cores from the soil without uprooting any plants by cutting around the mesh with a sharp knife. Wash the roots free of soil over a 2mm sieve. Collect all roots per pot in a paper bag, label it, dry it at 60˚C for at least 48 hours, and weigh it to the nearest milligram. The root free soil is replaced in the pit so a subsequent harvest can take place after one year. For vermiculite, this can be replaced from the bag. For local soil, make sure the soil is root-free prior to replacing.

4.5 Remove PRS probes, place all probes of one soil type into a sealable plastic bag, and send both bags back to Western Ag for analysis.

4.6 Carefully pull up the two data loggers. Download the data and place them back in the soil.

4.7 Following this, weed phytometers of any non-phytometer species.

5. Year two

5.1 Early in the growing season of the second year, weed the phytometers of any non-phytometer species.

5.2 A second harvest occurs one year following the first harvest. The ideal date of harvest is exactly one year after the first harvest; report any deviation from this date to the Bayreuth Group. Repeat all steps in section 4 except for 4.5.

5.3 Remove teabags from soil. If the label has degraded or is not readable, relabel it and place it in a plastic bag. Remove all soil and root debris from outside the bag. Dry the bags at 70°C for 48 hours, weigh them to the nearest milligram, and record this.

5.4 Report all data to Peter Wilfahrt (peter.wilfahrt@uni-bayreuth.de)

Outputs, benefits and data:

First, we hope that you find value in the phytometer project by gaining understanding of the characteristics of your research site(s) relative to others in the ClimMani network. To that end, all data will be cleaned and made available by the Bayreuth team after the first harvest to all participants. Second, we envision two papers emerging from this initiative. The first is a conceptual paper on the expanded use of phytometers in ecology. This would introduce our method to the broader ecological community, and advocate for its use by demonstrating its value. This paper is opt-in for those who are interested in developing the concept and method further. The second paper is the data-driven paper where we aim to examine the primary drivers of phytometer growth. For this paper, we offer automatic authorship to two members of each site that participates with a phytometer set.