Ecosphere

Disentangling climate from soil nutrient effects on plant biomass production using a multispecies phytometer

Appendix S1 - Protocol

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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 detail our phytometer approach, 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 have developed the phytometer as a living reference system to be used as a tool for community ecology for disentangling climatic and soil drivers.

In order for a standardized phytometer to be a useful tool for community ecologists, two major 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 is optimized when paired with a standardized substrate, which can then be compared to phytometers grown in local soil. Thus, an ideal phytometer is composed of a standardized plant mixture grown in standardized substrate, paired with the same plant mixture grown in local soils.

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 using the phytometer approach:

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. Single site phytometer installations following this protocol are also valuable in order to build a larger phytometer database and establish a geographic network of phytometer performance. Our phytometers are designed for herbaceous, grassland systems so should be placed in an area receiving 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. Following the protocol and sourcing material from our recommended providers when possible is highly recommended to ensure...
standardization. The minimum phytometer design (minimizing the amount of environmental data collected) is estimated at $\sim$€200 per site, though we recommend recording several additional environmental variables detailed in the protocol which may raise costs to $\sim$€750 per site. 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 59 days of greenhouse growth required prior to this 50-day period. For environmental data, sites should minimally have access to local climate information, with daily resolution for minimum and maximum temperature, and daily precipitation, as well as basic soil parameters (e.g. C:N, pH, SOM, texture). This document provides a short overview of the timeline of events, and a detailed section describing a step-by-step procedure.
Abbreviated protocol (follow line numbers for more details; blue items are optional):

1.1) Order materials for phytometers well in advance

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 two weeks, move quickpots outside to harden off. Keep soil moist at all times.

3.1) Retrieve local soil from site and fill pots to the rim.
3.2) Fill standard phytometer pots. Mix in Osmocote fertilizer.
3.3) Install root in-growth cores during 3.1 and 3.2
3.4) Dry teabags in 60°C oven for 48 hours. Weigh.
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.
3.8-3.9) Install add-ons: teabags, and TidbiT dataloggers.
3.10) Begin 10 days of watering; 1L per pot per day
3.11-3.13) Move pots to field site, dig them in up to the rim in checkerboard fashion.
3.14) Insert PRS probes into local and standard soil.

4.1) Record mortality and number flowering/seeding individuals in each pot.
4.2) Harvest aboveground biomass in pots. Individuals should be clipped 3cm above ground. Dry biomass at 60°C for 48h.
4.3) Remove root in-growth cores, extract roots from soil, replace cores with root free soil/substrate.
4.4) Remove PRS probe and return to Western Ag.
4.5) Dig up dataloggers, download data, and rebury.
4.6) 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
5.3) Remove teabags, dry in 60°C oven for 48 hours and weigh.

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Figure S1: 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:**

Note: certain line items are labeled as ‘per site’. A site consists of 10 phytometer pots and may be an observational area along an ecological gradient or a treatment within an already existing experiment.

1.1 **Seeds of three target species** (*Dactylis glomerata, Plantago lanceolata, Trifolium pratense*). Maintaining the genotype is important for standardization. Seeds should either be ordered directly from: www.rieger-hofmann.de

Alternatively, contact Anke Jentsch at the Department of Disturbance Ecology to have seeds sent to you at: anke.jentsch@uni-bayreuth.de

1.2 **Three trays for germination and 3 Quickpots for early growth** (separated by species). Provides enough individuals for two sites (20 pots).

1.3 **Greenhouse potting soil for germination and Quickpot growth.** Optimally potting soil for sowing and cultivating young plants, with a fine soil texture and high water holding capacity.

1.4 **Ten black plastic pots** (30 cm inner diameter, 23 cm height) per site

1.5 **Planting disc** (Allows planting at uniform density and size; can be printed from end of this document)

1.6 **Stamping tool** (makes uniform sized hole for inserting plant plugs)

1.7 **Fertilizer. Recommended: OSMOCOTE Exact Standard 8-9M slow-release fertilizer.** If an alternative is needed, Osmocote breaks down by weight and form as: 15% Nitrogen (NO$_3$-N: 6.6%, NH$_4$-N: 8.4%), 9% Phosphorus (P$_2$O$_5$), 11% Potassium (K$_2$O), 2% Magnesium (MgO), 0.03% Boron (B), 0.05% Copper (Cu), 0.45% Iron (Fe, 0.08% EDTA-Chelat), 0.06% Manganese (Mn), 0.02% Molybdenum (Mo), and 0.015% Zinc (Zn).

1.8 **40 L of Vermiculite per site** (Vermiculite G: K1 - 0-2mm grain size).

1.9 **40 L (~50 kg) of silica sand** (pure SiO$_2$; 0.6 – 1.3 mm grain size)

1.10 **Access to 60°C drying oven.**

1.11 **Paper bags** (for harvested plant mass).

**Optional materials**

2.1 **Rooibos and green teabags** for decomposition rates, 10 of each per site.

2.2 **Plastic mesh root ingrowth cores** for belowground biomass (3 cm diameter, 2 mm mesh size) 10 per site.

2.3 **TidbiT dataloggers.** Two per site. Ordered at: http://www.onsetcomp.com/products/data-loggers/utbi-001

Requires an additional device to download data and computer software. 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)

2.4 Soil moisture probes at 5 cm depth

2.5 Eight pairs of PRS probes per site. PRS probes can be ordered here:
https://www.westernag.ca/innovations/customer/order
2. **Planting and greenhouse management:**

2.1 Sow seeds of each species in separate trays (Fig. S2a) with potting soil. Each species should have its own tray, with about 10g of seeds per tray. This should be done 109 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 regularly 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 is ideally 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 3 weeks of germination, transplant the individuals into the quickpots (Fig 2b) with one individual per cell. Fill the quickpots with potting soil, 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. Transplant more individuals than are needed for the phytometers (see step 3.6)

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 S2a-b: Material for steps 2.1-2.3*

*Figure S3: Left to right: Trifolium pratense, Plantago lanceolata, and Dactylis glomerata in Quickpots prior to transferring to phytometers.*
3. **Pot installation:**

3.1 Prepare local soil phytometers. Transport local soil from your site to the greenhouse (~80L per site; can be stored in greenhouse until planting). 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. Place a paper towel at the bottom of the pot to prevent soil loss and fill with soil up to 2-3 cm below the rim. If it is not possible to transport soil to the greenhouse, pots can be prepared on-site but this requires daily visits to the field for ten days post-planting to water in the plants.

3.2 Prepare standard substrate phytometers. In a separate, larger container, mix 15 L of vermiculite with 15 L of quartz sand until it is evenly distributed. Place a paper towel at the bottom of the pot to prevent substrate loss and carefully fill the pot up to the rim; slight subsidence will occur over the following days. Add 4g of fertilizer mix evenly on top of the substrate surface and gently mix in so that it is still within the top 1 - 3 cm of the pot.

3.3 **(OPTIONAL) This step must occur during steps 3.1 and 3.2.** When filling pots, first fill them with a small layer of soil or substrate. Using the planting disc as a reference, use the stamping tool to create a hole for the ingrowth cylinder and place it in the soil (Position ‘C’; Fig. S5). Hold the root in-growth core in place while you fill soil around it, taking care to not get local soil in the core. Fill the open root in-growth core, a funnel is helpful here. For standard substrate pots, it should be filled with standard substrate. For local soil, it 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 with a 2 mm mesh sized sieve). The top of the in-growth core should be even with the local soil when finished, but standard substrate should be filled to the rim, burying the ingrowth core for now, as it will shrink over time. A plastic tag or stick can be used to mark the location.

3.4 **(OPTIONAL) Label the teabags (follow labeling from step 3.7).** 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.

![Figure S4a: Installing soil in-growth cores (local soil).](image-url)
3.5 Transplanting to the phytometers. At this step, take care that you do not plant non-target species (see Section 6) as seed mixes often contain a low rate of contamination from other species. Water the pots thoroughly as this helps with planting. Place the planting disc over the top of each pot, align the small notch in the disc with the hole drilled into the rim of the pot, and press holes into the soil using a wooden dowel, trowel handle, or some instrument capable of making a ~4-5 cm diameter hole that accommodates the quickpot soil plugs). 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), skipping over non-healthy individuals (browning, considerably smaller, etc). Cover the potting soil from the plug with the soil / substrate from the pot and gently press around the stem of the plant to minimize desiccation (Fig.S4b).

![Figure S4b: Transplanting species to phytometer pots.](image)

3.6 Clip 25 random, healthy individuals remaining in the quickpots at 3 cm above the soil, 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.

3.7 Affix the provided labels to a small hole drilled in the pot rims with the following labeling scheme (Fig.Sb): Site code - Soil type code - pot number. Site codes are two digit codes representing your site; 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.

3.8 **(OPTIONAL)** Dig a small hole for both teabags (Positions E and F; Fig. S5) in all ten pots at 5cm depth for measuring decomposition rates, insert a teabag, and cover.

3.9 **(OPTIONAL)** Data loggers should be placed at 5cm depth in one pot each for local and standard soil (Position A, Fig. S5). **Make sure to activate loggers before planting at a 30 minute logging interval!** 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.
3.10 After planting, water in pots for 10 days at a rate of 1 liter per pot per day. This is to avoid transplant shock and mortality of individuals unrelated to site conditions.

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. S6). 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 substrate phytometers at this step. We suggest inverting an empty pot over the standardized Vermiculite substrate pots to accomplish this. **Installation should occur in a fenced area that prevents vertebrate herbivory.**

3.12 If alternate pot arrangements are required to fit the available space, this should be diagrammed and recorded as such. 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 **(OPTIONAL)** PRS probes should be inserted 7 days prior to harvest. Samples consist of four pairs (anion/cation probes). One pair should be inserted into phytometer pots 01-04 for each soil type (Positions B and C; Fig. S4). Insert probes vertically, with the flat side facing the rim
of the pot by making a slot in the soil with a small spade or soil knife and pushing in the probe pointed-side down, **leaving the top 2cm of the probe exposed.** It is important to cut deeply enough into the soil, as the PRS probes will break if you try to force them through the soil. Once buried, make sure there is proper contact between the probe and substrate by firmly pushing the substrate back around where the probe was inserted. No substrate should be removed during this process.

![Figure S6: Preferred arrangement of pots in checkerboard fashion (left). Alternate arrangement showing one block necessary to fit in footprint of existing experiment (right)](image)
4. **Harvesting**

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

4.2 To harvest biomass, pull each individual erect and then clip 3 cm above the soil. Place the living biomass of the same species within each pot in one paper bag, and senesced or dead biomass of the same species in a separate paper bag. Thus, each pot should have six bags, and each site should have 60 bags. Reproductive biomass should be included with living biomass. 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 survivorship and biomass data into the provided data sheet.

4.3 **(OPTIONAL)** Carefully remove in-growth cores from the soil without uprooting any plants by cutting around the mesh with a sharp knife and empty interior contents into a plastic bag. Reinsert the empty root in-growth core into the same place and refill with root free soil/substrate so a subsequent harvest can take place after one year. In the lab, wash the roots free of soil over a fine 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. For local soil, make sure the soil is root-free by sieving over 2mm mesh prior to replacing.

4.4 **(OPTIONAL)** Remove PRS probes. After removal, probes must be cleaned, bagged properly (two bags per site; local soil probes and standard soil probes), and sent back to Western Ag. Instructions at: [https://www.westernag.ca/innovations/customer/submission](https://www.westernag.ca/innovations/customer/submission)

Note: One sample consists of all eight probes from each soil type per phytometer set.

4.5 **(OPTIONAL)** Carefully pull up the two data loggers. Download the data and place them back in the soil.

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

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*Figure S7 – (Left to Right) Pull plant erect and cut at 3cm above ground, cut away dead leaves and large senesced portions and put in separate bag; Remove core by cutting around and clear away soil and roots on outside of in-growth core; Put entire contents of in-growth core into plastic bag – make sure all roots within are removed.*
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; record any deviation from this date. Repeat all steps in section 4 (optional steps remain optional).

5.3 *(OPTIONAL)* 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 Record all data and send to Anke Jentsch (anke.jentsch@uni-bayreuth.de)
6.1 – *Dactylis glomerata* seed contamination with *Poa spec*

The most common and easy to confuse species contamination occurs in *D. glomerata* quickpots with *Poa* germinants. While it is best to avoid transplanting them to the quickpots from the germination tray, it can be quite difficult to distinguish at this stage. The guide below helps to avoid planting phytometer pots with *Poa*, which is critical and easily avoided when you know what to look for.

Figure S8a-c: Examples of *Dactylis glomerata* top plant in (a), shown closely in (b), and *Poa spec* lower plant in (a), and shown closely in (c).

Figure S8a shows the two species side by side, here you can see that *D. glomerata* has a single stem with a wider base and broader leaves, compared to the multiple tillered *Poa* with more sprawling architecture and thinner leaves. Figure S8b shows the upright, whitish green stem of *D. glomerata*. Figure S8c shows the multiple tillers of *Poa* that emerge at an angle to the soil, with a distinctive reddish white base.
7.1 – Washing roots

The roots from the in-growth core are very fine and may be difficult to separate from the soil. We suggest the following method. Stack two sieves; the top one with a coarse, 2 mm pore size, and the lower one with a finer pore size (for example, 0.45 mm). Then, place the contents of the in-growth core on the top sieve (large pore size) and gently wash with distilled water. This will separate the majority of the roots from most of the soil. Gather the roots in the top sieve and gently stir in a bowl of distilled water to remove all soil. Place on a paper towel to dry. Place the rest of the soil in the sieves into the bowl of distilled water and stir. The remaining roots should float to the top and can be removed with a forceps. In order to standardize effort, please take exactly five minutes per in-growth core for the last step.

*Figure S9:* (left to right) Stack sieves with largest pore size on top and add soil from in-growth core. Gently wash with distilled water until majority of soil has fallen through leaving roots. Rinse root mass in bowl of distilled water to separate from remaining soil. Place rest of soil in bowl of distilled water and spend 5 minutes removing root pieces with forceps.
Planted scheme print-out

The following print out can be used to construct a plant scheme for the phytometers. Print out both pages, align the pictures at the notch, and tape or glue them together. This can also be used to create a cardboard cutout, which would be more durable. The inner circles should be cut-out and used as a guide to stamp holes for the plant plugs.