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METHODS, TOOLS, AND TECHNOLOGIES

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

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Abstract. Plant community biomass production is co-dependent on climatic and edaphic factors that are often covarying and non-independent. Disentangling how these factors act in isolation is challenging, especially along large climatic gradients that can mask soil effects. As anthropogenic pressure increasingly alters local climate and soil resource supply unevenly across landscapes, our ability to predict concurrent changes in plant community processes requires clearer understandings of independent and interactive effects of climate and soil. To address this, we developed a multispecies phytometer (i.e., standardized plant community) for separating key drivers underlying plant productivity across gradients. Phytometers were composed of three globally cosmopolitan herbaceous perennials, *Dactylis glomerata, Plantago lanceolata,* and *Trifolium pratense*. In 2017, we grew phytometer communities in 18 sites across a pan-European aridity gradient in local site soils and a standardized substrate and compared biomass production. Standard substrate phytometers succeeded in providing a standardized climate biomass response independent of local soil effects. This allowed us to factor out climate effects in local soil phytometers, establishing that nitrogen availability did not predict biomass production, while phosphorus availability exerted a strong, positive effect independent of climate. Additionally, we identified a negative relationship between biomass production and potassium and magnesium availability. Species-specific biomass responses to the environment in the climate-corrected biomass were asynchronous, demonstrating the importance of species interactions in vegetation responses to global change. Biomass production was co-limited by climatic and soil drivers, with each species experiencing its own unique set of co-limitations. Our study demonstrates the potential of phytometers for disentangling effects of climate and soil on plant biomass production and suggests an increasing role of P limitation in the temperate regions of Europe.

Key words: aridity; climate gradient; nitrogen; nutrient availability; phosphorus; phytometer; plant productivity; resource limitation.

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INTRODUCTION

Understanding vegetation responses to multiple global change factors is a central goal in ecology (Franklin et al. 2016). Key to addressing this challenge is disentangling the co-occurring and covarying environmental factors that drive responses such as primary production (Dormann et al. 2013). Vegetation biomass production is co-dependent on precipitation (Huxman et al. 2004), temperature (Larcher 2003), soil nutrients (Fay et al. 2015), inherent characteristics of the vegetation (Michaletz et al. 2014), among other things. An additional challenge emerges as these drivers often have non-linear effects (Knapp et al. 2017) and non-additive interactions (Wang et al. 2017). Isolating drivers of primary production can improve our understanding of vegetation dynamics in the face of global change. Yet, controlling for multiple influential factors while allowing for natural variation in others is a challenging task, particularly along environmental gradients at continental scales.

Soils control productivity through water regulation (Vicca et al. 2012), the availability of essential soil nutrients, such as nitrogen (LeBauer and Treseder 2008), phosphorus (Vitousek et al. 2010), additional macro- and micronutrients (Fay et al. 2015), and soil properties such as texture and pH (Van Sundert et al. 2018). Temperate regions have long been considered nitrogen-limited systems (LeBauer and Treseder 2008), but anthropogenic nitrogen deposition and agricultural runoff may alleviate this limitation, potentially causing other nutrients to become limiting (Kaspari and Powers 2016). Increased soil N often shifts nutrient limitations to soil P (Vitousek et al. 2010), although K limitations are also common (Sardans and Peñuelas 2015). Furthermore, the ratio of soil N to P can strongly influence productivity (Penuelas et al. 2013) and community composition (Wassen et al. 2005) as pathways by which plants uptake soil nutrients, such as mycorrhizal associations, become more or less effective (Lambers et al. 2008). The interaction of soil properties in driving plant productivity makes isolating single soil resource effects challenging.

Climatic drivers, similarly to soil properties, are multi-faceted (Nemani et al. 2003) and interact with soil properties to drive plant productivity. Long-term climate–soil interactions, such as soil pH being linked to the climatic water balance (Slessarev et al. 2016), create uncertainty as to the relative importance of climate versus soil in driving productivity due to covariation between drivers. Moreover, more abrupt changes in climate increase the complexity. For instance, chronic fertilization can lead to decreased drought resistance in grassland ecosystems (Bharath et al. 2020), drought and fertilization can interactively change community composition (Van Sundert 2021) and decrease N and P availability to plants by altering water availability (He and Dijkstra 2014), and plant water availability from precipitation is regulated by soil properties (Vicca et al. 2012). Thus, to even begin to unravel the complex soil dynamics underlying plant productivity, the backdrop of climatic variability must be considered.

The species composition of a community is an additional layer of complexity that can influence primary production through changes in diversity (Loreau and Hector 2001). Though, even at static species richness levels, species in local plant communities react asynchronously to changes in the environment to stabilize productivity (Wilcox et al. 2017). Additionally, species composition and productivity can change concurrently in response to environmental changes (Owensby et al. 1999), creating additional uncertainty whether productivity changes directly with the environment or is mediated by species composition (Hautier et al. 2020).

Recently, coordinated experimental protocols and collaborative approaches have been used successfully to disentangle the effects of these drivers across continental to global gradients (Halbritter et al. 2020). Coordinated studies have shown how primary production is controlled by multiple soil nutrients (Fay et al. 2015), species diversity (Fraser et al. 2015), increased temperature (Peñuelas et al. 2007, Reinsch et al. 2017), and mediation of drought impacts by biodiversity (Kröel-Dulay et al. 2015, Kreyling et al. 2017, Craven et al. 2018). Nonetheless, drivers remain partially obscured due to inseparable covariation between climate, soil properties, and species composition across study gradients and sites. Common metrics use protocols and materials to provide standardized quantifications of ecosystem properties, helping link cross-site studies (Halbritter et al. 2020). Yet, standardized metrics for plant community ecology remain elusive. As the diversity of communities and their constituent species play a clear role in mediating ecosystems responses to environmental changes (Hautier et al. 2015), disentangling soil and climate effects on primary production may be facilitated by standardizing plant communities.

In order to improve understanding of how soil and climate drivers independently and interactively drive plant biomass production, we developed a living reference system in the form of a multispecies phytometer that integrates community-level processes and is designed to specifically separate climatic from edaphic effects. We expand on the traditional definition of a phytometer as a model plant community used within a single study (Clements and Goldsmith 1924) by introducing a standardized protocol and plant community grown in both local soil and a standardized substrate under different climate regimes. By testing this approach across a pan-European climate gradient, we address the following hypotheses: (1) Biomass production will decrease with aridity and increase with N and P availability, (2) biomass production in standard substrate will decrease with aridity but be unrelated to nutrient availability, (3) factoring out the biomass production from standard substrate in local soils (i.e., climate correction) will clarify soil relationships and potentially allow new relationships to biomass to emerge in local soils, and (4) species will have asynchronous responses to climate and soil drivers and the nature of these responses will be clearer using standard substrate and climate-corrected biomass.

MATERIAL AND METHODS

Site descriptions

Phytometers were installed at 18 experimental sites across 11 European countries (Table 1) in 2017. The habitats were predominantly grasslands. In the two shrubland and four forest habitats, the phytometers were installed in unshaded, open areas. The sites spanned a gradient in mean annual temperature from 2.9 to 15.5°C and in mean annual precipitation from 560 to 2005 mm. Local climate data were collected on-site or from weather stations at most 10 km away. All material necessary for germinating phytometer plants was distributed from the University of Bayreuth to ensure uniformity (see Appendix S1 for full list).

Phytometers were established in sets of 10 (five local soils, five standard substrates). Each phytometer consists of three species: *Dactylis glomerata* (grass), *Plantago lanceolata* (non-leguminous forb), and *Trifolium pratense* (leguminous forb). These species are cosmopolitan but non-aggressive perennial weeds. They naturally co-occur but have different sensitivities to environmental stressors, while also demonstrating sufficient survival rates across a range of climates, making them suitable candidates for global

Table 1. Summary of sites.

Site name	Country	Habitat	Latitude	Longitude	Elevation (m a.s.l.)	MAP (mm)	MAT (°C)	Start date of 50-d period	Precipitation (mm)	Aridity (mm)	Soil GDD (Standard)	Soil pH
Antwerp	Belgium	Semi-natural grassland	51° 9′ 36′′ N	4° 24′ 29′′ E	11	778	10.1	26 June 2017	104.1	110.2	712	8.1
Aveiro	Portugal	Mediterranean shrubland	40° 37′ 48′′ N	8° 39′ 0′′ W	4	916	13.9	3 July 2017	2	184.9	1059	6.9
Bayreuth	Germany	Semi-natural grassland	49° 55′ 19′′ N	11° 35′ 48′′ E	365	745	8.7	29 May 2017	144.9	115.3	830	4.1
Bílý Kříž	Czech Republic	Semi-natural grassland	49° 49′ 48′′ N	18° 54′ 30′′ E	890	1312	6.3	11 July 2017	105	70.8	663	3.6
Brandbjerg†	Denmark	Semi-natural grassland	55° 52′ 48′′ N	11° 58′ 12′′ E	15	742	9.7	29 June 2017	105	77.8	620	3.5
Domanínek	Czech Republic	Semi-natural grassland	49° 52′ 48′′ N	16° 23′ 60′′ E	530	609	7.2	10 July 2017	107.2	104.6	715	6.2
Esterberg	Germany	Semi-natural grassland	47° 31′ 12′′ N	11° 9′ 36′′ E	1250	1450	2.9	29 May 2017	219.7	21.4	608	5.6
FAHM	Estonia	Forest	58° 13′ 48′′ N	27° 18′ 0′′ E	44	650	5.4	14 June 2017	76.4	−49.0	576	4.5
Fendt	Germany	Semi-natural grassland	47° 49′ 45″ N	11° 3′ 58″ E	550	900	8.7	29 May 2017	241	2.8	765	5.3
GARRAF	Spain	Mediterranean shrubland	41° 18′ 8″ N	1° 49′ 5″ E	212	560	15.5	26 May 2017	27	296.8	1133	8.1
Graswang	Germany	Semi-natural grassland	47° 34′ 12′′ N	11° 1′ 48″ E	850	1300	6	29 May 2017	282.9	−43.1	716	6.8
Gumpenstein	Austria	Semi-natural grassland	47° 29′ 44″ N	14° 5′ 53″ E	700	1033	6.9	12 July 2017	424.1	−185.4	828	6.4
Kacka Suma	Serbia	Forest	45° 17′ 24′′ N	19° 53′ 24′′ E	86	647	11.4	20 July 2017	65.6	204.1	1022	7.2
Kiskunság	Hungary	Grassland	46° 52′ 16′′ N	19° 25′ 16′′ E	108	594	11	4 June 2017	114.2	208.3	1025	8.1
Mols	Denmark	Semi-natural grassland	56° 22′ 6″ N	10° 54′ 37″ E	56	669	8.7	29 June 2017	113.2	69.6	644	4.1
Oldebroek	Netherlands	Semi-natural grassland	52° 24′ 36′′ N	5° 55′ 12′′ E	25	2005	10.1	3 July 2017	115.4	80.2	626	4.3
Waldstein†	Germany	Forest	50° 8′ 24′′ N	11° 52′ 12′′ E	775	1165.5	5.3	29 May 2017	182.1	3.7	714	7.3
Zöbelboden†	Austria	Forest	47° 50′ 24′′ N	14° 26′ 24′′ E	950	1645	7.8	16 June 2017	371.9	−204.2	706	5.8

Notes: MAP, Mean annual precipitation; MAT, Mean annual temperature. Precipitation, aridity, and soil GDD are specific to the 50-d growth period of the phytometer trial. Aridity was the difference between site PET and precipitation during the 50-d trial, where PET was calculated using the Hargreaves-Samani equation. Growing Degree Days (GDD) is the area under the curve for temperature between 5 and 30°C. PRS adsorption values can be found in Appendix S2: Table S13.

† Site removed from analyses due to heavy herbivory.

distribution in such a phytometer (Joshi et al. 2001). By standardizing the plant community, we minimized variation from turnover in species, allowing a more focused inference of plant–environment relationships. Seeds for these species were provided from a single seed source located in Central Germany. Then, as early as March 2017, site partners began following a standardized protocol for plant germination, transplantation, phytometer installation, and data collection (Fig. 1a; Appendix S1).

Phytometer preparation

Seeds were germinated by the local site scientists in a standardized potting soil in a greenhouse or area with high natural light availability and regulated temperatures. Following an eight-week germination and growth period, plants were transplanted into the 30 cm diameter, 23 cm depth, phytometer pots, with 2–3 cm between the rim and substrate to ensure water penetration. Local soil pots consisted of soil from 5 to 30 cm below the surface at each site. The standard substrate was vermiculite (an inert, inorganic substance; 0–2 mm grain size), with 4 g of slow-release Osmocote fertilizer (see Appendix S1 for nutrient content) mixed evenly into the top 3 cm of substrate, a rate that likely removed nutrient limitations from these pots. Vermiculite was selected as a substrate to maximize the soil water holding capacity and isolate the water-related controls on plant performance derived from precipitation (i.e., the water supply to the soil) from the water controls derived strictly from the local soil properties (i.e., the soil capacity to store the water supplied by precipitation and to provide water to plants during rainless periods). Plants were planted systematically in exactly the same arrangement with an even density across the

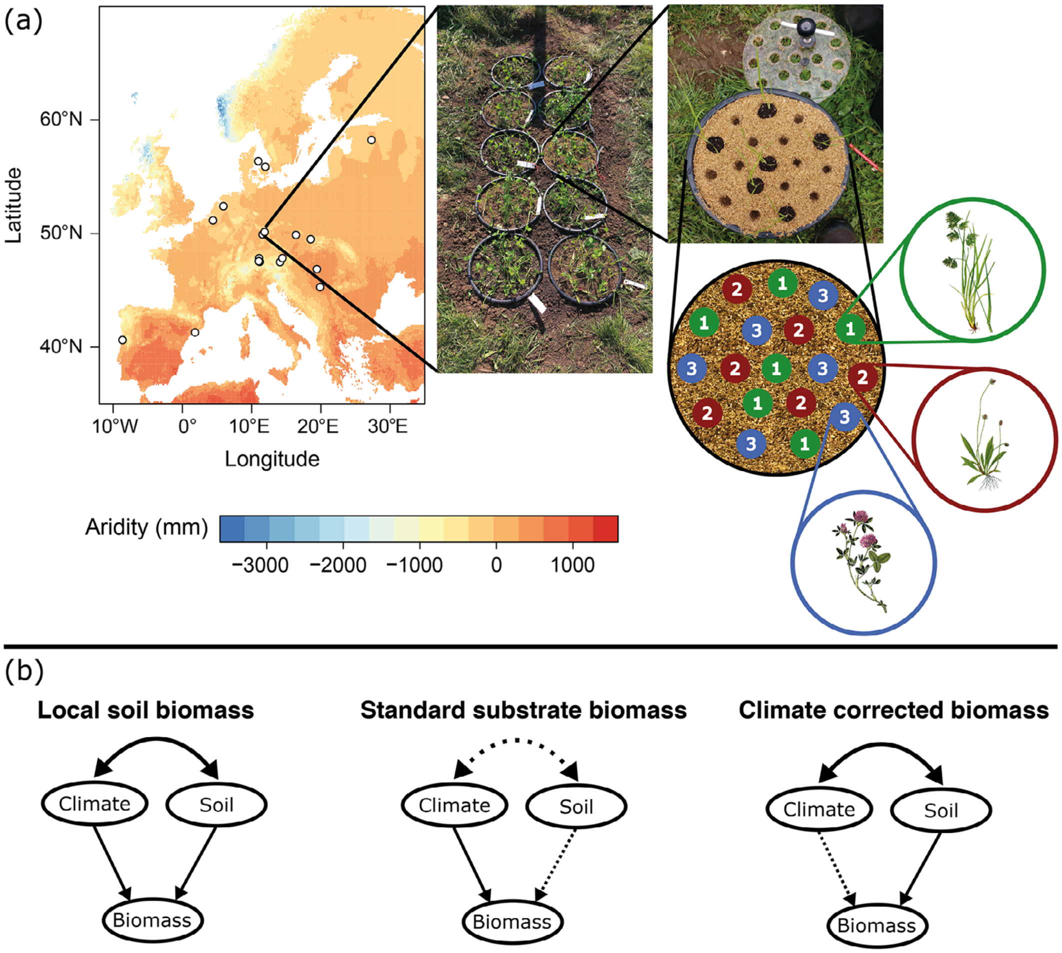

Fig. 1. (a) The phytometer workflow: from right to left, phytometers are planted in a standardized pattern with equal density of three globally distributed, perennial herbaceous species: *Dactylis glomerata, Plantago lanceolate*, and *Trifolium pratense*. Phytometer communities are planted in a standardized substrate and the local site soil. Study sites were distributed across an aridity (PET—precipitation; mm) and temperature gradient in Europe. The displayed gradient is calculated from mean annual PET and precipitation derived from WorldClim, while our study focused on 50-d values of this index (see Table 1). (b) The expected parsing of climate from soil effects, where local soils are influenced jointly by climate and soil. Standard substrate is influenced by only climate, which can be used to factor our climate influences from the local soil, clarifying the independent effects of soil. Dashed lines represent an expected absence of a relationship.

surface resulting in six individuals per species. A root-ingrowth core (3 cm diameter, 20 cm length, 2 mm mesh size) was installed in each pot in an area immediately surrounded by one of each species. To account for initial size differences due to varying greenhouse environments at the different sites, 25 individuals per species not planted in phytometer pots were clipped, dried at 60°C for 48 h, and weighed. Soil temperature data loggers (HOBO TidbiT v2 Temp Logger) were installed at 5 cm depth at this time. Following this, plants were watered for ten days at a rate of 1 liter per pot per day to avoid transplant shock; this also meant that phytometer pots at the beginning of the natural growth period were at or near field capacity. Then, pots were transferred out to the field and buried up to the rim.

Phytometer harvest

Phytometers were exposed to 50 d of growth in the local environment. This duration was chosen to accommodate possible sites with short growing seasons, but also limits inference to a juvenile stage of establishment where competition pressure may be lower than in more mature communities. Plants were inventoried for mortality (i.e., how many individuals were still present with green tissue), clipped 3 cm aboveground to avoid damaging roots, and all aboveground biomass was separated by species and each species was separated into green tissue and dead or senesced (i.e., brown) tissue to assess environmental stress. Root biomass in the root-ingrowth core was careful washed free of substrate and collected. All biomass was dried at 60°C for 48 h and weighed. More details on any of these steps may be found in the full protocol (Appendix S1). Three sites (Brandenbjerg, DK, Waldstein, DE, and Zöbelboden, AU) showed clear visual signs of heavy vertebrate herbivory and were excluded from the analyses.

Environmental variables

Plant Root Simulator (PRS) probes (Western Ag, Saskatoon, Canada) were installed in pairs in four local soil and four standard substrate pots for ten days prior to the harvest by placing them vertically in the top 10 cm of soil or substrate. These attract and adsorb soil ions, simulating plant root uptake, and indicate the availability of soil nutrients to plants in the final 10 d of the experiment when plant communities were most mature. Probes were analyzed in aggregate for each substrate type per site. This resulted in a single mean estimate for each ion per substrate type per site (i.e., variance was quantified only across sites). Soil pH was additionally measured in the local soil.

Climate data were matched to the 50-d growth period of the phytometer trial. We calculated two indices thought to be important to plant growth. First, we calculated an aridity index (PET—precipitation) (Thornthwaite 1948). PET was calculated using the Priestley-Taylor equation (Priestley and Taylor 1972), integrating daily minimum and maximum air temperature and relative humidity, daily clear sky radiation based on latitude and elevation, and solar radiation at ground level using the Hargreaves-Samani equation, which estimates cloudiness based on the difference between maximum and minimum daily temperature (Hargreaves and Samani 1982). Aridity indices have the desirable quality of integrating temperature and precipitation into a single variable (Maliva and Missimer 2012), but may still imperfectly integrate all climate variables (Stocker et al. 2018) before even considering its non-independencies with soil factors. Thus, the appeal of our standardized substrate phytometer lies in being an integrative proxy for both measured and unmeasured climate variables (e.g., microclimate, cloud cover, wind, climate-mediated plant–soil feedbacks). Second, we calculated growing degree days (GDD) per soil type per site by fitting a sinusoidal function to the maximum and minimum soil temperature each day, calculating the area under the curve that was above 5°C and below 30°C where plants are expected to be most photosynthetically active, and then summing each day to produce an integrated value per site. Here, we relied on temperature values taken from soil data loggers buried at 5 cm depth, as this more closely represents the temperature the plants experience than typical weather station data collected at 2 m aboveground (Körner and Hiltbrunner 2018).

Statistical analyses

*Empirical data.—*We explored how non-independence between climate and soil variables may influence patterns of biomass production. First, we used linear mixed-effects models to examine the relationship between biomass production at the community and species level to aridity, N availability, and P availability. Second, we used model selection to identify the most important predictors on biomass production. Third, we used interaction models to see how climate may have moderated soil effects on biomass production. In all three cases, we separately examined the response of biomass production in local soil phytometers, biomass production in standard substrate phytometers, and climate-corrected biomass. Climate-corrected biomass was calculated as local soil biomass production minus the mean of standard substrate biomass production at that site (Fig 1b).

In order to further understand causes of biomass changes in response to environmental drivers, we tested whether mortality, the proportion of brown tissue, or root:shoot biomass were predicted by aridity, N availability, and P availability in local soil and standard substrate. For mortality, we used generalized linear mixed-effects models with a negative binomial distribution link function to account for the large number of zeros in the data using site as a random effect. For the proportion of brown tissue, a sign of environmental stress, we took the mean proportion of brown tissue within a site and soil type and used generalized linear models with a beta distribution link function. As beta distributions cannot use true zeros and ones, any observed zero was set at 0.01 and any observed one was set at 0.99. We used linear mixed-effects models for root:shoot biomass using site as a random effect. We expected that environmental stress (i.e., low resource availability) would increase mortality, the amount of brown tissue, and root: shoot production.

*Model selection.—*We investigated abiotic drivers of community and species biomass production in our local soil phytometers by first using linear mixed-effect models with site as a random effect to determine how biomass production in each phytometer pot was driven by our principal climate variable, aridity, and our principal soil variables, N and P availability, at the community and species levels. We also ran separate models with aridity and N:P availability as predictors to test whether N:P availability was similarly or more predictive. The soil variables were log10-transformed to achieve normality in the residuals. In order to examine the importance of additional variables, we used the "dredge" function in R package MuMIn (Barton 2020) to test which combination of soil properties, PRS ion adsorption of macronutrients (N, P, K, Mg, S, and Ca availability), climate predictors (aridity, soil GDD), and whether between site differences in initial size caused by differences in growth during the greenhouse phase affected final biomass. For this analysis, all variables were scaled to have a mean of zero and standard deviation of 1 to allow direct comparison of coefficients. Site was included as a random effect in all models. We selected all models that were within two of the model with the lowest AICc (Burnham and Anderson 2003) and used model averaging to produce a final fit. We calculated the relative importance of each predictor by refitting a model with all of the selected variables and calculating the semi-partial R^2 for each term using the r2glmm package (Jaeger 2017), following the Nakagawa and Schielzeth (2013) method of calculating a pseudo-R^2 of fixed effects in random-effects models. Ca and S availability were additionally log10-transformed to achieve normally distributed residuals.

We repeated the process in the standard substrate phytometers, albeit with several different predictor variables. Aridity and soil GDD were used to represent climate and macronutrient availability was used to determine whether soil resource supply differed. In the standard substrate, macronutrient availability effects could indicate different rates of nutrient release from the fertilizer in different climatic conditions. Soil pH was unmeasured in the standard substrate as it was assumed to be homogenous at the start of the experiment by design, with any subsequent changes being mediated by differences in climate. We additionally tested whether observed subsidence of the vermiculite substrate affected our results. As the subsidence was not measured at two sites, it could not be included in the full models. Therefore, we tested whether the residual variation of the best standard substrate model of the subset with shrinkage data was related to shrinkage using standard linear regression.

We isolated the effect of soil drivers from climatic drivers in the local soil phytometer by subtracting away the site mean of standard substrate phytometer biomass from each local soil phytometer biomass to obtain a "climate-corrected biomass" value. Using linear mixed-effect models, we followed the same linear mixed-effects model and model selection procedure using the same set of predictors as the uncorrected local soil model.

*Interaction models.—*We explored whether climate and soil had non-additive interactions in our three response variables (biomass in local phytometers, biomass in standard phytometers, and climate-corrected biomass values). We examined how aridity and soil GDD interacted with both N and P availability using linear mixed-effect models with all four two-way interactions between the climate and soil variables with site as a random effect. These variables were selected as drought conditions are known to interfere with plant uptake of N and P (He and Dijkstra 2014). We calculated the relative importance of each group (soil predictors, climate predictors, and the four possible interactions) in predicting community biomass.

RESULTS

Empirical data

Linear regression of the principle biomass–environment relationships revealed that aboveground community biomass production in the local soil phytometers decreased with increasing aridity (pseudo-R^2 = 0.81, $P < 0.001$; Fig. 2; Appendix S2: Table S1), increased with P availability (pseudo-R^2 = 0.55, $P = 0.012$), and had no relationship with N availability ($P = 0.84$). Species-specific biomass production of *T. pratense* had the same relationships as community biomass with aridity ($P < 0.001$, pseudo-R^2 = 0.78) and P availability ($P < 0.01$, pseudo-R^2 = 0.52). *Dactylis glomerata* and *P. lanceolata* showed qualitatively similar, though non-significant relationships, with aridity and P availability (Appendix S2: Table S1). No species had a relationship with N availability. Aboveground community biomass production in the standard substrate phytometers also decreased with the aridity gradient (Fig. 3; Appendix S2: Table S2; pseudo-R^2 = 0.59, $P < 0.01$). Species-specific biomass production in standard substrate was negatively related to the aridity gradient for *T. pratense* ($P < 0.01$); *D. glomerata* ($P = 0.067$) and *P. lanceolata* ($P = 0.66$) showed no significant relationship with aridity (Fig. 3; Appendix S2: Table S2). No relationships between biomass production and N or P availability were found (Appendix S2: Table S2), confirming that any uneven soil supply rates of N and P from the fertilizer were not driving biomass production.

Using the standard substrate phytometers to factor out the independent influence of climate, we found that the community climate-corrected biomass was positively correlated with P availability of the local soil (Fig. 3; Appendix S2: Table S3; $P = 0.016$, pseudo-R^2 = 0.63), but showed no significant relationship with N availability (Fig. 3; $P = 0.47$). Species-specific climate-corrected biomass in the local soil phytometers had no significant relationships with N or P availability, though P availability had relatively high predictive power for all three as indicated by the semi-partial pseudo-R^2 (Appendix S2: Table S3; all pseudo-$R^2 > 0.3$).

Aridity was not significantly correlated with N or P availability in local soil or standard substrate. N:P availability was not a significant predictor of local soil (Appendix S2: Table S1), standard substrate (Appendix S2: Table S2), or climate-corrected (Appendix S2: Table S3) biomass production, though it often had similar predictive power to P availability.

Individual mortality increased with aridity in local soil ($P < 0.01$; Appendix S2: Table S4) and in standard substrate ($P < 0.001$). Percentage of brown tissue increased with aridity in local soil ($P < 0.01$; Appendix S2: Table S5) and in standard substrate ($P < 0.001$). Neither mortality nor percentage brown tissue showed a relationship with P or N availability in local or standard substrate (Appendix S2: Tables S4, S5) Root:shoot biomass showed no relationship to aridity, P availability, or N availability in either local soil or standard substrate (Appendix S2: Table S6).

Model selection

In the local soil biomass production, top models for community biomass included 9 of 10 predictors and model averaging included aridity and soil GDD (50-d thermal radiance), P availability, Mg availability, and soil pH as significant predictors (pseudo-R^2 = 0.92, Appendix S2: Table S7). Best-fit models for the species all had unique sets of predictors in the local soil phytometers, though each species had at least one climatic and one soil variable that were both significant and high in predictive power (Appendix S2: Table S7).

The top models in standard substrate biomass production of communities contained 7 of 9 predictors, but only aridity and soil GDD were significant (pseudo-R^2 = 0.68, Appendix S2: Table S8). This demonstrated that potential variability in nutrient release from the applied fertilizer may have had some impact in driving biomass production but was relatively small compared with the direct effect of climatic drivers. In the standard substrate phytometers, species-specific top models all contained unique sets of predictors (Appendix S2: Table S8). Notably, no predictor was significant after model averaging for *P. lanceolata*, although the 8 predictors chosen in top models were able to account for 54.7% of its variation in biomass (i.e., semi-partial pseudo-R^2).

For climate-corrected community biomass in the local soil phytometers, the top model

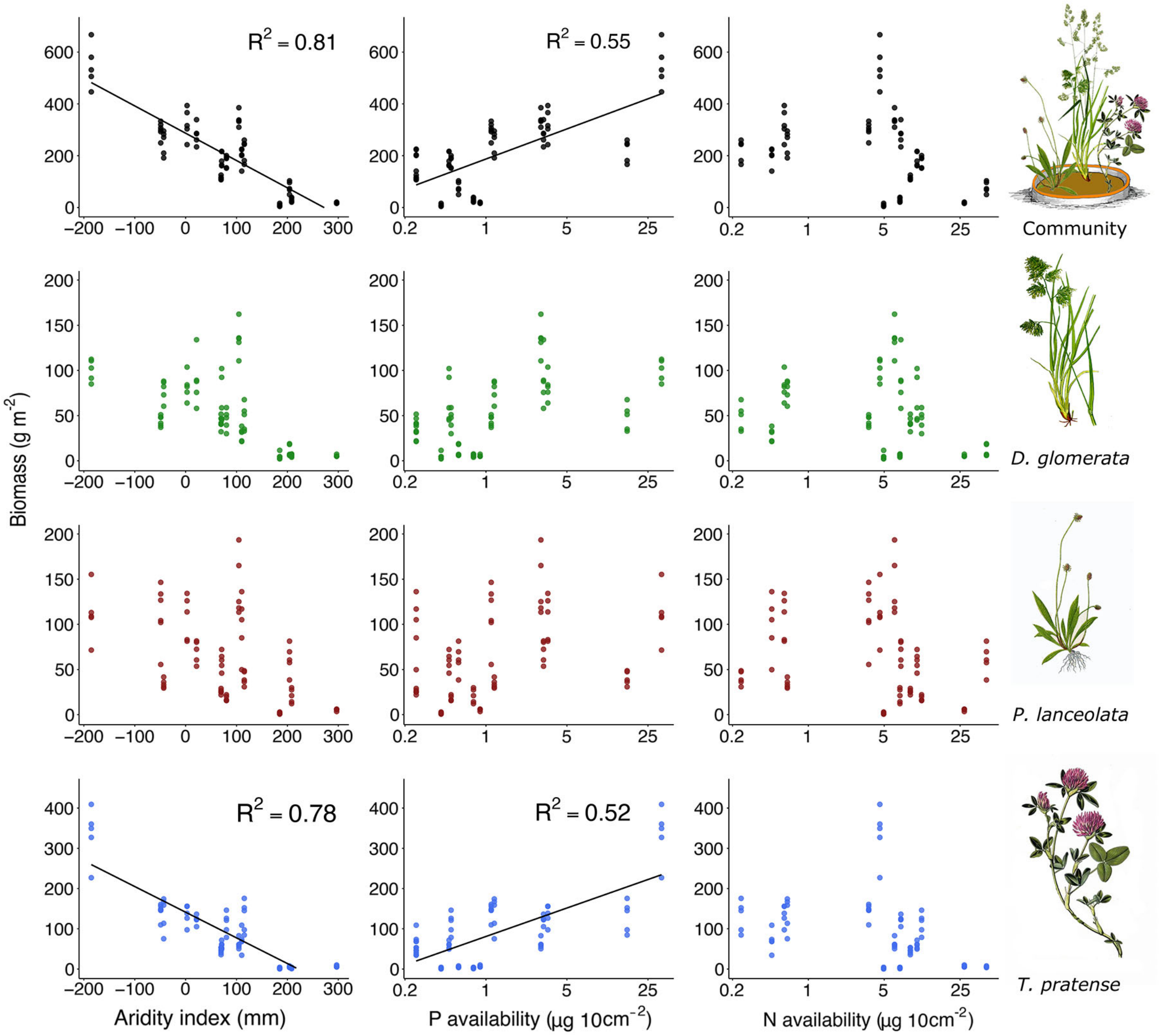

Fig. 2. Fifty-day aboveground biomass production of community and species in local soil across an aridity index (50-d PET—precipitation) and soil N and P availability. Soil predictor variables are log-transformed and black lines are the best-fit line from a mixed-effects linear model fit to the transformed data when $P < 0.05$. Significant relationships have the semi-partial pseudo-R^2 displayed for significant relationships.

contained all variables, though only P, K, and Mg availability were significant (pseudo-R^2 = 0.76; Appendix S2: Table S9). Notably, the climate-corrected biomass model revealed potassium as a significant predictor, which was not selected in the local soil model; potassium was also significant for *D. glomerata* and *P. lanceolata*. Top models for species-specific climate-corrected biomass all contained unique sets of predictors and significant predictors; P availability was the only consistent significant predictor for all species and *D. glomerata* and *T. pratense* contained significant climatic variables despite the climate correction (Appendix S2: Table S9).

Interaction models

Linear models with two-way interactions between the two climate variables and P and N availability revealed no significant interactions in local soil (Appendix S2: Table S10), standard substrate (Appendix S2: Table S11), or climate-corrected biomass responses (Appendix S2:

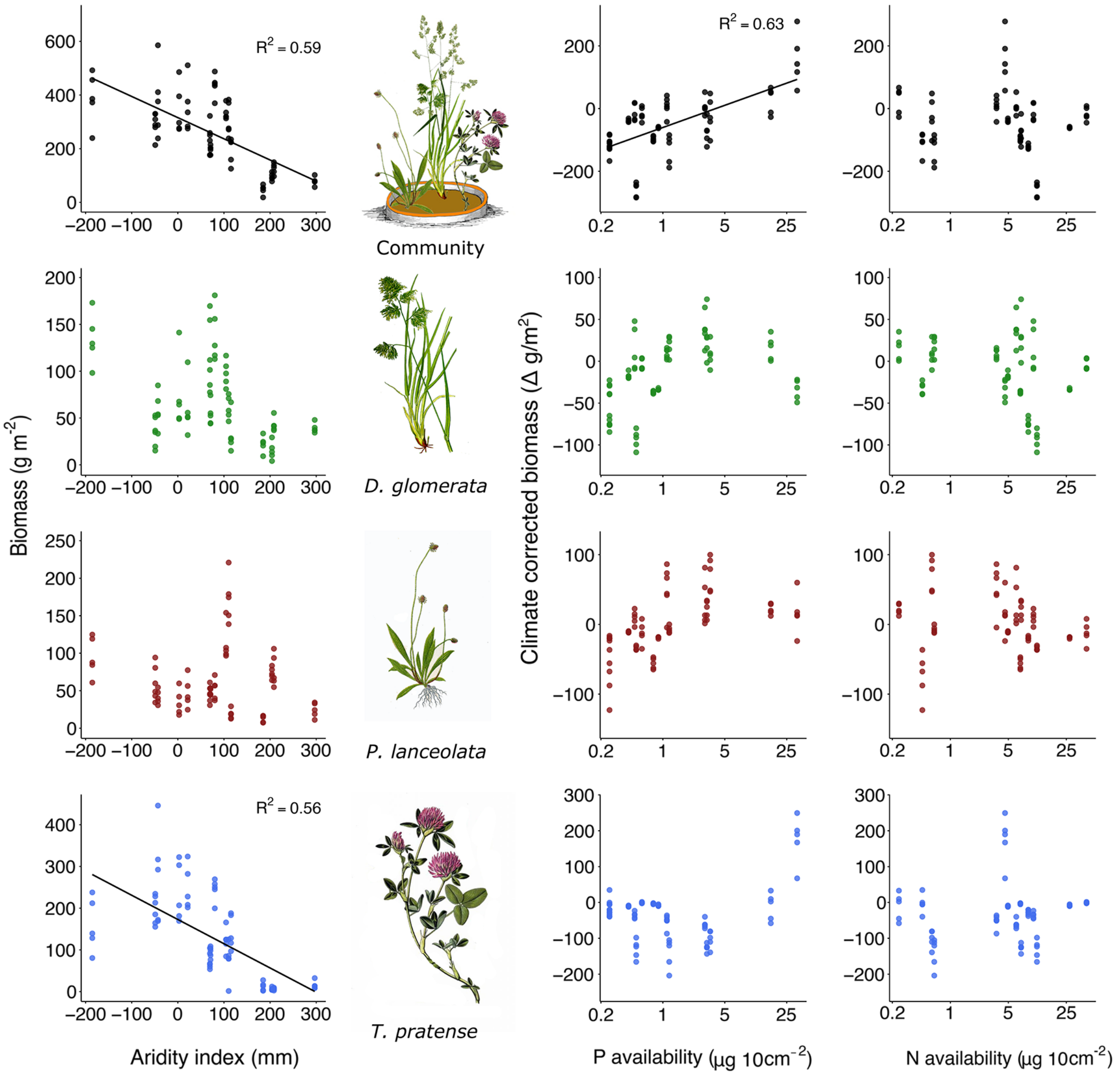

Fig. 3. Biomass response to aridity in the standard substrate and climate-corrected biomass response to P and N availability in the local soil phytometers at the community and species levels. Climate-corrected values are the 50-d biomass production in the local soil minus the mean biomass produced in the standard substrate during the same period. Negative climate-corrected values indicate sites where the standard substrate was more productive than the local soil and vice versa. Soil predictor variables are log-transformed and black lines are the best-fit lines from mixed-effect linear models fit to the transformed data when $P < 0.05$. Significant relationships have the adjusted R^2 displayed.

Table S12). Including interactions in models also masked the previously detected significance of main effects (i.e., aridity and P availability; Figs. 2, 3). Post hoc model simplification also did not reveal any significant interactions.

DISCUSSION

Our phytometer trial showed biomass production strongly decreased with aridity and increased with P availability, but surprisingly had no relationship to N availability. Phytometers grown in local soil revealed strong effects of aridity, soil temperature, and several soil parameters on biomass production, but failed to clearly separate the effects. Phytometer biomass in standard substrate was explained predominantly by climate variables. Climate-corrected biomass in local soil reacted strongly to soil parameters, with reduced climatic influence. The emergence of a climate-corrected biomass relationship with potassium that was not evident in the local soil analysis demonstrates that studies crossing large climate gradients may unintentionally mask relationships with soil nutrients (Bruelheide et al. 2018). This experimental separation of climatic and soil influences advances our ability to understand mechanistic controls on primary production, such as multiple-resource limitation (Fay et al. 2015) or non-linearity in climate responses (Knapp et al. 2017).

Aridity was a strong determinant of community biomass production across our European gradient in both local soil and standard substrate phytometers. High aridity led to increased mortality and senesced tissue, factors which would reduce aboveground biomass production. The weaker relationship of biomass with aridity in standard substrate may be partly explained by larger within site variance compared with local soil phytometers, which could result from the observed subsidence of the vermiculite substrate. Additionally, standard substrate phytometers had an overall lower range of biomass values across sites, which may artificially constrain R^2 values. We recommend a modified standard substrate using quartz sand in combination with vermiculite in our revised protocol to mitigate these effects in future trials (Appendix S2: Fig S2). There is additionally the possibility that plant–soil feedbacks led to changes in pH or microbial communities in the standard substrate (van der Putten et al. 2013), though given the isolation from the surrounding soil any such changes should themselves be largely mediated by climate, and we did not detect any effect of climate on soil nutrient availability in the standard substrate. The upshot is that standardized substrate phytometers could be an important tool even in the absence of paired local soil phytometer, by detailing non-linearities of biomass production in response to climatic influences when employed regularly along natural climatic gradients (Schweiger et al. 2016, Knapp et al. 2017, Kreyling et al. 2018). Additionally, this makes the use of standard substrate phytometers appropriate for clarifying soil influences as we do in this study.

Biomass production was responsive to multiple soil nutrients and properties, suggesting co-limiting factors (Fay et al. 2015). In the local soil phytometers, the role of multiple soil nutrients was clear. Correcting biomass values using the standardized substrate phytometers strongly shifted the predictive power of environmental variables from climate to soil nutrients and exposed potassium as a driver of biomass. Temperate grassland productivity is generally limited by N (LeBauer and Treseder 2008), but we surprisingly observed neither a direct nor an indirect relationship between biomass production and N availability in local soil or climate-corrected soil. High atmospheric deposition of N and fertilization of sites has the potential to shift soil nutrient limitations toward non-nitrogen sources, such as P (Vitousek et al. 2010) and K (Sardans and Peñuelas 2015). P limitation was apparent in both the local soil biomass production and climate-corrected biomass, as biomass production was highest in the presence of large P pools. K relationships became evident only after correcting for climate effects. However, increased K availability was actually associated with decreased biomass, potentially indicating that larger K pools were a result of decreased uptake resulting from decreased biomass. Plant nutrient uptake, and therefore nutrient demand, is not linear across plant ontogeny and resource gradients (Coleman et al. 1993), suggesting shifts to different resource limitations such as N would be possible as our phytometer communities mature.

Communities of species are more likely to experience multiple-resource co-limitation (Harpole et al. 2011). Here, species-specific biomass production was responsive to different soil nutrients and properties, suggesting that species were themselves uniquely co-limited by sets of resources (Harpole et al. 2016). *Trifolium pratense* reacted more strongly to climate, while the deeper rooting *Plantago lanceolata* showed more responsiveness to soil properties after climate correction, suggesting a more fundamental split between climate and soil as the dominant source of variation between the species. Community-level data displayed clearer responses to drivers, supporting the use of multiple species that may asynchronously respond to environmental conditions to stabilize biomass production (Loreau and de Mazancourt 2013). The discrepancy between community and species-specific biomass responses is likely due in part to confounding biotic interactions, which are impossible to separate from abiotic factors in this current approach. For instance, in the climate-corrected biomass, there is a notable uptick in biomass production of *T. pratense* at the highest P available site, while *D. glomerata* and *P. lanceolata* appear to decrease or level off at this site. P may limit the rate of nitrogen fixation, which is demanding in terms of ATP, leading to high P soils benefiting N-fixers like *T. pratense* (Dynarski and Houlton 2018), which could in turn competitively suppress other species at this site, though we lack the data to test this. The initial starting biomass (i.e., from the greenhouse growth phase), appeared as a significant contributor in several species models, particularly for *D. glomerata*, implying this species may have benefitted from an unintended advantage of being bigger relative to its competitors at some sites. However, while this clouds the species-level responses, communities are by definition networks of interacting species, and the underlying trade-offs between species are integrated into the emergent processes that interest ecologists.

In conclusion, our phytometer approach for parsing climatic and edaphic contributions to biomass production revealed that aridity and P availability were the strongest determinants of biomass production. While our focus was on climate–soil interdependencies, we believe the phytometer approach offers a flexible protocol for investigating additional drivers. For instance, soil microbes undoubtedly drive plant biomass production, and this could be tested by titrating local soil microbes into standardized soil pots. Similarly, plant competition and environmental filters could be explored more thoroughly through the addition of monocultures and single individual pots. Our study adds to a growing body of evidence suggesting increasing P limitation in temperate zones and climate-dependent N effects on biomass production. As ecosystems continue to face multiple global change pressures, unraveling independent and dependent effects of these drivers will only increase in importance.

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DATA AVAILABILITY STATEMENT

Data and code used for analyses are available from Figshare: https://doi.org/10.6084/m9.figshare.14675640.v2

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3719/full