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Temporal and small-scale spatial variation in grassland productivity, biomass quality, and nutrient limitation

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Abstract Characterization of spatial and temporal variation in grassland productivity and nutrition is crucial for a comprehensive understanding of ecosystem function. Although within-site heterogeneity in soil and plant properties has been shown to be relevant for plant community stability, spatiotemporal variability in these factors is still understudied in temperate grasslands. Our study aimed to detect if soil characteristics and plant diversity could explain observed small-scale spatial and temporal variability in grassland productivity, biomass nutrient concentrations, and nutrient limitation. Therefore, we sampled 360 plots of 20 cm × 20 cm each at six consecutive dates in an unfertilized grassland in Southern Germany. Nutrient limitation was estimated using nutrient ratios in plant biomass. Absolute values of, and spatial variability in, productivity, biomass nutrient concentrations, and nutrient limitation were strongly associated with sampling date. In April, spatial heterogeneity was high and most plots showed phosphorous deficiency, while later in the season nitrogen was the major limiting nutrient. Additionally, a small significant positive association between plant diversity and biomass phosphorus concentrations was observed, but should be tested in more detail. We discuss how low biological activity e.g., of soil microbial organisms might have influenced observed heterogeneity of plant nutrition in early spring in combination with reduced active acquisition of soil resources by plants. These early-season conditions are particularly relevant for future studies as they differ substantially from more thoroughly studied later season conditions. Our study underlines the importance of considering small spatial scales and temporal variability to better elucidate mechanisms of ecosystem functioning and plant community assembly.

Keywords Nitrogen · Phosphorus · Biodiversity Exploratories Project · Nutrient concentrations · Biodiversity–ecosystem functioning · Growth limitation

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Introduction

An important issue in ecology is the question of what drives variability in ecosystem productivity. Grassland ecosystems are one of the most globally widespread habitats, providing many functions and services on which we rely, such as livestock production, erosion prevention, and carbon sequestration (Lemaire et al. 2011; Allan et al. 2015), making them a focus of ecosystem research. While many studies use inter-site data, within-site variation has received less attention, although it can significantly contribute to a mechanistic understanding of ecosystems (Maestre and Cortina 2002; Fridley et al. 2011).

Biotic and abiotic factors influencing grassland productivity and plant nutrition such as soil moisture, microbial activity, and nutrient supply are known to vary spatially and temporally during the growing season (e.g., Gilliam and Dick 2010; Kleinebecker et al. 2011a; Parker et al. 2012; Regan et al. 2014). Recently, spatiotemporal heterogeneity has emerged as an important component of ecosystem processes and co-existence of species (e.g., Reynolds et al. 2007; Gross et al. 2009; Koorem et al. 2014). Small-scale spatial patterns of vegetation and related ecosystem functions have been well researched for arid and semi-arid ecosystems (e.g., Whitford 2002; Maestre and Cortina 2002; Osem et al. 2002; Maestre et al. 2005). For instance, Maestre and Cortina (2002) found that aboveground plant distribution (at 625 cm^2) of a semi-arid steppe depended on soil properties such as biological and physical crusts, which influenced site hydrology. Despite this, such patterns have been much less often studied in temperate grasslands, which widely differ from (semi-)arid ecosystems for example in terms of limiting resources, plant community structure, and productivity. Nevertheless, first analyses found for example a significant effect of small-scale differences in substrate properties (at 100 cm^2) on the resistance of a species-rich temperate grassland plant community to the effects of climate change (Fridley et al. 2011). Thus, small-scale variability in productivity and nutrition in over a growing season can be expected to give important insights in the functioning of temperate grassland ecosystems.

The chemical composition of aboveground plant biomass, especially the concentrations of nitrogen (N), phosphorus (P), and potassium (K), is an established proxy for integrating plant nutrient supply and uptake conditions during the growth phase (Marschner 2011). Ratios of nutrient concentrations (i.e., N:P, P:K, and N:K) have been frequently used to determine growth-limiting nutrients in plant communities (Koerselman and Meuleman 1996; Güsewell 2004). The type of nutrient limitation was found to be mechanistically linked to ecosystem properties such as differences in soil characteristics, productivity, plant diversity, plant functional traits, and land use (e.g., Olde Venterink et al. 2003; Wassen et al. 2005; Fujita et al. 2014). Olde Venterink et al. (2001) found the type of nutrient limitation to vary among years, changing from N to P limitation from drier to wetter seasons. This small-scale spatiotemporal heterogeneity in resource supply can significantly broaden the niche space for grassland plants and may therefore facilitate species co-existence e.g., by partitioning of nutrient acquisition (Harpole and Tilman 2007). Furthermore, several studies, mostly experimental, have found plant diversity to have positive effects on grassland productivity, nutrient cycling, and plant nutrition (Hector et al. 1999; Balvanera et al. 2006; Abbas et al. 2013). However, within-site differences in plant diversity on small spatial scales have rarely been assessed, although Gross et al. (2009) found evidence for a positive biodiversity–productivity relationship in 14 cm $\times$ 14 cm plots in grasslands.

Our study aimed to detect and explain spatiotemporal variability in productivity, biomass nutrient concentrations, and nutrient limitation in an unfertilized grassland in southwestern Germany. The 10 m $\times$ 10 m grassland plot was divided into 30 subplots, which were intensively sampled at six consecutive dates within a growing season, providing 360 samples in total. Previous analysis of this plot found that soil properties most strongly influenced spatiotemporal variation in microbial characteristics (Regan et al. 2014). The present study focuses on variability in productivity and chemical composition of the plant biomass. In detail, we addressed the following hypotheses:

(i) Absolute values and spatial variation in productivity and nutrient concentrations vary among sampling dates during the season. Accordingly, the type of nutrient limitation in the plant community also shows high spatiotemporal variability.
(ii) Spatiotemporal variation found in plant productivity and biomass composition can be

explained by abiotic soil characteristics and/or plant diversity.

Materials and methods

Study site and sampling design

The studied grassland (48°27′31.37″N, 9°27′36.26″E; 728 m a.s.l.) is a part of the *Biodiversity Exploratories*, a large interdisciplinary research project examining the relationships between land use, biodiversity, and ecosystem functioning (Fischer et al. 2010). The grassland is located in the Schwäbische Alb, a low limestone mountain range in southwest Germany. It has never been plowed or fertilized and is situated on rather nutrient-poor substrate. The soil type is characterized as a Rendzic Leptosol (FAO classification), a shallow soil on calcareous bedrock. The grassland is mown once a year in summer and briefly grazed by sheep in early autumn. The vegetation is dominated by three species: two grasses, *Festuca rubra* L. (mean cover 22 %) and *Helictotrichon pubescens* (Huds.) Pilg. (15 %), and one forb, *Plantago lanceolata* L. (14 %). Additional species with relatively high mean cover were the two forb species, *Galium mollugo* agg. (5 %) and *Geranium pratense* L. (4 %). A vegetation record from a plot adjacent to our study plot revealed 41 vascular plant species in 16 m^2.

The experiment, known as SCALEMIC, was established in spring 2011 by establishing a 10 m × 10 m plot and dividing it into 30 blocks (each 2 m × 1.67 m; Fig. 4 in Appendix; for further details see Regan et al. 2014). Each block was further subdivided into six rectangular areas, each of which contained pairs of 20 cm × 20 cm subplots. Thus, for each sampling date the dataset consisted of sixty samples (30 blocks × 2 subplots). Samples were collected in 2011: on April 5th at the beginning of the vegetation period, May 17th during the main growth phase, June 27th at peak plant biomass, August 16th 2 weeks after the grassland was mown, October 5th 2 weeks after it was slightly grazed, and November 21st after the first frost. Annual precipitation in 2011 did not differ from the mean value of 2005–2014 (2011: 931 mm; mean: 926 mm), but values at the sampling dates varied (Fig. 5 in Appendix; Deppe 2015). Soil texture measurements indicated that texture was fairly uniform throughout the plot with less than 1 % sand, 84.3 % silt, and 14.7 % clay. Average soil pH_{CaCl2} was 6.7, and organic carbon (C) and nitrogen (N) contents of 66 and 7 mg g^{-1}, respectively. Soil pH and soil C:N ratio were uniform over the sampling period (Regan et al. 2014).

Fieldwork and lab analyses

At each sampling date, plant biomass was sampled by cutting all plants at ground level from each of sixty 20 cm × 20 cm subplots. We then separated bryophytes and plant litter from living plant biomass, while plants that had senesced leaves but remained rooted were included. Biomass samples were dried for 48 h at 80 °C and weighed to the nearest 10 mg. We used total aboveground biomass as a measure of grassland productivity. Furthermore, in May, June, and October, all vascular plant species were recorded and their cover was visually estimated in percent prior to biomass harvest. From this data, we calculated the Shannon Index H as a measure of plant diversity (Shannon 1948). At the other dates, plants were too small because it was too early (April), too soon after mowing (August) or too late (November) to allow reliable plant species identification.

For chemical analysis of plant biomass, material was milled to a fine powder with a disk mill (TS 250, Siebtechnik, Mühlheim, Germany) and nutrient concentrations were determined by near-infrared spectroscopy (NIRS) (Kleinebecker et al. 2011b). After a second drying procedure (12 h at 80 °C), we scanned all samples with a SpectraStar 2400 (Unity Scientific, Columbia, MD, USA). Each spectrum is an average of 24 single scans, which were recorded at one nm intervals over a range of 1250–2350 nm. Spectral data were recorded as log $1/R$ (R reflectance). For predicting nitrogen (N), phosphorus (P), and potassium (K) concentrations from the spectral data, calibrations for small sample quantities were used (see Kleinebecker et al. 2011b for details). Four of the measured samples had values exceeding the range of the respective calibration model. These values were excluded from further analyses. To estimate the type of nutrient limitation for plant growth, we calculated N:P, N:K, and K:P mass ratios of the aboveground biomass. Following Koerselman and Meuleman (1996), Olde Venterink et al. (2003) and Güsewell (2004), we used the following critical values: *N limitation* = N:P ≤ 10; *NP co-limitation* = 10 < N:P < 16; *NK co-limitation* = N:K > 2.1 and K:P < 3.4; *P limitation* =

N:P > 16. These ratios were developed specifically for peak standing biomass, but as no critical values for earlier or later seasonal biomass have been reported, we carefully used the existing ratios throughout the season.

Belowground samples were collected with core augers (diameter 58 mm) to a depth of 10 cm after the vegetation was removed and recorded. The top one cm, consisting entirely of litter, was removed from each soil core to avoid introducing surface plant residues into the soil. Soil samples were stored at 4 °C immediately after sampling and sieved (<5 mm) with stones; roots and macrofauna removed within 24 h of collection. The soil was then subdivided for further analyses, with aliquots stored at 4 °C or frozen at −20 °C. Soil water content, reported as % soil dry weight, was determined gravimetrically after drying at 105 °C overnight. Ammonium (NH_4^+) and nitrate (NO_3^-), which together yield N_{min} concentrations, were extracted with 1 M KCl from soil samples (soil to extractant ratio of 1:4 w/v). Phosphate was determined as plant-available inorganic P ($NaHCO_3$ extractable Pi) after Hedley and Stewart (1982) modified by Kuo (1996). Briefly, 0.5 g soil was shaken with 0.5 M $NaHCO_3$ (adjusted to pH 8.5) for 30 min before decantation and filtration (13 P Munktell & Filtrak GmbH, Bärenstein, Germany). Elemental N concentrations (soil N_{total}) were analyzed with a MACRO CNS Elemental Analyzer (Elementar Analysensysteme, GmbH, Hanau, Germany). To determine the inorganic phosphorus concentrations in the extracts we used the ammonium molybdate-ascorbic acid blue method (Murphy and Riley 1962). Extracts were measured with a continuous flow analyzer (CFA, AA3, XY2, Seal Analytical, Norderstedt, Germany) at $\lambda = 660$ nm. Soil properties were analyzed by Regan et al. (2014); see "Methods" section there for detailed descriptions of all analyses.

Statistical analyses

To assess spatial variation in plant community properties at each sampling date, we used the standard deviation (SD) and the coefficient of variance (CV). The CV has the clear advantage to be less dependent on the mean value of the respective property compared with the SD. We used principal component analysis (PCA) to explore patterns in spatial and temporal variation in nutrient concentrations in plant biomass. PCA ordination was performed with z-transformed data using PC-ORD 5 (McCune and Grace 2002). To assess whether biomass, biomass nutrients, nutrient ratios, and plant diversity differed among the sampling dates, we used the glht() function from the *multcomp* package, which computes pairwise comparisons while accounting for heteroscedasticity using a heteroscedastic-consistent covariance estimation (Hothorn et al. 2008). These tests were also used to explore differences in soil characteristics relative to the types of nutrient limitation. As pre-tests revealed that spatial autocorrelation was likely to occur within our dataset, we calculated generalized least square models (Pinheiro et al. 2015) to assess how soil parameters together with biomass and plant diversity affected nutrient concentrations and ratios. This approach allows automatic correction for spatial dependency when testing for effects of environmental variables on biomass nutrients. We used data from May, June, and October ($n = 180$) and employed the following models: $y \sim$ silt percent + soil pH + soil C:N ratio + N_{min} + PO_4^{3-} + soil N_{total} + sampling date + biomass + Shannon diversity of vascular plants. The best model was selected according to the lowest AIC (Akaike Information Criterion). Furthermore, we used pairwise.wilcox.test() for non-normally and pairwise.t.test() for normally distributed variables together with *Holm* adjustment of significance level to search for differences in parameters among limitation types within single months. Results were obtained using the "summary" call, which accounts for shared variance and thus removes effects of the order the single predictors enter the model. All statistical analyses except the ordination were performed with R version 3.1.0 (R Development Core Team 2014).

Results

Variation in productivity, nutrients, and plant diversity

We detected considerable temporal and spatial variation in nearly all analyzed variables as reflected by mean values, standard deviation (SD), and the coefficient of variance (CV; Fig. 1; Table 2 in Appendix). Aboveground biomass showed a typical increase during the growing season with reductions due to

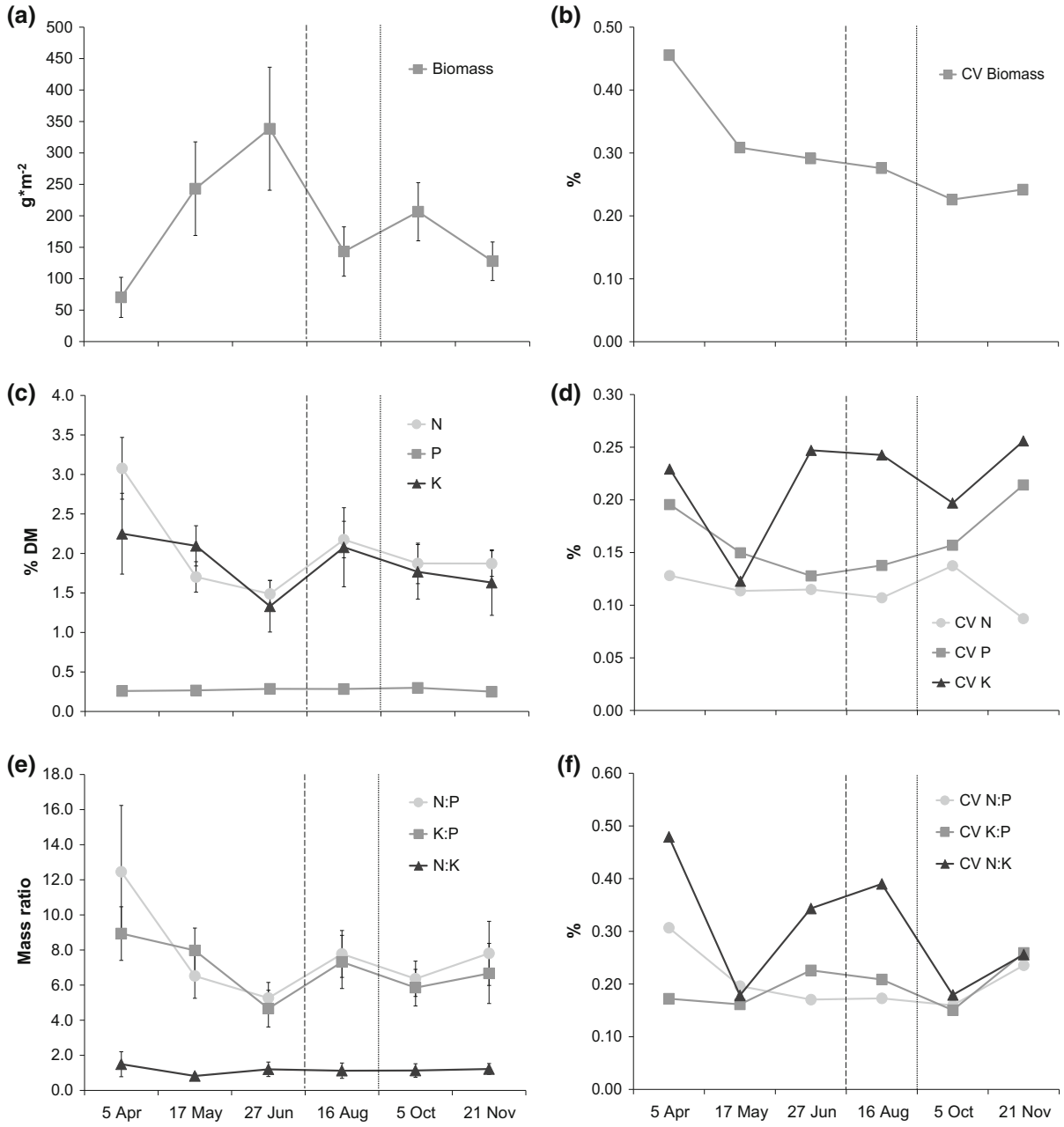

Fig. 1 Temporal changes in mean values (±SD) and the coefficients of variation (CV) in **a**, **b** aboveground biomass, **c**, **d** biomass nutrient concentrations, and **e**, **f** biomass nutrient ratios at six consecutive dates. *Vertical lines* indicate management events. *Broken line* mowing, *dotted line* sheep grazing

management, especially mowing in early August. Spatial variation expressed as SD increased with increasing biomass production; highest values were recorded in June, at peak standing biomass. If spatial variation is expressed as the CV, the result was somewhat different: highest at the first sampling in April and lowest in October (April 46 %, May 31 %, June 29 %, August 39 %, October 23 %, November 26 %; Fig. 1). Biomass nutrient concentrations, especially N and K, but also N:P and K:P ratios, decreased from April to June and then slightly increased in August after the grassland was mown (Fig. 1). In

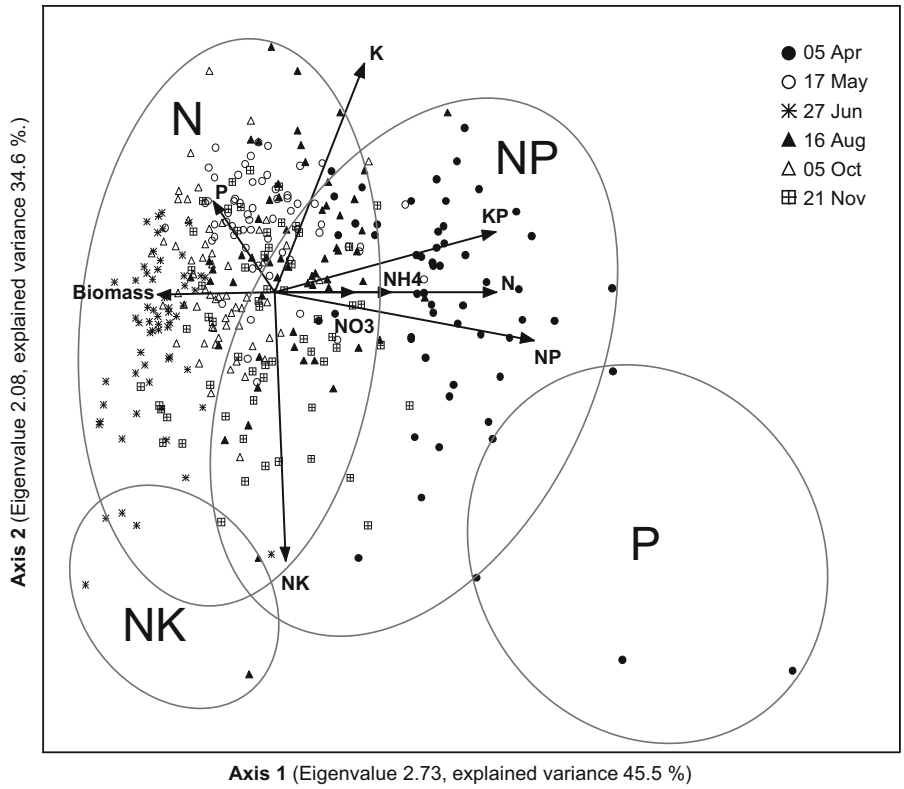

Fig. 2 PCA ordination of biomass nutrient concentrations and nutrient ratios of all subplots at six sampling dates ($n = 356$). Vectors of NO_3^-, NH_4^+ in soil and biomass are a subsequent overlay. *Circles* with *large letters* represent position of different types of nutrient limitation (see "Methods" section for details on estimations of nutrient limitation)

contrast, P concentrations and N:K ratios showed little temporal variation. Among the nutrient concentrations, P showed the smallest relative (proportional) variation over the sampling period (17 %), while N and K varied more strongly (28 %; Fig. 1). The widest range (min–max), describing the maximal spatial heterogeneity, was found for all nutrient concentrations and all nutrient ratios at the first sampling date in April, where also a high CV could be observed (Table 2 in Appendix). Mean plant diversity was constant over the three dates when vegetation was recorded. Nevertheless, it showed considerable spatial variability at each sampling date (Table 2 in Appendix). Mean number of plant species per subplot was 12.2 ± 2.7 SD (min 6, max 19).

PCA ordination of nutrient concentrations in biomass revealed the same trends and patterns as found by separate analyses of single nutrients (Fig. 2). The first ordination axis was predominantly positively correlated with N concentrations, N:P, and K:P ratios, as well as N_{min} in soil (NO_3^- and NH_4^+). This axis was also negatively correlated with biomass production and (less strongly) P concentrations. The second ordination axis was negatively correlated with N:K ratios and positively with P and K concentrations. Although samples from different dates overlapped, samples from the first to the third samplings clearly moved along the first axis, suggesting that this axis partly represented an inverse time axis. In contrast, subplots of later samplings scattered strongly but barely overlapped with those from the first sampling. April samples also covered the largest ordination space (most pronounced variation along the first and second axes), while spatial heterogeneity expressed as dissimilarity in the ordination plot declined later in the season (Fig. 2).

Variability in nutrient limitation

Different types of nutrient limitation occurred within the subplots (Fig. 3). Over the whole season, 294 samples showed N limitation, 53 samples NP co-

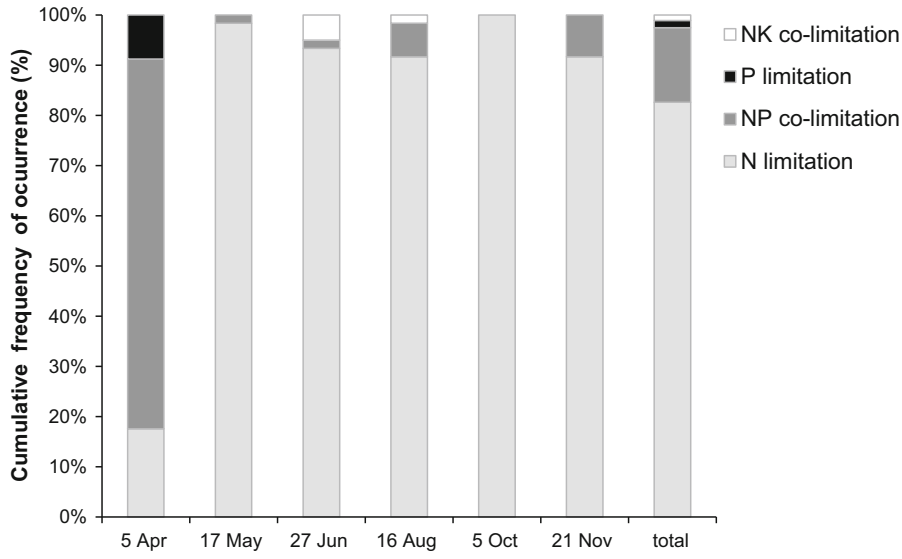

Fig. 3 Nutrient limitation of grassland subplots during the growing season in 2011. Different types of limitation derived from nutrient ratios in plant biomass harvested at six dates of the year and pooled over the dates ($n = 356$)

limitation, 4 samples P limitation, and 4 samples NK co-limitation. While N limitation turned out to be the dominant limitation from May onwards, in April P was similar in importance to N and the majority of subplots revealed NP co-limitation (Fig. 3). This shift in nutrient limitation early in the season was also reflected by the arrangement of samples along the first PCA ordination axis (Fig. 2). At the right side of Axis 1, samples indicated P and NP co-limitation (almost all of them taken in April), while later in the season only N (and in very few cases NK co-limitation) limitation was observed (Fig. 2). A comparison among N, NP co-limited, and P-limited subplots in April revealed no differences in abiotic soil characteristics except for N_{min} concentrations, which were significantly higher for P-limited compared to N and NP co-limited subplots. PO_4^{3-}, soil C:N ratio, silt content, pH, soil N_{total}, and biomass did not differ among the observed limitation types ($p > 0.05$). Testing for differences in plant diversity among limitation types was not possible because detailed vegetation records were not available in April when different nutrient limitation types were observed.

Relationships between biomass nutrients, productivity, soil parameters, and plant diversity

We found that plant diversity was significantly positively associated with P concentrations in plant biomass (Table 1). However, no other biomass parameter appeared related to plant diversity. Nevertheless, soil characteristics partly explained nutrient concentrations, contents, and ratios (Table 1). Soil N_{total} was negatively associated with N concentrations, plant N content, N:P, and N:K ratios, while the silt content of the soil was positively associated with K concentrations and K content in plant biomass. Neither plant biomass nor diversity could be related to any of the soil characteristics. Equally, biomass and plant diversity were not interrelated (Table 1).

Discussion

In accordance with our hypotheses, we observed considerable spatiotemporal variability in plant productivity, biomass nutrient concentrations, and nutrient limitation during the growing season within the studied temperate grassland, consistent with Gross et al. (2009) and Fridley et al. (2011). While the observed variability was strongly dependent on sampling date, abiotic soil characteristics were by far less important, except for specific nutrient concentrations or ratios. Overall, mean plant productivity (biomass) and biomass nutrients were low, but in the range of values reported for nutrient-poor calcareous dry grasslands (Kleinebecker et al. 2011a) as well as for more nutrient-rich, fertilized mesic grasslands (Klaus et al. 2011).

Table 1 Summary of generalized least square models of aboveground biomass, biomass nutrients, and plant diversity in relation to biotic and abiotic site characteristics sampled in 2011

	Correction	df	Intercept	Date	Soil N_{total}	Silt content	Biomass	Shannon diversity
Biomass (g m^{-2})	Null	170		***			×	
N%	Linear	169		**	* ↘			
P%	Linear	169		**			** ↘	* ↗
K%	Spherical	168		***		** ↗	*** ↘	
N:P ratio	Exponential	169	**	***	* ↘		* ↗	
K:P ratio	Linear	168		***				
N:K ratio	Exponential	168		***	* ↘		** ↗	
Shannon diversity	Null	170	**					×

Only significant variables are shown. Note that in the cases of biomass and Shannon diversity respective values were not used as an explanatory variable for themselves (indicated by ×). Data from May, June, and October were included

Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; no arrow $p > 0.05$. Upward arrows indicate a positive, downward arrows a negative effect. Correction: accounting for spatial autocorrelation in best model (null = no significant spatial autocorrelation detected). Variables which were included in the calculation of models but were not significant in any case: soil pH, soil C:N ratio, soil N_{min}, and PO_4^{3-}. See "Methods" section for further details

Patterns in temporal and spatial variation of productivity and nutrients

Our study showed that both mean values and spatial variability of plant productivity and biomass nutrients depended on sampling date. This was related in part to land use, particularly mowing, but in April, especially, conditions for plant growth appeared to differ significantly from subsequent months. At this date, many biomass parameters and nutrient ratios exhibited great spatial variation, indicating pronounced heterogeneity in plant chemical composition and plant nutrition early in the season. This pattern suggests a strong relationship to soil nutrient concentrations (NH_4^+, NO_3^-, PO_4^{3-}), which also showed highest spatial heterogeneity in April (Regan et al. 2014). From May onwards, nutritional conditions for plants appeared more homogeneous, perhaps due to enhanced biological activity of both soil organisms and plants. Plants can, for example, compensate for small-scale variations in soil conditions by increasing root growth, especially with respect to rather immobile nutrients such as P (Mullen and Schmidt 1993). Fungal biomass, as determined by the fungal PLFA marker, increased considerably from April to June (Regan et al. 2014) indicating better support of P acquisition for plants later in the season due to increased mobilization of P by fungi (Mullen and Schmidt 1993). Comparatively high variability in N, P, and K concentrations in plant biomass early in the season as well as decreasing nutrient concentrations from April to July were reported by Kleinebecker et al. (2011a) at a higher spatial scale (16 m^2) for a calcareous dry grassland in northwest Germany.

Generally, decreasing nutrient concentrations in plant biomass with time can be attributed to differences in plant tissues, especially young vs. old (senescent) tissues (Ågren 2008). Furthermore, N and K concentrations tend to decrease with increasing biomass production, a result of what is known as the dilution effect (Hejcman et al. 2010); that is, concentrations of nutrients in plant tissue decrease with further biomass production when plants increase the proportion of supporting tissue. This effect also explains higher nutrient concentrations after mowing, because the young tissue after plant cutting has higher nutrient concentrations than older tissue (Kleinebecker et al. 2011a).

Changes in nutrient limitation

While in April P (co-)limited growth in most subplots, later in the season N limitation dominated. This is in partial agreement with other studies, some of which identified N limitation most frequently in agricultural grasslands (Vitousek and Howarth 1991; Güsewell 2004; Klaus et al. 2013), while others demonstrated that P limitation arises in nutrient-poor, mostly

calcareous grasslands often associated with low productivity (Wassen et al. 2005; Klaus et al. 2011). Phosphorus and NP co-limitation were not accompanied by lower extractable PO_4^{3-} concentrations compared with N-limited subplots, however, possibly because P acquisition by plants due to e.g., exudates may not be related to generally higher PO_4^{3-} concentrations in calcareous soils. Instead, soil N_{min} concentrations tended to be higher under P than under N limitation. This indicates that plants may not have been able to maximize their use of available N due to P shortage. This early-season P shortage paralleled low soil fungal biomass (PLFA data, Regan et al. 2014). PLFA data from that study used only the marker for saprotrophic fungi, but in fact P limitation could also have been the effect of delayed mycorrhizal activity, because mycorrhizal fungi show a severe temperature dependency with lower activity during colder periods (Gavito et al. 2003; Koorem et al. 2014). Nevertheless, as our results clearly showed seasonal variability of nutrient limitation, future studies on nutrient cycling and limitation should pay more attention to the seasonal aspect of nutrient availability, perhaps especially focusing on the spatial and temporal variability in various fungi in soil.

Associations between plant diversity and plant biomass nutrients

Biomass production was not significantly related to plant diversity, in contradiction to the positive effect of diversity on productivity at the small scale found by Gross et al. (2009). However, Gross et al. found this in only one out of four studied grassland systems and the positive effect was very likely due to low-diversity plots with down to two species only, which is much less than in our study. In our study, plant diversity was not negatively related to any biomass parameters, although in observational inter-site studies at larger plot scales (16 m^2), productivity as well as biomass nutrient concentrations have often been negatively associated with plant diversity (e.g., Socher et al. 2012; Klaus et al. 2011, 2013). On the contrary, we found a positive association between P concentrations and plant diversity. This suggests that either more species-rich plant assemblages might serve to acquire P more effectively due to different interspecific mechanisms for P uptake (niche differentiation/resource partitioning hypothesis; Bazzaz and Catovsky 2001) or that with increasing diversity the probability of having species with naturally high P concentration increases (sampling effect hypothesis; Hector et al. 2002). However, due to the screening approach in this study, we cannot state definitively that this is a relevant ecological mechanism for plant nutrition. Nonetheless, Abbas et al. (2013) found positive effects of plant species richness on P stoichiometry along an experimental diversity gradient. As an underlying mechanism, higher functional diversity could promote nutrient acquisition by complementary resource utilization (e.g., Oelmann et al. 2007; Gubsch et al. 2011; Kleinebecker et al. 2014), although this mechanism is still debated (e.g., Kahmen et al. 2006).

Effects of plant diversity, such as the mechanism of complementary resource utilization, may well be important for nutrient cycling in ecosystems such as unfertilized semi-natural or natural grasslands. Under agricultural use, in contrast, the complementarity effect can be overridden by intense management, particularly fertilization, resulting in low-diversity stands with high P concentrations due to high P additions (Klaus et al. 2011).

Conclusions

Our results indicated distinct spatial heterogeneity in measures of plant nutrition and nutrient limitation in spring, most likely due to temperature-induced low biotic activity in the soil. Consequently, early growing season environmental conditions could play a major role in plant community assembly, with subsequent importance for related ecosystem processes. However, to clarify this issue more research is needed involving a higher number of grasslands differing in management type and intensity. Likewise, the positive association between plant diversity and biomass P concentrations should be investigated in more detail. Finally, our study has demonstrated both the importance of smaller spatial scales and temporal variation for elucidating mechanisms of ecosystem functioning.

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Compliance with ethical standards

Conflict of interest Thus, the authors declare that they have no conflict of interest.

Field work permit Field work permits were issued by the responsible state environmental offices of Baden-Württemberg.

Research involving human and animal rights The study did not involve any experiments with humans or animals.

Appendix

See Figs. 4, 5 and Table 2.

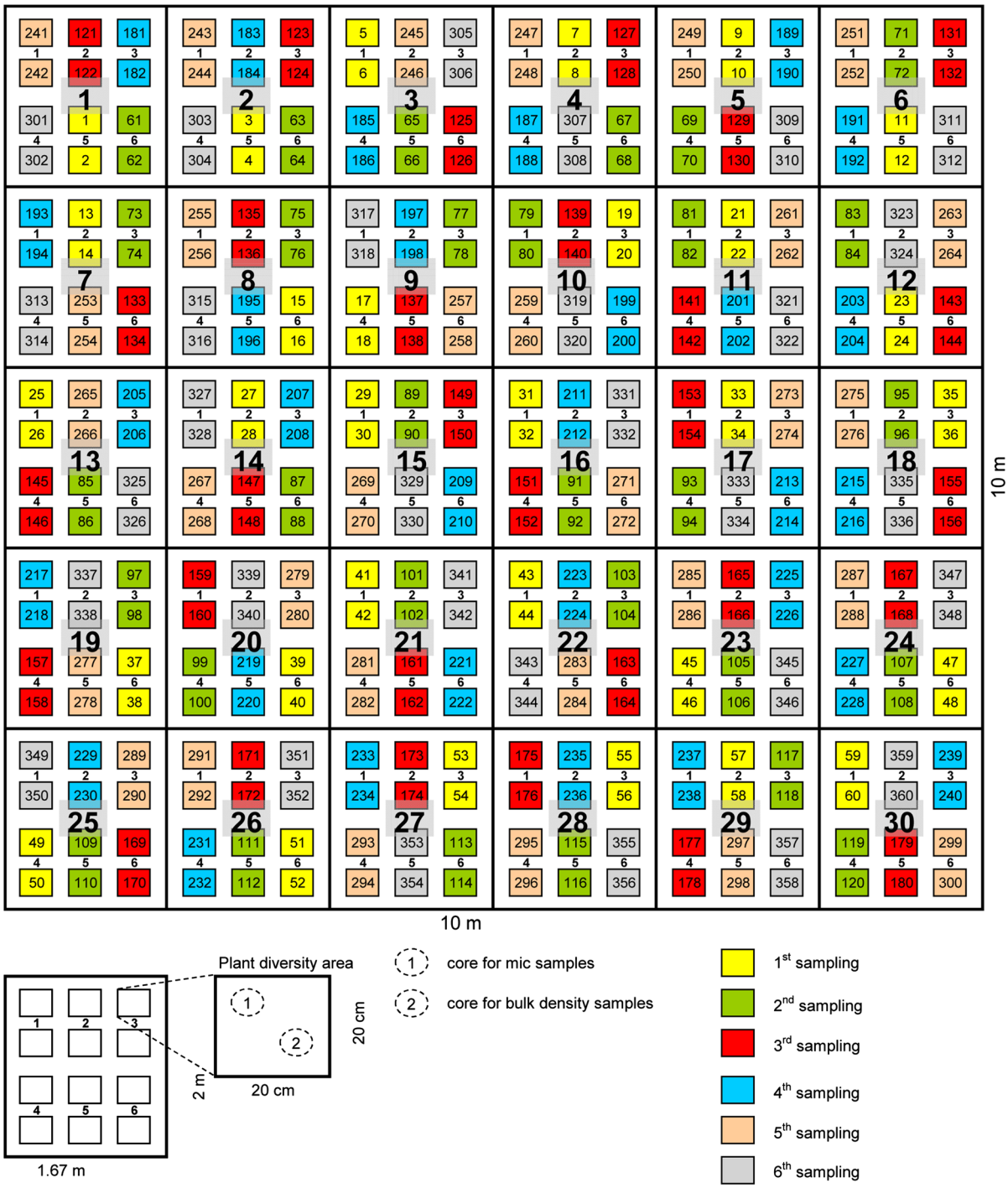

Fig. 4 Sampling design of the study site (taken from Regan et al. 2014)

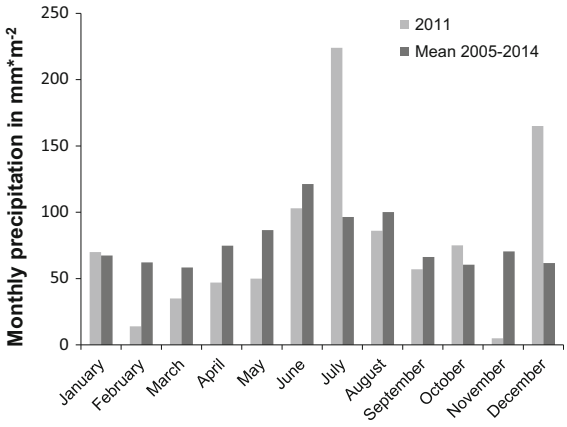

Fig. 5 Monthly precipitation in 2011 compared to means of the time period 2005–2014 (Deppe 2015)

Table 2 Descriptive statistics of aboveground biomass, biomass nutrients per square meter, biomass nutrient concentrations, biomass nutrient ratios, and Shannon diversity of plants in all subplots grouped according to sampling date in 2011

	5th of April					17th of May				
	n	Range	Min/Max	Mean	SD	*n*	Range	Min/Max	Mean	SD
Biomass (g m^{-2})	60	132.8	24.3/157.5	70.3	32.0e	60	332.2	116.3/448.5	243.3	75.0b
N%	59	2.04	2.20/4.24	3.08	0.39a	60	0.99	1.28/2.27	1.70	0.19d
P%	57	0.30	0.08/0.38	0.26	0.05b	60	0.19	0.18/0.37	0.27	0.04bc
K%	59	2.80	0.52/3.32	2.25	0.52a	60	1.16	1.41/2.57	2.10	0.26a
N:P ratio	57	25.36	7.87/33.23	12.45	3.82a	60	6.19	4.9/11.08	6.52	1.28c
K:P ratio	57	9.11	4.64/13.75	8.94	1.54a	60	6.84	5.17/12.01	7.97	1.29b
N:K ratio	59	5.02	0.95/5.97	1.49	0.72a	60	0.78	0.60/1.38	0.83	0.15c
Shannon diversity	–	–	–	–	–	60	1.27	1.16/2.43	1.74	0.31
	27th of June					16th of August				
	n	Range	Min/Max	Mean	SD	*n*	Range	Min/Max	Mean	SD
Biomass g*m^{-2}	60	582.5	167.5/750.0	338.5	98.5a	60	212.0	58.5/270.5	143.3	39.5d
N%	60	0.83	1.17/2.00	1.49	0.17e	60	1.23	1.60/2.83	2.18	0.23b
P%	60	0.19	0.18/0.37	0.29	0.04ac	60	0.18	0.21/0.39	0.28	0.04ac
K%	60	1.45	0.53/1.98	1.33	0.33c	60	2.50	0.79/3.29	2.08	0.50a
N:P ratio	60	6.33	3.95/10.28	5.27	0.90d	60	6.16	5.2/11.38	7.78	1.34b
K:P ratio	60	5.14	1.67/6.81	4.67	1.05e	60	8.38	2.48/10.86	7.32	1.53bc
N:K ratio	60	2.08	0.72/2.80	1.21	0.41b	60	2.95	0.63/3.58	1.13	0.44b
Shannon diversity	60	1.49	0.84/2.33	1.71	0.28	–	–	–	–	–
	5th of October					21st of November				
	n	Range	Min/Max	Mean	SD	*n*	Range	Min/Max	Mean	SD
Biomass (g m^{-2})	60	208.8	105.5/314.3	206.5	46.8c	60	129.2	63.8/193.0	127.8	31.0d
N%	60	1.54	1.41/2.95	1.87	0.26c	60	0.92	1.48/2.40	1.87	0.16c
P%	60	0.21	0.20/0.41	0.30	0.05a	60	0.23	0.12/0.35	0.25	0.05b

Table 2 continued

	5th of October					21st of November				
	n	Range	Min/Max	Mean	SD	*n*	Range	Min/Max	Mean	SD
K%	59	1.70	1.16/2.86	1.77	0.35b	60	1.53	0.87/2.40	1.63	0.42b
N:P ratio	60	3.98	4.46/8.44	6.36	1.02c	60	8.17	5.35/13.52	7.81	1.84b
K:P ratio	59	3.81	4.53/8.34	5.93	0.88d	60	7.54	3.42/10.96	6.67	1.73c
N:K ratio	59	1.17	0.63/1.80	1.09	0.20b	60	1.19	0.79/1.98	1.22	0.31b
Shannon diversity	60	1.62	1.02/2.64	1.78	0.32	–	–	–	–	–

Different letters indicate significant group differences

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