Soil properties as key predictors of global grassland production: have we overlooked micronutrients?

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Abstract

Fertilization experiments have demonstrated that nutrient availability is a key determinant of biomass production and carbon sequestration in grasslands. However, the influence of nutrients in explaining spatial variation in grassland biomass production has rarely been assessed. Using a global dataset comprising 72 sites on six continents, we investigated which of 16 soil factors that shape nutrient availability associate most strongly with variation in grassland aboveground biomass. Climate and N deposition were also considered. Based on theory-driven structural equation modelling, we found that soil micronutrients (particularly Zn and Fe) were important predictors of biomass and, together with soil physicochemical properties and C:N, they explained more unique variation than climate and N deposition (32% vs 24%, respectively). However, the association between micronutrients and biomass was absent in grasslands limited by N(P). These results highlight soil properties as key predictors of global grassland biomass production and point to serial co-limitation by N(P) and micronutrients.

Introduction

Climatic factors, particularly precipitation, have long been recognized as major determinants of grassland aboveground productivity at a global scale (Sala *et al.* 1988; Huxman *et al.* 2004). The important role of soil nutrients in determining biomass production patterns has likewise long been acknowledged (Chapin 1980) and extensively studied in native and managed grassland ecosystems. Fertilization experiments repeatedly demonstrate that grassland productivity can be significantly limited by two macronutrients in particular: nitrogen and phosphorus (Craine & Jackson 2010; Harpole *et al.* 2011; Ågren *et al.* 2012; Niu *et al.* 2018). In line with this, modelled anthropogenic N deposition has been shown to predict 16% of the variation in global grassland biomass production (Stevens *et al.* 2015).

Recent research has revealed that co-limitations by nutrients other than N and P can also occur in many grasslands (Olde Venterink *et al.* 2001; Elser *et al.* 2007; Borer *et al.* 2014b; Fay *et al.* 2015; Lannes *et al.* 2016). For example, Fay et al. (2015) demonstrated that half of the 42 investigated grasslands responded to a mixture of less-studied nutrients (potassium, calcium, magnesium, sulphur) and elements found in trace amounts in plants – micronutrients (iron, boron, copper, manganese, zinc). This points to a potentially significant oversight of these nutrients, particularly micronutrients, given that they are rarely measured across large spatial scales. Even though micronutrients are needed in much smaller quantities for plants than N and P, they are constituents of prosthetic groups that catalyse redox processes, form enzyme-substrate complexes, enhance enzyme reactions or play a role in protein synthesis (Fageria *et al.* 2002; Broadley *et al.* 2011). They also indirectly influence plant production by regulating aspects of plant defence (e.g., tissue palatability) and reproduction, e.g. by contributing to the manufacture of floral structures (Römheld & Marschner 2018). While agronomists have long understood the potentially subtle but significant role of nutrients other than N and P for crops (Fageria *et al.* 2002), their importance for plant production in non-agricultural grasslands globally is mostly an unexplored frontier.

Thus far, it has been challenging to comprehensively examine the role of nutrient availability in global grassland productivity, not only because the concentrations of many soil nutrients are not systematically measured but also because, besides nutrients concentrations, nutrient availability is strongly driven by soil physicochemical properties such as pH, texture, organic matter and soil cation exchange capacity (Lehmann & Schroth 2005; Vicca *et al.* 2018; Van Sundert *et al.* 2019). The question thus remains: which soil properties governing nutrient availability are of the most widespread importance for grassland aboveground biomass production and what is their relative contribution compared to atmospheric drivers?

Here, we use the comprehensive and harmonized grassland biomass and soil dataset from NutNet – a globally distributed network of grasslands (Borer *et al.* 2014a) – to examine the relationship between the in-situ variation in soil properties and nutrient concentrations and the variation of global grassland aboveground biomass production (hereafter referred to as biomass). The data on biomass, measured in a consistent manner in 72 sites around the globe (Fig. 1), were collected along with soil physicochemical properties, the concentrations of 12 different soil nutrients and integrated modelled data on atmospheric N deposition and climatic conditions. This dataset thus contained information about a wide set of soil nutrients across globally distributed grassland sites with contrasting climatic conditions and levels of N deposition.

In line with the conventional knowledge, we expected that, besides climate and N deposition, soil physicochemical properties would have a predominant influence on plant biomass production due to their decisive effect on overall soil fertility (Bünemann *et al.* 2018). Part of the effect of atmospheric factors and soil physicochemical properties was expected to occur via their influence on the concentrations of N and P, which are well-known limiting factors in grasslands (Filippelli 2008; LeBauer & Treseder 2008). Less clear was whether variation in K and a range of other nutrients would have any detectable influence globally, despite their acknowledged importance in plant metabolic processes. We hypothesized that the influence of these nutrients might emerge in situations where N and P availability does not limit biomass production (Kaspari 2021). To test these hypotheses, we used structural equation models (SEM) where the variables hypothesized to be key biomass drivers were given the advantage in model construction (Grace *et al.* 2010). We also tested if the relationship between important nutrients other than N and P identified in the SEM and biomass persists in grasslands with low N availability and those previously shown to be NP (co-) limited.

Materials and methods

Experimental design and biomass sampling

Plant aboveground biomass was sampled from 72 Nutrient Network (NutNet) grassland experimental sites (www.nutnet.org) (Table S1). Sites were located on six continents and spanned a wide range of peak biomass (58 – 1602 g/m^2), mean annual precipitation (211 – 2813 mm) and mean annual temperature (-2.7 – 27.8℃) (Fig. 1). At each site, standing crop (live biomass and recently senescent material) was measured by destructively clipping aboveground vegetation at the peak of the growing season from two 0.1 m^2 (10 x 100 cm) strips for a total of 0.2 m^2 within 5 x 5 m permanent plots. More details on experimental design for NutNet sites are described in Borer et al. (2014a). At each site, the data were collected from non-fertilized plots. Total live biomass was then dried at 60℃ and weighed to the nearest mg. Single-time-point biomass measurements were performed between 2007 and 2017, depending on the site (Table S1). Most sites contained 30 plots while 12 sites contained fewer than 10 plots (a minimum of three). We calculated average standing biomass from all the plots within a site to obtain a proxy of aboveground grassland biomass production [g/m^2] per site. While peak standing crop is not a perfect measure of biomass production (Scurlock *et al.* 2002), it has been shown that this method can be a fairly good indicator for the general ranking of grassland biomass production and that it can produce similar estimates compared to those obtained by more complex methods (Lauenroth *et al.* 2006). Some of the sites were subject to different management practices within one year before biomass sampling. To assess the potential effect of different management practices on biomass production estimates (management was present in 25 out of 63 sites for which the data was available), we created a land-use intensity index based on grazing intensity, mowing intensity and the presence of burning. Low-intensity grazing was assigned with score 1, medium with 2 and high with 3. Low-intensity mowing with 1 and higher intensity mowing with 2 and burning with the score 1. These scores were then summed into a land-use intensity index (following a similar approach as in Blüthgen et al. (2012)). Moreover, to examine the effect of the longer-term management history, we divided the sites into relatively pristine (unmanaged for more than 20 years before the sampling; 23 sites) and more recently managed sites (39 sites).

Soil sampling and analyses

Soil sampling was conducted in the same 5 x 5 m plots where biomass was measured by taking three soil cores (2.5 cm diameter) at a depth of 0-10 cm. The soil was subsequently pooled in one sample per plot, air-dried and analysed for different nutrients (total N and total C, extractable soil P, K, Ca, Mg, Na, S, Zn, Fe, B, Cu, Mn), pH, soil organic matter (SOM), and cation exchange capacity (CEC). Except for the latter two at a few sites, all measurements were performed in the same years of biomass sampling. Total soil C and N [mass per g of soil] were determined using dry combustion gas chromatography on an Elemental Analyzer (Costech ECS 4010 CHNSO Analyzer, Valencia, CA USA). pH was determined by a pH meter in 1:1 soil: water v:v suspension (A&L Analytical Laboratory, Memphis, TN USA). The concentrations of extractable P, K, Ca, Mg, Na, S, Zn, Fe, B, Cu and Mn [mass ppm] were analysed using the Mehlich-3 extraction method with Inductively Coupled Plasma Mass Spectrometry (A&L Analytical Laboratory, Memphis, TN USA). Mehlich-3 analysis is considered suitable for the determination of both macro- and micronutrients in a wide range of soil types (Mehlich 1984; Jones 1990). The measured concentrations were in all cases above the minimum detection level for different micronutrients. While Mehlich-3 was designed for acid to neutral soils, it has been shown to give reliable results in calcareous soil for most micronutrients analysed in this study, except for Mn (Friedericks 1994; Iatrou *et al.* 2015). Effective cation exchange capacity [meq/100g] (referred to as CEC) was estimated based on the concentrations of Ca, Mg and K using the method described by Ross & Ketterings (1995). This method of determining cation exchange capacity is reliable for soils with pH < 7.5 (Ross & Ketterings 1995). The percentage of soil organic matter was determined using the loss on ignition method, by performing soil combustion at 400 °C. Soil texture, expressed as the percentage sand, percentage silt, and percentage clay, was measured for 45 sites on 100 g dry soil using the Bouyoucos method (A&L Analytical Laboratory, Memphis, TN USA). The values of soil parameters were averaged per site. Given that some of the methods might have limitations in calcareous soils, we repeated the original analyses excluding six sites with pH higher than 7.5 and we found comparable results (Fig. S2) .

Climatic and N deposition data

We obtained climatic data based on the site locations using global databases. Mean annual precipitation (MAP) and temperature (MAT) estimates for the period between 1979 and 2013 were derived using the 'Climatologies at high resolution for the earth's land surface areas' database (Karger *et al.* 2017); hereafter referred to as 'CHELSA'. We compared CHELSA precipitation estimates with long-term weather-station measurements available for 41 sites and we used the measured values instead of CHELSA-estimates for nine sites where the latter were more than 15% off. In all other cases, CHELSA-estimated and measured values were very similar (Fig. S1). We further calculated the length of the growing season as the number of months with a mean monthly temperature higher than 5^{o}C. This threshold is considered to be appropriate especially for mid-latitudes (Frich *et al.* 2002), where the majority of our sites are located, but it was used here as a rough indicator of growing-season length for all the sites. Based on this, mean precipitation and mean annual temperature during the growing season (MAPgs and MATgs, respectively) were calculated and included in the analyses in addition to MAP and MAT because they might better represent the conditions plants are exposed to during the period of their activity. The aridity and potential evapotranspiration (PET) data were obtained using the CGIAR-CSI Global-Aridity and PET Database (Zomer *et al.* 2008). Data on total inorganic nitrogen deposition [kg/ha/yr] was derived from Ackerman et al. (2018). We used the average values over the period of years available in the database (1984-1986, 1994-1996, 2004-2006, and 2014-2016) to account for long-term patterns of N fertilization via atmospheric deposition.

Statistical analyses

Disentangling the predictors of aboveground biomass

To disentangle the direct and indirect role of different (often correlated, Fig. S3) predictors, we used structural equation modelling that incorporates prior knowledge in model building. With this approach, the variables that are expected to have the most important role on biomass production either directly or indirectly through other factors (e.g., climate through soil nutrients) were given the advantage in the model construction so that their potential direct and indirect effects could be explored (Fig. 2). Variables were $\log_e$-transformed prior to analyses in case of a skewed distribution to improve normality and linearity. All analyses were performed in R (version 3.3.2) (R Core Team 2015). Structural equation models were constructed using the *lavaan* package (Rosseel 2012).

We constructed SEMs representing the influence of different variables in three steps (Fig. 2c). Climate, atmospheric N deposition and soil physicochemical properties determining soil fertility (SOM, CEC, pH) were expected to be the main overarching drivers of global grassland biomass production (Sala *et al.* 1988;

Huxman *et al.* 2004; Stevens *et al.* 2015; Bünemann *et al.* 2018) and their influence was therefore tested first (Fig 2c). In addition, we hypothesized that the availability of the most limiting macronutrients (NP) and/or other nutrients explain additional variation due to their important role in (co)-limiting grassland productivity (Olde Venterink *et al.* 2001; Elser *et al.* 2007; Harpole *et al.* 2011; Fay *et al.* 2015; Lannes *et al.* 2020). Besides direct effects, we tested all possible indirect effects of climate on aboveground biomass through soil physicochemical properties (Zhao *et al.* 2019) and soil nutrients (Havlin 2004; Bünemann *et al.* 2018) (Fig. 2b). Precipitation was also expected to have an additional indirect influence on aboveground biomass through atmospheric N deposition as precipitation determines wet N deposition rates (Prado-Fiedler 1990; Kryza *et al.* 2011; Wałaszek *et al.* 2013). Moreover, given that atmospheric N deposition is typically high in regions with strong anthropogenic influences, we expected that precipitation could be related to increased anthropogenic deposition of other nutrients and thereby to soil nutrient concentrations (Deboudt *et al.* 2004; Vet *et al.* 2014).

Prior to SEM construction, automated model selection using *glmulti* (Calcagno & Mazancourt 2010) based on AICc was performed to determine the combination of atmospheric factors (MAPgs, MATgs, MAP, MAT, aridity, PET and N deposition) that best explained the variation in biomass. These were then used to build the 'core' SEM together with soil physicochemical properties SOM, CEC and pH (Fig. 2c, Table S2). The effect of soil texture was also tested on the subset of sites for which the data were available. Each of the soil physicochemical properties was added separately to the model containing atmospheric factors. All those that significantly contributed ($P < 0.05$) to explaining additional variation were retained and grouped into one composite variable (following a similar approach as in Grace et al. (2016)). This was done by summing the product of each soil property with their coefficient in the full SEM model including atmospheric factors and all retained soil physicochemical properties. The model was then reconstructed substituting the individual soil physicochemical properties with the composite variable. In the following steps, N, C/N and P were separately added to the previous model (Fig. 2b) and those that had a significant contribution were retained and grouped into one composite variable representing this group of macronutrients. The same procedure was applied in the next step for other nutrients (K, Ca, Mg, S, Na, Zn, Fe, Mn, Cu, B).

The fit was assessed using standard indices, where model chi-square (χ^2) $P > 0.05$, comparative fit index (CFI) > 0.95, Tucker-Lewis index (TLI) > 0.95, root mean squared error of approximation (RMSEA) < 0.08, and standardized root mean square residual (SRMR) < 0.08 were considered as indicators of a good fit (Hooper *et al.* 2008). In each step, the models with a good fit, significant (direct or indirect) paths and the highest R^2 was selected and reported.

We further constructed a multiple regression model using the variables with a significant direct path (i.e., black line in Fig. 2b) on biomass in the final SEM and partitioned the variance explained by atmospheric and soil factors. The model performance was evaluated via repeated (100 times) k-fold (k = 10) cross-validation using the *caret* package.

Examining the influence of N availability levels and N/NP (co)limitation on the relationship between other selected nutrients and biomass

We hypothesized that the influence of soil nutrients other than NP selected as important predictors of biomass in the prior step would depend on grassland N availability. To test this hypothesis, we first assigned each grassland site to two groups according to their C:N ratios (low and high) and N deposition levels (low and high) and combined them to obtain a variable with four categories (low C:N - low N deposition, low C:N - high N deposition, high C:N - low N deposition, high C:N - high N deposition). The threshold between 'low' and 'high' levels of N deposition and C:N was based on 50% quantiles (cut-offs of 3.64 kg/h/y and 13.2, respectively). The median value for C:N in our study was comparable to the average C:N value found in worldwide-distributed grasslands (Cleveland & Liptzin 2007) supporting its use to contrast relatively low and high C:N. Mean values of N deposition were 1.73 ± 0.78 / 8.38 ± 4.23, and of C:N = 11.36 ± 1.54 / 16.68 ± 4.08 in the low and the high group, respectively. The group with high C:N and low N deposition is here considered as the 'low N availability level'. This assumption is based on the general finding that C:N is a relatively robust indicator of spatial variation in N availability, where increasing C:N can indicate decreasing N availability (Andrianarisoa *et al.* 2009; Wang *et al.* 2014; Alberti *et al.* 2015; Vicca *et al.* 2018), while atmospheric N deposition can substantially increase N availability but it can take very long for this effect to be translated in a decrease of soil C:N (Vicca *et al.* 2018). We then performed linear regression analyses between selected nutrients and biomass for each group. To test the sensitivity of the chosen threshold and examine the potential influence of the values close to the median, we performed an additional analysis using the threshold of < 33% quantiles for the 'low' group (the threshold value for N deposition = 1.97 kg/ha/y and for C:N = 12.08; mean N deposition in the group = 1.27 ± 0.39, mean C:N = 10.7 ± 1.48) and > 66% quantiles in the 'high' group (the threshold value for N deposition = 5.34 kg/ha/y and for C:N = 14.4; mean N deposition in the group = 10.01 ± 4.0, mean C:N = 18.01 ± 4.3). These analyses provided very similar results (Table S6).

Given that soil C:N and N deposition may not be accurate indicators of soil N availability for all sites (Risch *et al.* 2019), the effect of N limitation on the relationship between selected soil nutrients and biomass was more explicitly examined using the results of the experimental study by Fay et al. (2015). To this end, we explored this relationship for NutNet sites that had previously been demonstrated to be N limited, co-limited by N and P, or without limitation by N alone or combined with P. The normality of residuals of the linear regression analyses was tested using the Shapiro-Wilk test (P > 0.05). Fay et al. (2015) assessed N (co-)limitation in 38 of the 72 sites included in our dataset. The N(co-)limitation status of the other 34 sites was not known and it thus was not possible to confirm that the groups that we designated as having low N availability generally contained N (co-)limited sites.

Results

Disentangling the predictors of aboveground biomass

Structural equation modelling revealed that, in the most parsimonious core model, a composite variable describing soil physicochemical properties (based on SOM and CEC, Table S3) had the strongest influence (factor loading) on biomass, followed by mean annual precipitation during the growing season (MAPgs) which additionally had an indirect effect through N deposition (Fig. 3a). In the second step, N, P and C:N were added but only C:N had a significant effect and was retained in the model (Fig. 3b). In the last step, other nutrients were sequentially added to the previous model out of which two micronutrients (Zn and Fe) were significantly associated with variation in biomass. These were retained and combined into a micronutrient composite variable (Table S3) which was significantly influenced by N deposition and soil physicochemical properties. The final model explained 61% of the variation in biomass (Fig. 3c).

The specific effect of soil texture (%sand, %silt, %clay and sand-to-silt ratio) on biomass was tested in separate analyses conducted on the subset of sites for which the data were available ($n = 45$). While silt had a significant positive and sand to silt ratio had a significant negative association with biomass ($R^2 = 17\%$, $R^2 = 16\%$, $P < 0.01$), this effect was already contained in other correlated core variables (mainly CEC) and the path from silt or silt:sand to biomass in the SEM model was not significant. Hence, the effect of soil texture on biomass was captured by the composite variable representing soil physicochemical properties. It was confirmed that the final SEM for the reduced dataset was similar to the one for the full dataset, such that removing the sites lacking texture data did not affect overall conclusions.

A multiple regression model composed of the variables with a significant direct effect on biomass in the final SEM (MAPgs, N deposition, soil physicochemical composite, C:N and micronutrient composite; the individual relationship between these variables and biomass are shown in Fig. S3) explained 58% of the variation in biomass. Repeated K-fold cross-validation demonstrated that this model predicted 56% of the variation in the validation dataset. Variance partitioning revealed that soil factors together explained a higher proportion of unique variation in biomass than atmospheric factors, i.e., precipitation and atmospheric N deposition (32% vs 24%, respectively).

We additionally tested the impact of land-use intensity and management history on biomass production across sites using linear regression and ANCOVA analyses and found no significant effects with or without accounting for the effect of the most important atmospheric predictors (Table S5).

The influence of N(P) limitation on the relationship between micronutrients and biomass

To investigate the potential influence of soil N availability on the micronutrient-biomass relationship, we created different 'N availability' levels by splitting the dataset into four classes, where the group with high C:N ratio and low N deposition was considered as the low 'N availability' group. Linear regression analyses for each of these four groups showed that the relationship between the micronutrient composite and biomass was significantly positive in all but the 'low N availability' group (Fig. 4, Table S6).

To corroborate these findings, we further explored the micronutrient-biomass relationship for the subset of sites previously demonstrated to be N limited or NP co-limited and those that had no N limitation / NP co-limitation in the fertilization study by Fay et al. (2015). In line with the previous results, the relationship between micronutrient composite and biomass was not detected in N(P)(co-)limited grasslands (Fig. 5a,c) as opposed to grasslands with no signs of N(P) (co-)limitation (Fig. 5b,d) (Table S7).

Discussion

Our results clearly demonstrate the importance of soil factors that govern nutrient availability, i.e., soil physicochemical properties, C:N, and concentrations of soil micronutrients, as predictors of global grassland production. Together, they explained 32% of the unique (non-shared) variation in the most parsimonious model predicting global grassland biomass, more than precipitation and atmospheric N deposition combined. It is noteworthy, however, that the atmospheric factors in this dataset were estimated rather than measured at each site which is why their perceived effect on biomass might be less accurate than for soil properties. Nonetheless, considering the large gradient in climatic conditions, the lower accuracy for atmospheric estimates does not preclude the conclusion that soil properties are important predictors of global biomass production. Moreover, some of the sites in this study were exposed to various types of (mainly low-intensity) management in years prior to biomass sampling. Although different land-use practices can substantially affect biomass production, we found no evidence that management had a consistent influence on biomass across worldwide distributed sites, where the large differences in soil properties and climate likely play a predominant role.

Soil organic matter content, soil texture and cation exchange capacity are key determinants of soil fertility and overall nutrient availability (Havlin 2004; Bünemann *et al.* 2018). Organic matter is a source of nutrients (Schroeder & Gething 1984; Roy *et al.* 2006), which also determines the CEC of soil, indicating its capacity to store and exchange important nutrients. In this study, the index of soil physicochemical properties was strongly correlated with concentrations of different soil nutrients. Overall, this index was a better predictor of grassland biomass than the concentrations of most nutrients. Nonetheless, C:N as one of the indicators of soil N availability (Vicca *et al.* 2018), explained additional variation in biomass together with the index of micronutrient availability based on Zn and Fe. SOM has been shown to play a critical role in driving the transformation and enhancing the accessibility of micronutrient cations (Obrador *et al.* 2003; Cakmak 2008; Chen *et al.* 2017) and our structural equation modelling revealed that the effect of soil physicochemical properties on biomass might partly be mediated by soil - micronutrients.

The potentially important contributing role of micronutrients for grassland productivity has been highlighted in fertilization experiments (Fay *et al.* 2015; Lannes *et al.* 2016), but few studies in non-agricultural grasslands focused on micronutrients additions specifically (however, see Lannes et al. (2020) for the role of B as limiting factor in Cerrado grasslands). Therefore, the role of micronutrient deficiency in the productivity of non-agricultural grasslands globally has not been explicitly considered. Even though micronutrients are only needed in relatively small concentrations and in high concentrations they can be toxic to plants, micronutrient deficiency has been well documented in arable systems (Sillanpää 1982, 1990) where it was found to influence plant growth and limit plant yield in many regions of the world (Rashid & Ryan 2004; Alloway 2008; Shukla *et al.* 2014). For instance, Sillanpää (1990) showed that Zn deficiency occurred in almost 50% out of 190 investigated agricultural soils.

Arable fields are typically subjected to long-term fertilization by macronutrients which can, in turn, induce and/or exacerbate micronutrient limitations. Similarly, in our study, the relationship between micronutrient availability and biomass was present only in grasslands with no signs of N (and P) co-limitations suggesting that when N and P are ample in grassland soils (either naturally or e.g., due to atmospheric fertilization), there might be an increased demand for micronutrients which become limiting for plant growth. These results provide support for serial co-limitation, in which the response to additional micronutrient resources occurs only after N and P have been added (Harpole *et al.* 2011; Kaspari 2021). This imbalanced need for macronutrients before growth-limitation by micronutrients builds from earlier work demonstrating substantial variation among sites in the combinations of elements limiting growth (Fay *et al.* 2015). The positive effect of micronutrient fertilization on the yield of agricultural plants grown in soils with low N(P) and micronutrient availability has been shown to be contingent on N(P) fertilization in several studies (Loneragan & Webb 1993; Cakmak *et al.* 2010; Sahrawat *et al.* 2010). Moreover, it is well acknowledged that N is important for uptake and translocation of certain micronutrients, particularly Zn (Cakmak *et al.* 2010; Shi *et al.* 2010; Erenoglu *et al.* 2011; Gupta *et al.* 2016) which could be another explanation for the lack of the relationship between micronutrients and biomass under low N availability found in this study.

Micronutrient deficiencies are not per se a consequence of low total concentrations of these nutrients in soil but rather as a result of soil factors that reduce their availability to plants (Sillanpää 1982). Our results show that the grasslands located in the regions with higher temperatures and potential evapotranspiration, with predominantly sandy soils poor in organic matter might be prone to Zn and Fe deficiencies while other micronutrients might be deficient in soils with low cation exchange capacity (Fig. S5). It has previously been shown that drylands and alkaline (calcareous) soils are particularly prone to micronutrient deficiencies (Chen & Barak 1982; Fageria *et al.* 2002). Our dataset included only few grasslands in arid regions with alkaline soils, but it is possible that the effect of micronutrients on biomass production in such grasslands would be even more pronounced. The expansion of aridity in grasslands might thus further exacerbate micronutrient deficiencies in future (Moreno-Jiménez *et al.* 2019). On the other hand, combined macronutrient and metallic micronutrient deposition (which are often tightly related to industrial activities (Pan & Wang 2015) might alleviate them.

This study emphasizes the importance of soil physicochemical properties and nutrients including micronutrients, for predicting grassland biomass production globally. Although observational studies cannot fully disentangle causal relationships, our results highlight the potential undervalued role of micronutrients in global plant productivity while motivating future experiments. Such manipulation experiments should focus on micronutrient (especially Zn) additions, alone and in combination with NP, particularly in the grasslands that are likely to be prone to micronutrient deficiencies (high sand content, low organic matter content, calcareous soils) to further unravel the role that nutrients play in determining grassland productivity. It would also be beneficial to measure soil properties and nutrients (Vicca *et al.* 2018), including micronutrients (both in plants and soil) in studies investigating grassland productivity. This would allow to determine the extent of deficiencies of these nutrients and their link with grassland productivity. Given the critical role of nutrient availability in mediating grassland responses to environmental changes (Van Sundert *et al.* 2021), information on soil properties and nutrients is essential to fully unravel the impact of global changes on grasslands and other ecosystems.

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Figure captions

Figure 1 The distribution of 72 NutNet grassland sites along the precipitation gradient. White points indicate the location of different sites and different sizes of pink circles correspond to the amount of aboveground biomass per site.

Figure 2 The scheme depicting the methodological approach used in the study to examine the predictors of aboveground biomass production **a)** Three groups of variables and hypothesized relationships between them used in the construction of SEM. **b)** The theoretical direct (black full lines) and indirect paths (dotted grey lines) from different variables to biomass that were tested in SEMs. The numbers indicate the order in which the influence of different factors was examined (1 refers to glmulti pre-selection of atmospheric variables). c) Step-by-step construction of SEMs. The core model explaining variation in biomass was constructed using atmospheric factors and soil physicochemical properties (pcp). Those pcps that had significant contributions in the model were grouped into one composite pcp variable. In the next step, N (C:N) and P were added followed by other nutrients in the final step.

* Due to missing data, soil texture was included in additional analyses on a smaller dataset. Atmospheric factors were not allowed to influence soil texture. ** Pre-selection of atmospheric factors was conducted based on automated model selection procedure.

Figure 3 a) 'Core' SEM depicting the direct (black lines) and indirect (grey lines) influence of different predictors that were hypothesized to be the most important drivers of biomass production. Dotted lines indicate which variables were used in the creation of the composite variable (soil physicochemical properties - pcp) represented by a hexagon. All the paths were significant and factor loadings are indicated for each path. **b)** The most parsimonious model after the addition of N (C:N) and P. **c)** The final SEM after the addition of all nutrients, where the micronutrient composite (mic) was created from Zn and Fe. All models had a good fit based on each of the goodness-of-fit criteria (Table S4).

Figure 4 The relationship between the micronutrient composite (based on Zn and Fe) and biomass ($\log_e$) under different levels of C:N and N deposition; from top-left to bottom-right: low C:N - high N deposition, high C:N - high N deposition, low C:N - low N deposition, high C:N - low N deposition. The median values of C:N and N deposition were taken as thresholds based on which the dataset was split into 4 equal groups. Different colours of the points represent different levels of growing season precipitation (ranging from 160 mm to > 1500 mm per year).

Figure 5 The relationship between the micronutrient composite (based on Zn and Fe) and biomass ($\log_e$) in the subset of NutNet sites (n=38) for which the effect of nutrient additions was assessed by Fay et al. (2015). Linear regression relationship in the soils that were shown to be **a)** N limited (n = 9); **b)** without N limitation (n = 29); **c**) NP co-limited (n = 23); **d**) without NP co-limitation (n = 15).