**Nutrients and herbivores impact grassland stability across multiple spatial scales through different pathways**

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**Abstract**

Nutrients and herbivores alone may impact the temporal stability of aboveground biomass in local grassland communities through changing species richness. Whether nutrients and herbivores jointly impact stability and whether their effects are scale-dependent remain unknown. In an experiment adding nutrients and excluding herbivores in 34 globally distributed grasslands, we found that nutrients and herbivores mainly had additive effects. Nutrient addition consistently reduced stability at the local and larger spatial scales (aggregated local communities), while herbivore exclusion weakly reduced stability at these scales. Moreover, nutrients and herbivores impacted stability at the larger spatial scale via different pathways. Nutrient addition reduced stability primarily by causing changes in local community composition over time, but also by reducing local species richness and evenness, pointing at the importance of turnover driven by species replacement in determining grassland stability. In contrast, herbivore exclusion weakly reduced stability at the larger scale mainly by decreasing asynchronous dynamics among local communities, but also by weakly decreasing local species richness. Our findings indicate that preserving the multiple facets of plant diversity (species richness, evenness, and community composition) may help stabilize grassland biomass across spatial scales from eutrophication and herbivore extirpation.

**Keywords**: biodiversity-stability; grazing; eutrophication; cross-scale; global change

**Introduction**

Grasslands are one of the largest terrestrial ecosystems on Earth (Suttie et al. 2005), providing many essential services to humanity such as feed for livestock, carbon storage, and climate mitigation (Bengtsson *et al.* 2019). However, human activities are altering environmental conditions significantly, threatening the sustainability of grassland services (Suttie et al. 2005). For instance, increasing nitrogen deposition (Galloway *et al.* 2004) and accelerating herbivore extirpation (Ripple *et al.* 2015; Atwood *et al.* 2020) are altering ecosystem functions and services in grasslands (Hautier *et al.* 2015; Blüthgen *et al.* 2016; Qin *et al.* 2019; Zhang *et al.* 2019; Borer *et al.* 2020; Hautier *et al.* 2020). Ensuring proper delivery of grassland ecosystem functions and services requires a good understanding of how nutrients and herbivores affect the stability of these ecosystems. Stability is a multi-dimensional concept (Donohue *et al.* 2013; Arnoldi *et al.* 2019). Here we define stability as temporal invariability, calculated as the mean of aboveground biomass (a proxy for productivity) through time divided by its standard deviation. Based on temporal invariability, metacommunity theory defines consistent measures of stability across spatial scales and clarifies that stability at larger scales (aggregated local communities; gamma stability) is determined by stability in local communities (alpha stability) and asynchronous dynamics among local communities (spatial asynchrony) (Wang & Loreau 2016; Wang *et al.* 2019).

Grassland studies have shown that nutrient addition usually decreases alpha stability (Hautier *et al.* 2015; Koerner *et al.* 2016; Zhang *et al.* 2016; Liu *et al.* 2019) and that these effects propagate to gamma stability (Zhang *et al.* 2019; Hautier *et al.* 2020). In contrast, herbivore exclusion has been found to have positive, neutral, or negative effects on grassland stability, depending on the herbivore species excluded and spatial scales studied (Halpern *et al.* 2005; Hautier *et al.* 2015; Blüthgen *et al.* 2016; Ren *et al.* 2018; Ganjurjav *et al.* 2019; Qin *et al.* 2019; Saruul *et al.* 2019; Liu *et al.* 2021). Effects of herbivores on stability may be more apparent at the larger spatial scale because herbivores usually promote vegetation heterogeneity in the landscape due to selective grazing, trampling, localized deposition of urine and dung (Glenn *et al.* 1992; Howison *et al.* 2017). However, to our knowledge, no study has investigated how nutrients and herbivores jointly regulate grassland stability across multiple spatial scales.

Considerable work has been conducted to assess the joint effects of nutrients and herbivores on grassland species richness (Proulx & Mazumder 1998; Worm *et al.* 2002; Bakker *et al.* 2006; Hillebrand *et al.* 2007; Alberti *et al.* 2010, 2011; Yang *et al.* 2013; Borer *et al.* 2014b; Beck *et al.* 2015; Koerner *et al.* 2018), community evenness (Hillebrand *et al.* 2007), community composition (Milchunas & Lauenroth 1993; Chase *et al.* 2000; Grellmann 2002; Hartley & Mitchell 2005; Hodapp *et al.* 2018), and aboveground biomass (Milchunas & Lauenroth 1993; Chase *et al.* 2000; Moran & Scheidler 2002; Alberti *et al.* 2010, 2011; Borer *et al.* 2020). The majority of these studies found strong interactive effects. For instance, herbivores typically decrease species richness at low nutrients or productivity, while increasing it at higher nutrients or productivity (Proulx & Mazumder 1998; Bakker *et al.* 2006; Hillebrand *et al.* 2007; Borer *et al.* 2014b). Herbivores can also consume extra aboveground biomass stimulated by nutrient addition particularly when their abundance is high (Borer *et al.* 2020). As plant diversity and biomass can impact stability indirectly and directly, it is likely that nutrients and herbivores also jointly impact grassland stability. For instance, nutrients and/or herbivores can indirectly impact gamma stability by regulating species richness at the local and larger spatial scales (alpha and beta diversity respectively) (Hautier et al. 2015; Zhang et al. 2019; Liang et al. 2020). Although less often tested, nutrients and/or herbivores can also impact alpha and gamma stability through community evenness (Grman *et al.* 2010; Liang et al. 2020) and community dissimilarity across time and space (Koerner *et al.* 2016; Zhang *et al.* 2019). See Table S1 for more details on how nutrients and/or herbivores regulate different facets of plant diversity (species richness, evenness, and community dissimilarity) and how these plant diversity metrics impact alpha and gamma stability.

Assessing the relative contribution of different facets of plant diversity to alpha and gamma stability can help prioritize conservation efforts. Local species richness is usually positively correlated with alpha stability (Tilman *et al.* 2006; Hautier *et al.* 2015), but studies have also shown that evenness and change in community composition over time sometimes better predict alpha stability than species richness (Grime 1998; Polley *et al.* 2007; Grman *et al.* 2010; Koerner *et al.* 2016). However, these results are usually based on single-site studies, and the relative importance is often qualitative. Therefore, the generality of the relative importance (in a quantitative way) of different facets of plant diversity to stability remains to be elucidated. Community dissimilarity metrics that integrate changes in species identities and abundance across time or space (i.e. temporal and spatial community dissimilarity) may better capture changes in community composition and structure, and thus may better predict alpha and gamma stability. Indeed, community dissimilarity across time (i.e. temporal community dissimilarity) is commonly used as an index of compositional stability (Hillebrand *et al.* 2018; Hillebrand & Kunze 2020; White *et al.* 2020), and higher compositional stability has been suggested to lead to higher alpha stability (Allan *et al.* 2011). However, recent studies using a consumer removal experiment in a marine system suggest that compositional stability is not necessarily positively correlated with alpha stability (e.g. White *et al.* 2020). Looking at the correlation of temporal and spatial community dissimilarity to alpha and gamma stability can therefore also provide a rigorous test of whether compositional stability corresponds to biomass stability across scales in grasslands, which to our knowledge has not been tested so far.

Here, we used a globally coordinated grassland experiment, Nutrient Network (NutNet) (Borer *et al.* 2014a) to assess (1) the joint effects of nutrient addition and herbivore exclusion on the temporal stability of aboveground biomass at the local and larger spatial scales (i.e. alpha and gamma stability); (2) the relative contribution of different facets of plant diversity in mediating the responses of alpha and gamma stability to nutrients and herbivores. Previous studies using subsets of NutNet data have found that nutrients and herbivores jointly impact species richness and aboveground biomass at sites where grazing intensity is high (Borer *et al.* 2014b, 2020). Nutrient addition not only decreases species richness (Hautier *et al.* 2020), but also increases temporal species turnover (Hodapp *et al.* 2018), and herbivore exclusion further increases temporal species turnover by increasing species replacement (Hodapp *et al.* 2018). We hypothesized that (1) nutrient addition decreases alpha and gamma stability, while herbivore exclusion worsens these decreases particularly at sites with high grazing intensity; (2) Nutrient addition decreases gamma stability via reducing alpha stability, which is regulated by all facets of plant diversity. Herbivore exclusion decreases gamma stability not only via alpha stability but also via spatial asynchrony, because herbivores often increase vegetation heterogeneity in the landscape (i.e. spatial community dissimilarity).

 **Methods**

**Sites selection**

We selected sites that met the following criteria: (1) ≥5 years of post-treatment; (2) ≥3 blocks; (3) each block containing a factorial design of nutrient addition and herbivore exclusion by fencing. All these criteria were met at 36 sites as of May 1, 2020, but 2 desert sites (ethamc.au and ethass.au) were excluded in this analysis due to statistical reasons (too many zeros in biomass). To maximize site number and to capture sufficient temporal dynamics, we focus on 1-5 years post-treatment. For sites with more than 3 blocks, we focused on the first three blocks. A block typically spreads over 320 m2, blocks typically spread over >1000 m2. Sites selected here spanned a broad envelope of climate and included a wide range of grassland types such as montane, alpine, semiarid grasslands, prairies, old fields, pastures, savanna, tundra, and shrub-steppe (Table S2).

**Experimental design**

Details on the experimental design of NutNet have been documented elsewhere (e.g. Borer *et al.* 2014b, a). Here we only provide a brief description. In each block at each site, two plots were fertilized with nitrogen (N), phosphorus (P), potassium (K), and micronutrients (Fe, S, Mg, Mn, Cu, Zn, B, and Mo in combination). Nutrients were added annually before the start of the growing season at rates of 10 gm-2 y-1. N was supplied as time-release urea ((NH2)2CO) or ammonium nitrate (NH4NO3), there were no detectable differences between these N sources on plant biomass or diversity (Seabloom *et al.* 2015). P was supplied as triple superphosphate (Ca(H2PO4)2), and K as potassium sulfate (K2SO4). The micronutrient mix was applied at 100 g m-2 at the start of the experiment. e Two plots (one in ambient conditions and the other receiving nutrient addition) were enclosed with fences to test the effects of herbivore exclusion (excluding mammalian herbivores > 50 g) on plant communities. Fences were around 230 cm tall, and the lower 90 cm were covered by 1cm woven wire mesh. To further exclude digging animals such as voles, an additional 30 cm outward-facing flange was stapled to the soil. Four strands of barbless wire were strung at similar vertical distances above the wire mesh. Six of the 34 sites deviated from this fence design (Table S2). Wild herbivores such as rodents, lagomorphs, ungulates, marsupials are present at all sites, while domestic herbivores such as sheep, yak, goats, and cattle are also present at a few sites (Table S2).

**Sampling protocol**

All NutNet sites followed standard sampling protocols. A 1×1m subplot within each 5×5 m plot was permanently marked and sampled annually at peak biomass. The number of species, species identity, and their covers was recorded. Species cover (%) was estimated visually for all species in the subplots. Aboveground biomass was measured adjacent to the permanent subplot, by clipping all aboveground biomass within two 1 × 0.1 m strips (in total 0.2 m2). For shrubs and subshrubs occurring in strips, we collected all leaves and current year’s woody growth. Biomass was dried at 60 °C (to constant weight) before weighing to the nearest 0.01 g. Dried biomass was multiplied by 5 to estimate grams per square meter. At some sites with strong seasonality, cover and biomass were recorded twice per year to include a complete list of species and follow typical management procedures at those sites. For those sites, the maximum cover for each species and total biomass were used in the following analyses. The taxonomy was adjusted within sites to ensure consistent naming over time. Specifically, when individuals could not be identified as species, they were aggregated at the genus level but referred to as “species” for simplicity.

**Grazing intensity**

FollowingBorer *et al.* (2020)and Anderson *et al.* (2018),we quantified grazing intensity from vertebrate herbivores at each site using a herbivore index. Specifically, herbivore species (>2 kg) that consume grassland biomass were documented at each site by site PIs, and PIs assigned an importance value for each species, from 1 (present, but low impact and frequency) to 5 (high impact and frequency). An index value was calculated for each site as the sum of herbivore importance values for all herbivores.

**Plant diversity metrics** **and stability across scales**

Following Hautier et al. (2020), we treated each 1 m2 subplot as a “community” and the replicated subplots within a treatment across blocks within a site as the “larger scale” sensu Whittaker (1972) (see an illustration in Fig. S1). Plant diversity metrics used in this study included alpha diversity, beta diversity, Pielou’s evenness, and community dissimilarity metrics. Alpha diversity is the average number of species (i.e. species richness) recorded in the three subplots in each treatment at each site. Beta diversity is calculated as gamma diversity/alpha diversity (i.e. multiplicative beta diversity), where gamma diversity is the total number of species recorded in three subplots in each treatment at each site. Pielou’s evenness was calculated as H/ln (S), where H is Shannon's diversity index, and S is alpha diversity.

We calculated community dissimilarity (temporal and spatial community dissimilarity) using Bray–Curtis dissimilarity metrics based on cover data. Note that some researchers also refer to temporal and spatial community dissimilarity as temporal and spatial beta diversity (e.g. Chalcraft *et al.* 2008; Dornelas 2014). A recent theoretical framework demonstrates that community dissimilarity can arise from two concurrent processes, abundance gradients and balanced variation in abundance (Baselga 2017). The abundance gradients arise from a simultaneous increase or decrease in the cover of each species, reflecting changes in total cover. The balanced variation arises from replacement among species (i.e. decreases in the cover of some species are compensated for by increases in other species), reflecting changes in relative cover. Temporal and spatial community dissimilarity may impact gamma stability via alpha and spatial asynchrony respectively, and their impact may depend on the driving processes (see Fig. S2 for more details). Therefore, we also look at which process is driving the temporal and spatial community dissimilarity, and their impact on alpha and gamma stability. The temporal community dissimilarity of each treatment was calculated as the dissimilarity of a community through the 5-year experiments and averaged over 3 blocks. Similarly, the spatial community dissimilarity of each treatment was calculated as the dissimilarity of 3 blocks in each treatment each year and averaged over the experimental years. The temporal/spatial community dissimilarity, and the partitioning of it into abundance gradients and balanced variation in abundance, were done using the function “beta.multi.abund” from the R package betapart (Baselga & Orme 2012) with the index.family of “Bray”. (Baselga & Orme 2012).

Stability at a given spatial scale was calculated as temporal invariability: $\frac{μ}{σ}$ , μ and σ are the mean and standard deviation of aboveground biomass over the experimental years. Alpha stability was the stability of aboveground biomass averaged over three subplots in each treatment at each site; gamma stability was the stability of the total aboveground biomass in three subplots in each treatment at each site (Wang et al. 2019; Hautier et al. 2020). Biomass was not detrended because no clear trends were shown over the 5-year experiment in most sites (Fig. S3). Also, previous studies using NutNet data show that treatment effects (i.e. nutrient addition) on stability is quantitatively the same with or without detrending (Hautier *et al.* 2020). Spatial asynchrony was calculated as 1/φ, φ = $\frac{\sum\_{i,j}^{}w\_{ij}}{\left(\sum\_{i}^{}\sqrt{w\_{ii}}\right)^{2}}$ , where wij is the temporal covariance of aboveground biomass between local communities i and j, and wii is the temporal variance of aboveground biomass of local community i. These variables were calculated using the function “var.partition” (Wang *et al.* 2019).

**Statistical analyses**

All the analyses were performed in R v.3.6.3 (R core team, 2020). We used linear mixed-effects models from the R package “nlme” (Pinheiro *et al.* 2017) to account for the nested structure of the data. We looked at the treatment effects on each plant diversity metric and stability using the formula lme (y ~ nut\*fen, random= ~1|site). Alpha stability, spatial asynchrony, and gamma stability were log-transformed to improve normality and homogeneity of variance. To look at whether the effects of herbivore exclusion and its interaction with nutrient addition on stability increase as the grazing intensity increases, we rerun the above models but adding the herbivore index as a covariate in the model.

We built a structural equation model (SEM) using the function “psem” from the R package piecewiseSEM (Lefcheck 2016) to evaluate the direct and indirect effects of nutrient addition, herbivore exclusion, and their interaction on alpha and gamma stability. An initial model (Fig. S4) was built based on prior knowledge (Grman *et al.* 2010; Wilcox *et al.* 2017; Zhang *et al.* 2019; Hodapp *et al.* 2018; Gilbert *et al.* 2020). Rationales for each link in the initial SEM are summarized in Table S1. To fit the SEM, we used the function “lme” with site as the random effect when the component models tested only the treatment effects. We used function “lm” when the component models tested the relative contribution of both treatments and plant diversity metrics to stability to take the diversity gradient among sites into account. Alpha stability, spatial asynchrony, and gamma stability were log-transformed to improve normality and homogeneity of variance. Several studies suggest that abiotic variables such as rainfall, temperature, and soil parameters impact grassland stability (Zelikova *et al.* 2014; García-Palacios *et al.* 2018; Zhang *et al.* 2018; Gilbert *et al.* 2020). We, therefore, performed another SEM including temporal variability in water balance and spatial variability in soil chemistry following Gilbert *et al.* (2020) to test the robustness of our results. See online supplementary text for more details.

We further analyzed whether alpha stability was correlated to the two components of temporal community dissimilarity and whether spatial asynchrony was correlated to the two components of spatial community dissimilarity. We fitted “lm” models where alpha stability was the response variable, temporal community dissimilarity (or each of its components), and its interaction with treatments as the explanatory variables. We fitted similar models for spatial asynchrony. We define that an effect is marginally significant when 0.05<p<0.1, while significant when p≤0.05.

**Results**

Nutrient addition and herbivore exclusion had no significant interaction on any variables measured except for spatial asynchrony (Fig. 1). Herbivore exclusion decreased spatial asynchrony under ambient conditions but not under fertilized conditions. Subsequently, although not statistically significant, herbivore exclusion reduced gamma stability by 11 % on average compared with that of the control under the ambient conditions. Additionally, effects of herbivores on spatial asynchrony and gamma stability tended to be more apparent at sites where herbivore index was higher under the ambient conditions (Fig. S5). Nutrient addition alone decreased alpha diversity and evenness, but it did not affect beta diversity. Nutrient addition increased temporal community dissimilarity by increasing temporal balanced variation and decreasing temporal abundance gradients whereas it decreased the spatial abundance gradients. Moreover, nutrient addition decreased alpha stability and marginally decreased gamma stability. Similarly, herbivore exclusion decreased alpha diversity and evenness, but it did not affect beta diversity. Herbivore exclusion increased spatial community dissimilarity by increasing spatial balanced variation, and it also marginally increased temporal balanced variation. Herbivore exclusion had no significant effects on alpha and gamma stability, but marginally reduced spatial asynchrony (Fig. 1; see Table S3 for test statistics).

The SEM clarified the direct and indirect effects of nutrient addition, herbivore exclusion, and their interaction on stability at the local and larger spatial scales (Fig. 2). Nutrient addition and herbivore exclusion alone influenced gamma stability through different pathways. Nutrient addition decreased gamma stability by decreasing alpha stability, but not spatial asynchrony. The negative effect of nutrient addition on alpha stability was mediated by decreased alpha diversity, evenness, and increased temporal community dissimilarity. Moreover, temporal community dissimilarity was the primary factor through which nutrient addition decreased alpha and gamma stability, contributing ca. 50 % of these stability decreases. In contrast, herbivore exclusion weakly decreased gamma stability via decreasing spatial asynchrony, although it also had a weak negative effect on alpha stability by decreasing alpha diversity (Fig. 2). However, the decrease in spatial asynchrony with herbivore exclusion was counteracted by its positive interaction with nutrient addition. Results were generally robust to the inclusion of environmental factors (see supplementary text and Fig. S6).

The variation in temporal community dissimilarity was mainly driven by temporal balanced variation, which was also negatively correlated with alpha stability (slope and 95 % confidence intervals (CI): -0.68, CI = [-1.12, -0.24]; Fig. 3A). In contrast, the temporal abundance gradients only accounted for a small portion of temporal community dissimilarity and were unrelated to alpha stability (-0.50, CI = [-1.78, 0.77]). Spatial asynchrony was negatively correlated with spatial abundance gradients (-0.86, CI = [-1.71, -0.01]), but was unrelated with spatial balanced variation (-0.02, CI = [-0.24, 0.21]). Because spatial community dissimilarity was mainly driven by spatial balanced variation, spatial asynchrony was also unrelated to spatial community dissimilarity (-0.09, CI = [-0.34, 0.16]; Fig. 3B).

**Discussion**

Based on a globally coordinated experiment in grasslands, our analyses demonstrate that (1) nutrient addition and herbivore exclusion mainly had additive effects, with nutrient addition consistently reduced stability at the local and larger spatial scales, while herbivore exclusion weakly reduced stability at both scales; (2) nutrient addition reduced stability primarily by shaping temporal community dissimilarity, but also by changing species richness and evenness. In contrast, herbivore exclusion reduced gamma stability mainly via reducing spatial asynchrony, but also weakly by decreasing local species richness. Temporal and spatial community dissimilarity was mainly attributed to balanced variation (i.e. change in relative abundance among species but not total abundance in communities across time or space), pointing at the importance of turnover driven by species replacement in determining grassland stability.

In contrast to our hypothesis 1, our analysis provides weak support for interactive effects of nutrients and herbivores on plant community properties (additive for plant diversity and stability but a synergistic effect for spatial asynchrony). Previous analyses using different subsets of NetNut data looking at the joint effects of nutrient and herbivores on species richness and aboveground biomass also found weak interactive effects (Borer *et al.* 2014b, 2020). Lack of the interactive effects may be attributed to relatively low grazing intensity at many sites or because the duration of the experiment was not long enough to capture the changes in plant communities (Chen *et al.* 2019, 2020). Indeed, we found that the effects of herbivores on spatial asynchrony and gamma stability tended to be more apparent at sites with high grazing intensity under the ambient conditions (Fig. S5). Moreover, Borer *et al*. (2020) found that both the separate and joint effects of nutrients and herbivores on biomass persisted across a decade, suggesting that duration may not play an important role in mediating treatment effects on stability. Alternatively, lack of interaction may be because the effects of nutrients and herbivores on plant communities act at different spatial scales, where nutrient addition influences local communities and herbivores modulate spatial heterogeneities (see next paragraph). Consistent with previous analyses (Zhang *et al.* 2019; Hautier *et al.* 2020), our results show that the negative effects of nutrient addition alone cascaded to larger spatial scales. Herbivore exclusion alone had weak negative effects on stability at the two scales investigated. Again, this may be due to low grazing intensity at many sites (Table S2).

Confirming our hypothesis 2, nutrients and herbivores impacted gamma stability via different pathways. Nutrient addition reduced alpha and gamma stability probably because nutrient addition intensified interspecific competition within local communities, causing a decline in alpha diversity, a decrease in evenness, and an increase in temporal community dissimilarity. Moreover, increased temporal community dissimilarity contributed to decreased alpha and gamma stability as much as the combined effects of decreasing alpha diversity and evenness. This confirms previous results showing a stronger contribution of temporal community dissimilarity to alpha stability compared to alpha diversity (Koerner *et al.* 2016). In contrast, herbivore exclusion weakly decreased gamma stability primarily via decreasing spatial asynchrony, and weakly via decreasing alpha diversity. It suggests that the effects of herbivores may be more apparent at the larger spatial scale probably via impacting habitat and vegetation heterogeneity (Glenn *et al.* 1992; Howison *et al.* 2017). Surprisingly, we found that herbivore exclusion increased spatial community dissimilarity (Fig. 2), indicating an increased vegetation heterogeneity. Such a counterintuitive pattern has also been found in other systems (Collins & Smith 2006; Alberti *et al.* 2017) and maybe because herbivore exclusion benefits different dominant species in different local communities (Alberti et al. 2017). Our results suggest that maintaining stability from nutrient addition and herbivore exclusion in grasslands may need to focus on different processes across spatial scales.

Additionally, nutrient addition decreased alpha and gamma stability via increasing the temporal balanced variation, while its negative effect on temporal abundance gradients does not translate into changes in stability. In contrast, the effects of herbivore exclusion on spatial asynchrony and gamma stability were not related to spatial community dissimilarity. Thus, the relationship between compositional stability and biomass stability may depend on the spatial scale considered. This necessitates looking at the multidimensional stability (Donohue *et al.* 2013). As cover is usually easier to measure in the field, being non-destructive compared with biomass harvesting, many researchers evaluate stability based on the total cover (i.e. the mean of the total cover through time divided by its standard deviation; e.g. Post 2013; Beck *et al.* 2015; Blüthgen *et al.* 2016; Wilcox *et al.* 2017). Our results suggest that using cover data to calculate stability may fail to capture changes induced by balanced variation. For instance, two communities (in different years) can change distinctively in biomass due to species replacement even when their total cover remains the same (Fig. S2E). As a result, a cover-based metric of stability may overestimate ecosystem stability relative to its biomass-based counterpart (Fig. S7). That said, community dissimilarity (and its partitioning) is a useful index to predict biomass stability, but spatial scales need to be considered.

In this study, we regarded aggregated local communities within treatments across blocks as “larger spatial scale” following previous analyses (Chalcraft *et al.* 2008; Wilcox *et al.* 2017; Zhang *et al.* 2019; Hautier *et al.* 2020). However, subplots belonging to different treatments within blocks are closer to each other compared with subplots belonging to the same treatments across blocks (i.e. larger spatial scale), so dispersal may be stronger for subplots within blocks than the larger spatial scale used here and dispersal may bias the results. Despite this limitation, we argue that dispersal may not influence our results for two reasons. First, blocks within sites typically spread around 1000 m2, possibly that all plots within sites are connected in dispersal in such a small area (Zhang *et al.* 2019). Second, we found that subplots belonging to different treatments within blocks had higher spatial community dissimilarity (0.61 average across sites) than those belonging to the same treatments across blocks (0.55, 0.58, 0.53, 0.57 for control, fence, NPK, and NPK+fence). This suggests that treatments are the dominant filter for plant community assembly.

Our results—based on 34 grasslands across four continents—advance our knowledge in that (1) nutrients and herbivores mainly have additive effects on stability in grasslands; (2) nutrients and herbivores impact stability across multiple spatial scales through different pathways, wherein turnover driven by species replacement is more important than species richness in determining grassland stability. Our results highlight cross-scale monitoring and management are needed to maintain ecosystem stability in the face of global environmental changes. More importantly, our results highlight the need to reduce nitrogen deposition while preserving or reintroducing herbivores to ensure the stable provisioning of grassland biomass.

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**Author Contributions**

QC, SW, and YH developed and framed research questions. QC analyzed the data with help from YH. QC wrote the paper with contributions and input from all authors. EWS and ETB are Nutrient Network coordinators. The author contribution matrix is provided in Table S6 and Table S7.

**Competing interests**

The authors declare no competing interests.

**Data availability**

Data will be made publicly available upon acceptance on the Dryad Digital Repository.

**References**

Alberti, J., Bakker, E.S., van Klink, R., Olff, H. & Smit, C. (2017). Herbivore exclusion promotes a more stochastic plant community assembly in a natural grassland. *Ecology*, 98, 961–970.

Alberti, J., Canepuccia, A., Pascual, J., Pérez, C. & Iribame, O. (2011a). Joint control by rodent herbivory and nutrient availability of plant diversity in a salt marsh-salty steppe transition zone. *J. Veg. Sci.*, 22, 216–224.

Alberti, J., Casariego, A.M., Daleo, P., Fanjul, E., Silliman, B., Bertness, M., *et al.* (2010). Abiotic stress mediates top-down and bottom-up control in a Southwestern Atlantic salt marsh. *Oecologia*, 163, 181–191.

Allan, E., Weisser, W., Weigelt, A., Roscher, C., Fischer, M. & Hillebrand, H. (2011). More diverse plant communities have higher functioning over time due to turnover in complementary dominant species. *Proc. Natl. Acad. Sci. U. S. A.*, 108, 17034–17039.

Anderson, T.M., Griffith, D.M., Grace, J.B., Lind, E.M., Adler, P.B., Biederman, L.A., *et al.* (2018). Herbivory and eutrophication mediate grassland plant nutrient responses across a global climatic gradient. *Ecology*, 99, 822–831.

Arnoldi, J.F., Loreau, M. & Haegeman, B. (2018). The inherent multidimensionality of temporal variability: How common and rare species shape stability patterns. *bioRxiv*.

Arnoldi, J.F., Loreau, M. & Haegeman, B. (2019). The inherent multidimensionality of temporal variability: how common and rare species shape stability patterns. *Ecol. Lett.*

Atwood, T.B., Valentine, S.A., Hammill, E., McCauley, D.J., Madin, E.M.P., Beard, K.H., *et al.* (2020). Herbivores at the highest risk of extinction among mammals, birds, and reptiles. *Sci. Adv.*, 6.

Bakker, E.S., Ritchie, M.E., Olff, H., Milchunas, D.G. & Knops, J.M.H.H. (2006). Herbivore impact on grassland plant diversity depends on habitat productivity and herbivore size. *Ecol. Lett.*, 9, 780–788.

Baselga, A. (2017). Partitioning abundance-based multiple-site dissimilarity into components: balanced variation in abundance and abundance gradients. *Methods Ecol. Evol.*, 8, 799–808.

Baselga, A. & Orme, C.D.L. (2012). Betapart: An R package for the study of beta diversity. *Methods Ecol. Evol.*, 3, 808–812.

Beck, J.J., Hernández, D.L., Pasari, J.R. & Zavaleta, E.S. (2015). Grazing maintains native plant diversity and promotes community stability in an annual grassland. *Ecol. Appl.*, 25, 1259–1270.

Bengtsson, J., Bullock, J.M., Egoh, B., Everson, C., Everson, T., O’Connor, T., *et al.* (2019). Grasslands-more important for ecosystem services than you might think. *Ecosphere*, 10, e02582.

Blüthgen, N., Simons, N.K., Jung, K., Prati, D., Renner, S.C., Boch, S., *et al.* (2016). Land use imperils plant and animal community stability through changes in asynchrony rather than diversity. *Nat. Commun.*, 7, 1–7.

Borer, E.T., Harpole, W.S., Adler, P.B., Arnillas, C.A., Bugalho, M.N., Cadotte, M.W., *et al.* (2020). Nutrients cause grassland biomass to outpace herbivory. *Nat. Commun.*, 11, 1–8.

Borer, E.T., Harpole, W.S., Adler, P.B., Lind, E.M., Orrock, J.L., Seabloom, E.W., *et al.* (2014a). Finding generality in ecology: A model for globally distributed experiments. *Methods Ecol. Evol.*, 5, 65–73.

Borer, E.T., Seabloom, E.W., Gruner, D.S., Harpole, W.S., Hillebrand, H., Lind, E.M., *et al.* (2014b). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature*, 508, 517–520.

Chalcraft, D.R., Cox, S.B., Clark, C., Cleland, E.E., Suding, K.N., Weiher, E., *et al.* (2008). Scale-dependent responses of plant biodiversity to nitrogen enrichment. *Ecology*, 89, 2165–2171.

Chase, J.M., Leibold, M.A., Downing, A.L. & Shurin, J.B. (2000). The Effects of Productivity, Herbivory, and Plant Species Turnover in Grassland Food Webs. *Ecology*, 81, 2485.

Chen, Q., Bakker, J.P., Alberti, J. & Smit, C. (2020). Long-term management is needed for conserving plant diversity in a Wadden Sea salt marsh. *Biodivers. Conserv.*, 29, 2329–2341.

Chen, Q., Howison, R.A., Bakker, J.P., Alberti, J., Kuijper, D.P.J., Olff, H., *et al.* (2019). Small herbivores slow down species loss up to 22 years but only at early successional stage. *J. Ecol.*, 107, 2688–2696.

Collins, S.L. & Smith, M.D. (2006). Scale-dependent interaction of fire and grazing on community heterogeneity in tallgrass prairie. *Ecology*, 87, 2058–2067.

Donohue, I., Petchey, O.L., Montoya, J.M., Jackson, A.L., Mcnally, L., Viana, M., *et al.* (2013). On the dimensionality of ecological stability. *Ecol. Lett.*, 16, 421–429.

Dornelas, M. (2014). Assemblage Time Series Reveal Biodiversity Change but Not Systematic Loss. *Science (80-. ).*, 344, 296–300.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., *et al.* (2004). *Nitrogen cycles: Past, present, and future*. *Biogeochemistry*.

Ganjurjav, H., Zhang, Y., Gornish, E.S., Hu, G., Li, Y., Wan, Y., *et al.* (2019). Differential resistance and resilience of functional groups to livestock grazing maintain ecosystem stability in an alpine steppe on the Qinghai- Tibetan Plateau. *J. Environ. Manage.*, 251, 109579.

García-Palacios, P., Gross, N., Gaitán, J. & Maestre, F.T. (2018). Climate mediates the biodiversity–ecosystem stability relationship globally. *Proc. Natl. Acad. Sci. U. S. A.*, 115, 8400–8405.

Gilbert, B., MacDougall, A.S., Kadoya, T., Akasaka, M., Bennett, J.R., Lind, E.M., *et al.* (2020). Climate and local environment structure asynchrony and the stability of primary production in grasslands. *Glob. Ecol. Biogeogr.*, 29, 1177–1188.

Glenn, S.M., Collins, S.L. & Gibson, D.J. (1992). Disturbances in tallgrass prairie: local and regional effects on community heterogeneity. *Landsc. Ecol.*, 7, 243–251.

Grellmann, D. (2002). Plant responses to fertilization and exclusion of grazers on an arctic tundra heath. *Oikos*, 98, 190–204.

Grime, J.P. (1998). Benefits of Plant Diversity to Ecosystems: Immediate, Filter and Founder Effects. *J. Ecol.*, 86, 902–910.

Grman, E., Lau, J.A., Schoolmaster, D.R. & Gross, K.L. (2010). Mechanisms contributing to stability in ecosystem function depend on the environmental context. *Ecol. Lett.*, 13, 1400–1410.

Halpern, B.S., Borer, E.T., Seabloom, E.W. & Shurin, J.B. (2005). Predator effects on herbivore and plant stability. *Ecol. Lett.*, 8, 189–194.

Hartley, S.E. & Mitchell, R.J. (2005). Manipulation of nutrients and grazing levels on heather moorland: Changes in Calluna dominance and consequences for community composition. *J. Ecol.*, 93, 990–1004.

Hautier, Y., Tilman, D., Isbell, F., Seabloom, E.W., Borer, E.T. & Reich, P.B. (2015). Anthropogenic environmental changes affect ecosystem stability via biodiversity. *Science (80-. ).*, 348, 336–340.

Hautier, Y., Zhang, P., Loreau, M., Wilcox, K.R., Seabloom, E.W., Borer, E.T., *et al.* (2020). General destabilizing effects of eutrophication on grassland productivity at multiple spatial scales. *Nat. Commun.*, 11, 1–9.

Hillebrand, H., Gruner, D.S., Borer, E.T., Bracken, M.E.S., Cleland, E.E., Elser, J.J., *et al.* (2007). Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. *Proc. Natl. Acad. Sci.*, 104, 10904–9.

Hillebrand, H. & Kunze, C. (2020). Meta-analysis on pulse disturbances reveals differences in functional and compositional recovery across ecosystems. *Ecol. Lett.*

Hillebrand, H., Langenheder, S., Lebret, K., Lindström, E., Östman, Ö. & Striebel, M. (2018). Decomposing multiple dimensions of stability in global change experiments. *Ecol. Lett.*, 21, 21–30.

Hodapp, D., Borer, E.T., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., *et al.* (2018). Spatial heterogeneity in species composition constrains plant community responses to herbivory and fertilisation. *Ecol. Lett.*, 21, 1364–1371.

Howison, R.A., Olff, H., Van De Koppel, J. & Smit, C. (2017). Biotically driven vegetation mosaics in grazing ecosystems: the battle between bioturbation and biocompaction. *Ecol. Monogr.*, 87, 363–378.

Koerner, S.E., Avolio, M.L., La Pierre, K.J., Wilcox, K.R., Smith, M.D. & Collins, S.L. (2016). Nutrient additions cause divergence of tallgrass prairie plant communities resulting in loss of ecosystem stability. *J. Ecol.*, 104, 1478–1487.

Koerner, S.E., Smith, M.D., Burkepile, D.E., Hanan, N.P., Avolio, M.L., Collins, S.L., *et al.* (2018). Change in dominance determines herbivore effects on plant biodiversity. *Nat. Ecol. Evol.*, 2, 1925–1932.

Lefcheck, J.S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol. Evol.*, 7, 573–579.

Liu, J., Li, X., Ma, Q., Zhang, X., Chen, Y., Isbell, F., *et al.* (2019). Nitrogen addition reduced ecosystem stability regardless of its impacts on plant diversity. *J. Ecol.*, 107, 2427–2435.

Liu, J., Yang, X., Ghanizadeh, H., Guo, Q., Fan, Y., Zhang, B., *et al.* (2021). Long-term enclosure can benefit grassland community stability on the loess plateau of China. *Sustainability*, 13, 1–19.

Milchunas, D.G. & Lauenroth, W.. W. (1993). Quantitative Effects of Grazing on Vegetation and Soils Over a Global Range of Environments. *Ecol. Monogr.*, 63, 327–366.

Moran, M.D. & Scheidler, A.R. (2002). Effects of nutrients and predators on an old-field food chain: Interactions of top-down and bottom-up processes. *Oikos*, 98, 116–124.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & Team, R.-C. (2017). nlme: Linear and Nonlinear Mixed Effects Models. *R Packag. version 3.1-131*.

Polley, H.W., Wilsey, B.J. & Derner, J.D. (2007). Dominant species constrain effects of species diversity on temporal variability in biomass production of tallgrass prairie. *Oikos*, 116, 2044–2052.

Post, E. (2013). Erosion of community diversity and stability by herbivore removal under warming. *Proc. R. Soc. B*, 280, 20122722.

Proulx, M. & Mazumder, A. (1998). Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. *Ecology*, 79, 2581–2592.

Qin, J., Ren, H., Han, G., Zhang, J., Browning, D., Willms, W., *et al.* (2019). Grazing reduces the temporal stability of temperate grasslands in northern China. *Flora Morphol. Distrib. Funct. Ecol. Plants*, 259, 151450.

Ren, H., Taube, F., Stein, C., Zhang, Y., Bai, Y. & Hu, S. (2018). Grazing weakens temporal stabilizing effects of diversity in the Eurasian steppe. *Ecol. Evol.*, 8, 231–241.

Ripple, W.J., Newsome, T.M., Wolf, C., Dirzo, R., Everatt, K.T., Galetti, M., *et al.* (2015). Collapse of the world’s largest herbivores. *Sci. Adv.*, 1, e1400103.

Saruul, K., Jiangwen, L., Jianming, N., Qing, Z., Xuefeng, Z., Guodong, H., *et al.* (2019). Typical steppe ecosystems maintain high stability by decreasing the connections among recovery, resistance, and variability under high grazing pressure. *Sci. Total Environ.*, 659, 1146–1157.

Seabloom, E.W., Borer, E.T., Buckley, Y.M., Cleland, E.E., Davies, K.F., Firn, J., *et al.* (2015). Plant species origin predicts dominance and response to nutrient enrichment and herbivores in global grasslands. *Nat. Commun.*, 6.

Tilman, D., Reich, P.B. & Knops, J.M.H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, 441, 629–632.

Valencia, E., de Bello, F., Galland, T., Adler, P.B., Lepš, J., E-Vojtkó, A., *et al.* (2020). Synchrony matters more than species richness in plant community stability at a global scale. *Proc. Natl. Acad. Sci.*, 117, 201920405.

Wang, S., Lamy, T., Hallett, L.M. & Loreau, M. (2019). Stability and synchrony across ecological hierarchies in heterogeneous metacommunities: linking theory to data. *Ecography (Cop.).*, 42, 1200–1211.

Wang, S. & Loreau, M. (2016). Biodiversity and ecosystem stability across scales in metacommunities. *Ecol. Lett.*, 19, 510–518.

White, L., O’Connor, N.E., Yang, Q., Emmerson, M.C. & Donohue, I. (2020). Individual species provide multifaceted contributions to the stability of ecosystems. *Nat. Ecol. Evol.*, 4, 1594–1601.

Wilcox, K.R., Tredennick, A.T., Koerner, S.E., Grman, E., Hallett, L.M., Avolio, M.L., *et al.* (2017). Asynchrony among local communities stabilises ecosystem function of metacommunities. *Ecol. Lett.*, 20, 1534–1545.

Worm, B., Lotze, H.K., Hillebrand, H. & Sommer, U. (2002). Consumer versus resource control of species diversity and ecosystem functioning. *Nature*, 417, 848–851.

Wright, C.K. & Wimberly, M.C. (2013). Recent land use change in the Western Corn Belt threatens grasslands and wetlands. *Proc. Natl. Acad. Sci.*, 110, 4134–4139.

Yang, Z., Guo, H., Zhang, J. & Du, G. (2013). Stochastic and deterministic processes together determine alpine meadow plant community composition on the Tibetan Plateau. *Oecologia*, 171, 495–504.

Zelikova, T.J., Blumenthal, D.M., Williams, D.G., Souza, L., LeCain, D.R., Morgan, J., *et al.* (2014). Long-term exposure to elevated CO2 enhances plant community stability by suppressing dominant plant species in a mixed-grass prairie. *Proc. Natl. Acad. Sci. U. S. A.*, 111, 15456–15461.

Zhang, Y., Feng, J., Loreau, M., He, N., Han, X. & Jiang, L. (2019). Nitrogen addition does not reduce the role of spatial asynchrony in stabilising grassland communities. *Ecol. Lett.*, 22, 563–571.

Zhang, Y., Loreau, M., He, N., Wang, J., Pan, Q., Bai, Y., *et al.* (2018). Climate variability decreases species richness and community stability in a temperate grassland. *Oecologia*, 188, 183–192.

Zhang, Y., Loreau, M., He, N., Zhang, G. & Han, X. (2017). Mowing exacerbates the loss of ecosystem stability under nitrogen enrichment in a temperate grassland. *Funct. Ecol.*, 31, 1637–1646.

Zhang, Y., Loreau, M., Lü, X., He, N., Zhang, G. & Han, X. (2016). Nitrogen enrichment weakens ecosystem stability through decreased species asynchrony and population stability in a temperate grassland. *Glob. Chang. Biol.*, 22, 1445–1455.