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

Q. Q. Chen[1], Shaopeng Wang[1], Eric Seabloom[2], Andrew MacDougall[3], Elizabeth Borer[2], Jonathan Bakker[4], Ian Donohue[5], Johannes Knops[6], John Morgan[7], Oliver Carroll[3], Michael Crawley[8], Miguel Bugalho[9], Sally A Power[3], Anu Eskelinen[10], Risto Virtanen[11], Anita Risch[12], Martin Schuetz[13], Carly Stevens[14], Maria Caldeira[15], Sumanta Bagchi[3], Juan Alberti[16], and Yann Hautier[2]

[1]Peking University
[2]University of Minnesota
[3]Affiliation not available
[4]University of Washington
[5]Trinity College Dublin
[6]Xi'an Jiaotong-Liverpool University
[7]La Trobe University
[8]Imperial College
[9]University of Lisbon Centre for Applied Ecology Prof Baeta Neves
[10]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig
[11]University of Oulu
[12]Swiss Federal Institute for Forest Snow and Landscape Research
[13]Swiss Federal Institute for Forest, Snow and Landscape research
[14]Lancaster University
[15]University of Lisbon
[16]Instituto de Investigaciones Marinas y Costeras (IIMyC; UNMDP-CONICET)

April 16, 2021

Abstract

Nutrients and herbivores have independent effects on the temporal stability of aboveground biomass in grasslands; however, their joint effects may not be additive and may also depend on spatial scales. 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, nutrient addition reduced stability primarily by causing changes in local community composition over time and by reducing local species richness and evenness. 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 disentangling the influences of processes operating at different spatial scales may improve conservation and management in stabilizing grassland biomass.

Introduction

Grasslands are one of the largest terrestrial ecosystems on Earth (Suttie et al. 2005). Aboveground biomass production is one of the important functions in grasslands, providing many essential services to humanity such as feed for livestock, carbon storage, and climate mitigation (Bengtsson *et al.* 2019). However, increasing nitrogen deposition (Galloway *et al.* 2004) and accelerating herbivore extirpation (Ripple *et al.* 2015; Atwood *et al.* 2020) are altering aboveground biomass and stable provision (i.e. stability) of aboveground biomass in grasslands (Hautier *et al.* 2015, 2020; Blüthgen *et al.* 2016; Qin *et al.* 2019; Zhang *et al.*2019; Borer *et al.* 2020). Stability is a multi-dimensional concept (Donohue *et al.* 2013; Arnoldi *et al.* 2019); here we examine temporal invariability, calculated as the mean of aboveground biomass through time divided by its standard deviation in local communities (alpha stability) and larger spatial scales (aggregated local communities; gamma stability). Metacommunity theory clarifies that alpha stability and asynchronous dynamics among local communities (spatial asynchrony) determine gamma stability (Wang & Loreau 2016; Wang *et al.* 2019).

In grassland ecosystems, nutrient addition often decreases alpha stability (Hautier *et al.* 2015; Koerner *et al.* 2016; Zhang*et al.* 2016; Liu *et al.* 2019), and these effects may 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, and 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.

In contrast to stability, a range of studies have examined 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; Alberti *et al.* 2017; 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 between nutrients and herbivores. 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 also can 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 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 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). Our understanding of plant diversity (including alpha and beta diversity, evenness, community dissimilarity across time and space) on stability are limited because different studies usually focus on different plant diversity metrics (Grime 1998; Tilman *et al.* 2006; Polley*et al.* 2007; Grman *et al.* 2010; Hautier *et al.*2015; Koerner *et al.* 2016). Therefore, it remains unclear which plant diversity metrics are the major factors mediating the effects of nutrient and herbivores on alpha and gamma stability. Assessing the relative contribution of different facets of plant diversity to alpha and gamma stability can deepen our understanding of the underlying mechanisms for stability across spatial scales and help prioritize conservation efforts.

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. 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).

Material and methods

Experimental Design

We replicated a factorial combination of nutrient addition and herbivore exclusion by fencing at 34 sites, which were part of the Nutrient Network distributed experiment (NutNet; Borer et al. 2014a). These sites were the subset of 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. A block typically spreads over 320 m^2, and all three blocks typically spread over $>$1000 m^2. These sites 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 S1).

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 ($(NH_2)_2CO$) or ammonium nitrate (NH_4NO_3), there were no detectable differences between these N sources on plant biomass or diversity (Seabloom *et al.* 2015). P was supplied as triple superphosphate ($Ca(H_2PO_4)_2$), and K as potassium sulfate (K_2SO_4). The micronutrient mix was applied once at the start of the experiment at a rate of 100 g m^{-2}. 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). Further details on the design are available in Borer et al. (2014b).

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 were 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 m^2), which were moved each year to avoid resampling the same location. 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 mass) 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

Following Borer *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 each species was assigned an importance value 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 m^2subplot 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). Community dissimilarity can arise from two concurrent processes, namely abundance gradients and balanced variation in abundance (Baselga 2017). Abundance gradients arise from a simultaneous increase or decrease in the cover of all species, reflecting changes in the total cover. 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 the 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 temporal and spatial community dissimilarity, and their impact on alpha and gamma stability. Temporal community dissimilarity of each treatment was calculated as dissimilarity of a community through the 5-year experiments and averaged over 3 blocks. Similarly, spatial community dissimilarity of each treatment was calculated as dissimilarity of 3 blocks in each treatment each year and averaged over the experimental years. Temporal/spatial community dissimilarity and the partitioning of it into abundance gradients and balanced variation were done using the function "beta.multi.abund" from the R package betapart with the index.family of "Bray" (Baselga & Orme 2012).

Stability at a given spatial scale was calculated as temporal invariability: $\frac{\mu}{\sigma}$, where 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 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 are quantitatively the same with or without detrending (Hautier *et al.* 2020). Spatial asynchrony was calculated as $1/\varphi$, $\varphi = \frac{\sum_{i,j} w_{\mathrm{ij}}}{\left(\sum_i \sqrt{w_{\mathrm{ii}}}\right)^2}$, where w_{ij} is the temporal covariance of aboveground biomass between local communities i and j, and w_{ii} 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 R syntax: 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 grazing intensity increases, we rerun the above models but adding the herbivore index as a covariate in the models.

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 S2. To fit the SEM, we used the function “lme” with site as the random effect when the component models tested only the treatment effects, and we used function “lm” when the component models tested the relative contribution of both treatments and plant diversity metrics to stability to take into account the diversity gradient among sites. 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 which component (abundance gradients or balanced variation) of temporal community dissimilarity was more related to alpha stability and which component of spatial community dissimilarity was more related to spatial asynchrony. 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 interactive effects on any variables measured except for spatial asynchrony (Fig. 1). That is, herbivore exclusion decreased spatial asynchrony under ambient conditions but not under fertilized conditions. Although nutrient addition and herbivore exclusion had no significant interactive effects on gamma stability, herbivore exclusion reduced gamma stability by 11 % on average compared with that of the control under the ambient conditions. Additionally, under the ambient conditions, the effects of herbivores on spatial asynchrony and gamma stability tended to be more apparent at sites where herbivore index was higher (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 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 by which nutrient addition decreased alpha and gamma stability, contributing ca. 50 % of the decline in stability. In contrast, herbivore exclusion weakly decreased gamma stability by decreasing spatial asynchrony, although it also had a weak negative effect on alpha stability by decreasing alpha diversity (Fig. 2). A decrease in spatial asynchrony with herbivore exclusion was counteracted by its positive interaction with nutrient addition. Spatial asynchrony was positively correlated with beta diversity but not with spatial community dissimilarity. The effects of herbivore exclusion and the interactive effects of nutrient addition and herbivore exclusion on spatial asynchrony were not mediated by beta diversity or spatial community dissimilarity. 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 negatively correlated with alpha stability (slope and 95 % confidence intervals (CI): -0.68, CI = [-1.12, -0.24]; Fig. 3A). In contrast, 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]). The variation in spatial community dissimilarity was mainly driven by spatial balanced variation, which was unrelated with spatial asynchrony (-0.02, CI = [-0.24, 0.21]). In contrast, spatial abundance gradients only accounted for a small portion of spatial community dissimilarity but were negatively correlated with spatial asynchrony (-0.86, CI = [-1.71, -0.01]).

Discussion

Based on a globally coordinated experiment in grasslands, our analysis demonstrates that (1) nutrient addition and herbivore exclusion mainly had additive effects, with nutrient addition consistently reducing stability at the local and larger spatial scales, while herbivore exclusion weakly reduced stability at both scales; (2) nutrient addition reduced stability primarily by increasing temporal community dissimilarity and decreasing species richness and evenness. In contrast, herbivore exclusion reduced gamma stability mainly by 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 stability across spatial scales and other 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). Several non-exclusive processes may explain the lack of the interactive effects of nutrients and herbivores found here. First, relatively low grazing intensity at many sites. 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). Second, duration of the current experiment may not be long enough to capture changes in plant communities (Chen *et al.* 2019, 2020). Borer*et al* . (2020) found that the interactive effects of nutrient addition and herbivore exclusion on aboveground biomass became stronger using 8-10 years post-treatment data compared with those using 2-4 years post-treatment data. Third, the effects of nutrients and herbivores on plant communities may 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 S1).

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 by decreasing spatial asynchrony, and weakly by decreasing alpha diversity. It suggests that the effects of herbivores may be more apparent at the larger spatial scale probably by impacting vegetation heterogeneity, particularly in aboveground biomass (Glenn *et al.* 1992; Howison *et al.* 2017). Our results suggest that maintaining stability from nutrient addition and herbivore exclusion in grasslands needs to focus on different processes across spatial scales.

Additionally, nutrient addition decreased alpha and gamma stability via increasing 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, which is consistent with previous analyses (Wilcox*et al.* 2017; Zhang *et al.* 2019). Community dissimilarity across time 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 biomass stability (Allan *et al.* 2011). Thus, the relationship between compositional stability and biomass stability may depend on the spatial scale considered. This necessitates looking at multidimensional stability (Donohue *et al.* 2013). As cover is usually easier to measure in the field and less destructive compared with biomass harvesting, many researchers evaluate stability based on total cover (i.e. mean of total cover through time divided by its standard deviation; e.g. Post 2013; Beck *et al.* 2015; Bluthgen*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 or places) can differ markedly 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) serves as 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), thus dispersal may be stronger for subplots within blocks than the larger spatial scale used here and dispersal may bias the results. We argue that dispersal may not influence our results for two reasons. First, blocks typically spread within a relatively small area at each site (around 1000 m^2), thus all subplots within sites may be connected by dispersal (Zhang *et al.*2019). Second, we found that subplots belonging to different treatments within blocks had relatively 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 point to the need to reduce nitrogen deposition while preserving or reintroducing herbivores to ensure the stable provisioning of grassland biomass. More importantly, our results highlight that maintaining grassland stability in the face of increasing nutrient addition and herbivore extirpation requires a multi-scale framework to disentangle the influences of processes operating at different scales to guide conservation and management practices.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31988102). This work was generated using data from the Nutrient Network (http://www.nutnet.org) experiment, funded at the site-scale by individual researchers. Coordination and data management have been supported by funding to E. Borer and E. Seabloom from the National Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (NSF-DEB-1234162 and NSF-DEB-1831944 to Cedar Creek LTER) programs, and the Institute on the Environment (DG-0001-13). We also thank the Minnesota Supercomputer Institute for hosting project data and the Institute on the Environment for hosting Network meetings. Nitrogen fertilizer was donated to the Nutrient Network by Crop Production Services, Loveland, CO. We thank Maowei Liang for helpful discussion.

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., Perez, C. & Iribame, O. (2011). 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., Hernandez, 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.

Bluthgen, 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.

Garcia-Palacios, P., Gross, N., Gaitan, 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). Supplementary for 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., Lindstrom, E., Ostman, O. & 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.

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.

Figures

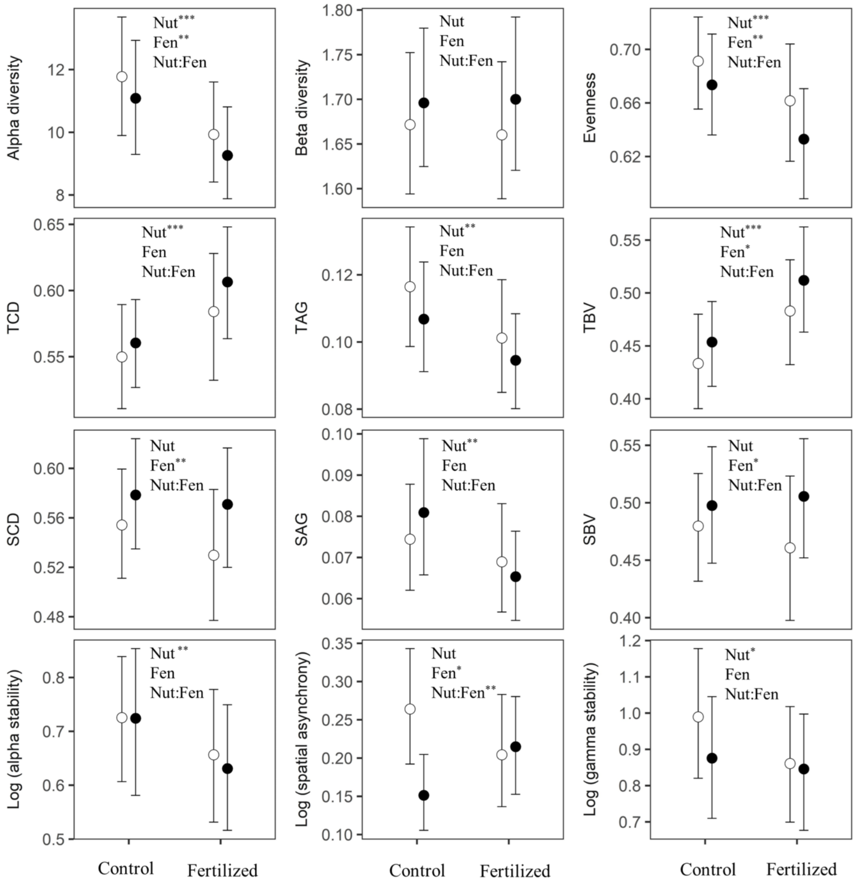

Fig. 1. The average response of plant communities (n = 34) to nutrient addition, herbivore exclusion by fencing, and their interaction. Values show means of raw data, error bars are bootstrapped 95% confidence interval. See Table S3 for test statistics. Asterisks indicate significance of treatment effects: * $P<0.1$; ** $P<0.05$; *** $P<0.001$. TCD: temporal community dissimilarity; TAG: temporal abundance gradients; TBV: temporal balanced variation in abundance; SCD: spatial community dissimilarity; SAG: spatial abundance gradients; SBV: spatial balanced variation in abundance.

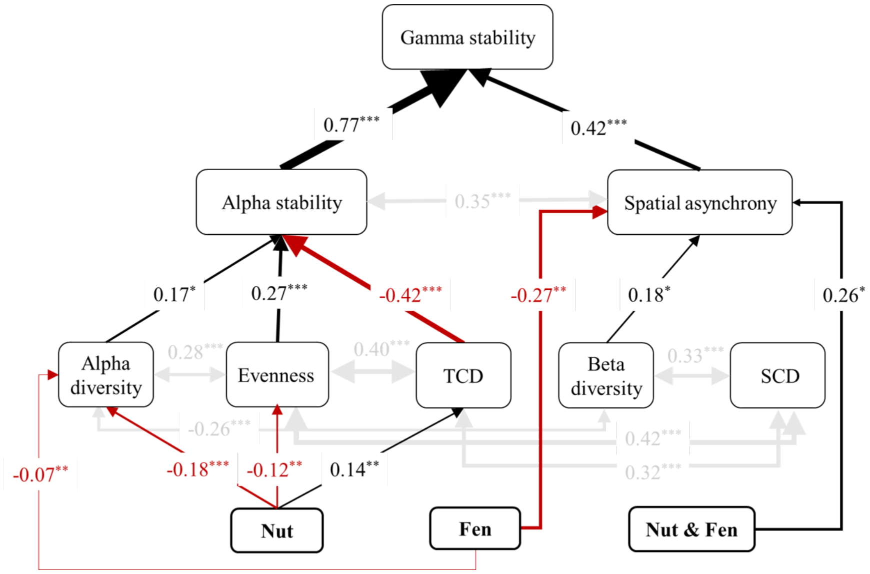

Fig. 2. Direct and indirect effects of nutrient addition, herbivore exclusion by fencing, and their interaction on stability at the local (i.e. alpha stability) and larger spatial scales (i.e. gamma stability). Model fit the data well (Fisher's C = 34.08, P = 0.368, d.f. = 32, N = 136). Boxes represent measured variables and arrows represent relationships among variables. The displayed numbers are standardized path coefficients. Width of the arrows indicates the strength of the pathways. Line color represents positive (black), negative (red) path coefficients. Grey arrows and numbers show correlated errors. Asterisks indicate significant paths: * P<0.1; ** P<0.05; *** P<0.001. See Table S4 for R^2 for each component model. Alpha stability, spatial asynchrony, and gamma stability were on the log scale to improve normality and homogeneity of variance. TCD: temporal community dissimilarity; SCD: spatial community dissimilarity.

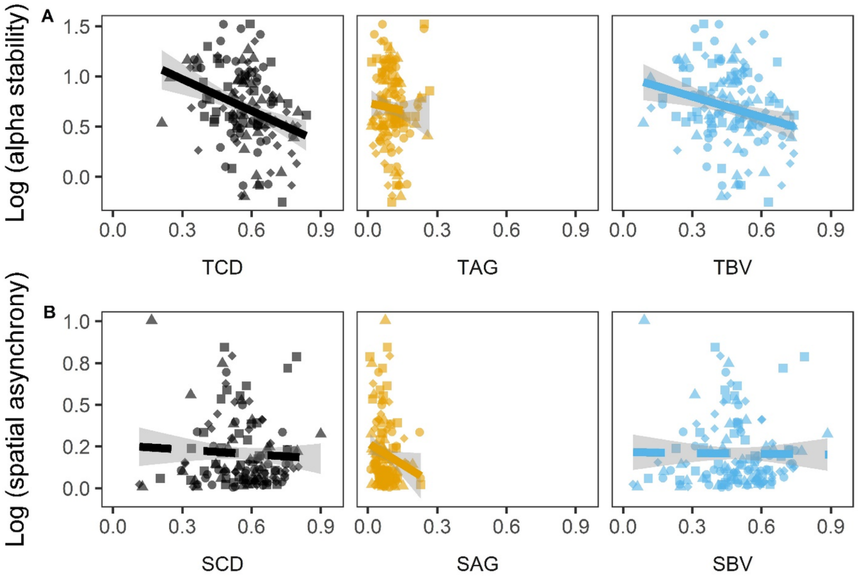

Fig. 3. Relationships between alpha stability and temporal community dissimilarity and its

components (A); spatial asynchrony and spatial community dissimilarity and their components (B). Solid lines show significant relationships, dashed lines show non-significant relationships. Lines are linear model fits across treatments, because the interaction is not significant. See Table S5 for test statistics. Different shapes represent different treatments (square: Control.Control; circle: Control.Fenced; triangle: Fertilized.Control; Diamond: Fertilized.Fenced). TCD: temporal community dissimilarity; TAG: temporal abundance gradients; TBV: temporal balanced variation in abundance; SCD: spatial community dissimilarity; SAG: spatial abundance gradients; SBV: spatial balanced variation in abundance.