

Grazing-induced biodiversity loss impairs grassland ecosystem stability at multiple scales

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31 **Abstract**

32 Livestock grazing is a major driver shaping the functioning and stability of grasslands. Although
33 previous studies have documented the effect of grazing on grassland stability, whether this effect
34 is scale-dependent remains unclear. Here, we conducted a sheep-grazing experiment in a
35 temperate grassland to test grazing effects on biomass stability across scales and organizational
36 levels. We found that an increase of grazing intensity increased species stability, but it
37 substantially decreased local ecosystem stability due to reduced asynchronous dynamics among
38 species. Moreover, grazing reduced ecosystem stability at larger spatial scales, but to a lesser
39 extent. By decreasing biodiversity within and across communities, grazing impairs the insurance
40 effects of biodiversity and hence the up-scaling of stability from species to ecosystem and further
41 to larger scales. Our study provides the first evidence for the context-dependence of grazing
42 effects on grassland stability via shaping biodiversity and contributes to bridging fine-scale
43 experiments and broad-scale ecosystem management.

44

45 **Introduction**

46 Occupying more than one-third of earth's terrestrial land surface and contributing about 40 % of
47 global agricultural domestic products, grassland is the largest coupled human and natural
48 systems on Earth (Herrero *et al.* 2013). The sustainable delivery of functions and services by
49 grassland is critical for human society and wildlife living upon it. However, human activities are
50 altering earth's environment with extreme events becoming more frequent worldwide, which
51 threatens the ability of grassland ecosystems to reliably provide functions and services to
52 humanity (Sloat *et al.* 2018; Liang & Gornish 2019). Understanding the stability of grassland
53 ecosystems in the face of environmental changes is thus critical for sustainable ecosystem
54 management and decision-making.

55 Livestock grazing is the most intensive land-use activity in grasslands, which presents a
56 major driver shaping the functioning and stability of grasslands (Fetzel *et al.* 2017; Wang *et al.*
57 2019a; Filazzola *et al.* 2020). Recent studies experimentally testing the effect of grazing on
58 grassland ecosystem stability revealed differential effects at different spatial scales (Post 2013;
59 Beck *et al.* 2015; Hautier *et al.* 2015; Bluthgen *et al.* 2016; Hallett *et al.* 2017; Ganjurjav *et al.*
60 2019; Qin *et al.* 2019). At smaller spatial scales (e.g., ~tens of m²), studies have shown that
61 herbivore exclusion decreases the temporal stability of productivity or cover in grassland
62 communities (Post 2013; Beck *et al.* 2015; Hautier *et al.* 2015; Hallett *et al.* 2017), indicating
63 that herbivory could promote community stability. At intermediate scales (e.g., from ha to km²),
64 two recent studies found that livestock grazing did not affect the stability of total plant cover
65 across European grasslands (Bluthgen *et al.* 2016) or aboveground plant biomass on the Tibetan
66 Plateau (Ganjurjav *et al.* 2019). At even broader scales (e.g., ~hundreds of km²), studies in
67 temperate grasslands found that livestock grazing decreased the stability of plant community

productivity (Qin *et al.* 2019). These results suggest that the effect of grazing on stability might be scale dependent. Yet, previous studies have mostly focused on a particular scale, and whether grazing effects differ across scales remains to be tested.

The impact of grazing on stability may be mediated by its effect on plant species diversity. A large body of experimental and theoretical work has demonstrated that biodiversity can provide insurance effects to enhance ecosystem temporal stability (Loreau 2010; Tilman *et al.* 2014). While these studies have mostly been conducted at small spatial scales, recent studies started to understand whether these results can be extended to understand biodiversity and stability at larger spatial scales, where landscape heterogeneity and habitat structures may interact with biodiversity patterns (Donohue *et al.* 2013; Isbell *et al.* 2017; Kefi *et al.* 2019; Gonzalez *et al.* 2020). Such a potential scale dependency is particularly relevant in grazing systems, which often exhibit greater heterogeneity as grazing area increases (Adler *et al.* 2001). Moreover, herbivore behavior (e.g., foraging selectivity and trampling) is an important mechanism impacting plant diversity in grassland ecosystems (Collins *et al.* 1998; Koerner *et al.* 2018) with scale-dependent effects on plant species diversity (Olff & Ritchie 1998; de Bello *et al.* 2007; Tonn *et al.* 2019). However, to our knowledge, no study has explored whether changes in plant diversity with grazing intensity mediate its impact on stability across spatial scales.

Recently, a new theoretical framework has been developed to study ecological stability across scales (Wang & Loreau 2014, 2016). This framework proposes consistent measures of temporal stability (i.e. the inverse of temporal variability) across organizational levels and spatial scales (Fig. 1). Specifically, in a landscape consisting of a number of communities, γ stability is defined as the temporal stability of total ecosystem function at large spatial scales (i.e. a landscape or aggregation of communities), α stability is defined as a weighted average of

91 temporal stability across local communities, and *species stability* is defined as a weighted
92 average of local population stability across species within local communities (Fig. 1). Theory
93 predicts a consistent increase of stability from species to local communities and further to larger
94 spatial scales, with the amounts of increase determined by *species asynchrony* and *spatial*
95 *asynchrony*, respectively (Wang *et al.* 2019b). Specifically, γ *stability* can be expressed as the
96 product of species stability, species asynchrony, and spatial asynchrony. Thus, asynchronous
97 responses among species and local communities to environmental fluctuations provide species
98 and spatial insurance, respectively, to maintain the stability of ecosystem function at larger
99 organizational level and spatial scales (Yachi & Loreau 1999; Loreau *et al.* 2003). Recent studies
100 showed that local species diversity (α diversity) and spatial turnover (β diversity) could
101 contribute to increasing species and spatial asynchrony, respectively (Wang & Loreau 2016;
102 Hautier *et al.* 2020; but see Wilcox *et al.* 2017; Zhang *et al.* 2019). Thus, grazing may regulate
103 ecosystem stability at larger scales by altering local and spatial turnover of biodiversity, but this
104 hypothesis has not been tested previously.

105 In this study, we conducted a grazing experiment to quantify the impact of grazing intensity
106 on stability at multiple scales and assess whether these effects are mediated by changes in plant
107 diversity. Specifically, we asked the following questions: (1) Does grazing affect the stability of
108 plant biomass at species, local communities, and large spatial scales? (2) Does grazing affect
109 species and spatial insurance effects by changing plant diversity at multiple spatial scales? Our
110 analyses revealed context-dependent effects of grazing on stability, which shifted from a positive
111 effect on species stability to negative effects on community stability at both local and larger
112 spatial scales. Such effects were modulated by grazing-induced decreases in both α and β
113 diversity. Our findings provide novel insights for a mechanistic understanding of grassland

ecosystem stability across scales and have useful implications for landscape-scale ecosystem management.

Material and methods

Study site

The study site is located at the Xilin Gol Grassland Nature Reserve, Inner Mongolia, China (44°08'N, 116°19'E, 1 129 m) (Fig. S1a). The biome type is the typical steppe grassland, which is dominated by rhizome grass (*Leymus chinensis* (Trin.) Tzvel.) and bunchgrass (*Stipa grandis* P. Smirn) (Liang *et al.* 2019). Soil taxonomy was classified as the Calcic-Orthic Aridisol based on USDA soil classification. The study area is characterized as the semiarid climate (BSk) in the Köppen climate classification. During the past six decades, the mean annual air temperature was 2.6 °C and mean annual precipitation (MAP) was 266.9 mm, with more than 90 % the precipitation falling in the growing season (<http://data.cma.cn/>).

Grazing experiment

In 2011, we fenced 12 equal-sized (120 m × 120 m) paddocks to exclude natural grazing activities and implemented four grazing intensity treatments from 2013 to 2018, including 0, 2, 4, and 8 sheep·ha⁻¹, referred to as no- (NG), light- (LG), medium- (MG), and heavy-grazing (HG), respectively (Fig. S1b). Each treatment had three replicates. We implemented sheep-grazing in four bouts during the growing season of each year, from June through September. Each bout was exposed to some grazing treatment for 21 days. Species biomass data was collected in the last week of each month after grazing since June 2014. More detailed experiments descriptions can be found in Liang *et al.* (2019).

Plant sampling strategies

137 We regarded each 120 m × 120 m paddock as a community at the large scale, which consisted of
 138 five subplots (120 m × 20 m) (Fig. S1c). For each subplot, we used scissors to clip the residual
 139 living aboveground tissue of all vascular plants (i.e., green plant biomass) above the soil surface
 140 in a 1 m × 1 m quadrat, which was used to represent the community at local spatial scale. After
 141 these tissues of plants has been oven-dried at 65 °C in 48 hours, we weighted them and estimated
 142 the biomass for each species (g·m⁻²). We collected the data in June, July, August, and September
 143 between 2014 and 2018. In total, we measured more than 7, 000 plant species biomass values in
 144 1, 200 quadrats from 2014 to 2018, i.e. five years × four months (or bouts) × four treatments ×
 145 three replicates × five quadrats.

146 *Biodiversity, stability, and asynchrony across scales*

147 We used abundance-based metrics to calculate species diversity across scales (Fig. 1 and Fig.
 148 S1c). At the local spatial scale (e.g., a local community l), we calculated the Simpson index:

149 $\phi_l = \sum_i^S p_{il}^2$, where p_{il} represents the relative biomass of species i in the local community l and S is

150 the number of species in the local community l . We then defined α diversity as the inverse of the

151 weighted average of Simpson index: $\alpha_{simp} = 1 / \sum_{l=1}^5 \omega_l \phi_l$, where ω_l is the ratio of total biomass of

152 the local community l to that of the aggregate community (i.e. aggregation of the five quadrats)

153 at the large scale (Wang & Loreau 2016). The γ diversity was defined as: $\gamma_{simp} = 1 / \sum_i p_i^2$, where

154 $p_i = \sum_{l=1}^5 \omega_l p_{il}$ denotes the relative abundance of species i in the aggregate community at the large

155 scale. β diversity was defined multiplicatively, i.e. $\beta_{simp} = \gamma_{simp} / \alpha_{simp}$. To test the robustness of our

156 results, we also calculated the Bray-Curtis dissimilarity among the five quadrats in each plot as
 157 an alternative measure of β diversity. Besides, we also calculated species richness at the local
 158 quadrat (α_{rich}) and larger (γ_{rich}) scales, and the multiplicative beta diversity as: $\beta_{\text{rich}} = \gamma_{\text{rich}} / \alpha_{\text{rich}}$.

159 Furthermore, we defined the species stability, as well as community stability at α and γ
 160 scales (Wang & Loreau 2014; Wang *et al.* 2019b). For the stability metrics, we calculated its
 161 temporal stability by the inverse of coefficient of variation (i.e. the ratio of mean to standard
 162 deviation). Species stability was defined as the weighted average of local species stability across
 163 species and local communities; α stability was calculated as the weighted average of community
 164 stability across local communities; γ stability was calculated as the community stability at large
 165 spatial scale (Fig. 1). The mathematical formulas for these definitions are (Wang *et al.* 2019b):

$$166 \quad \text{Species stability} = \frac{\sum_{i,k} \mu_{i,k}}{\sum_{i,k} \sqrt{v_{ii,kk}}} \quad (1)$$

$$167 \quad \alpha \text{ stability} = \frac{\sum_{i,k} \mu_{i,k}}{\sum_k \sqrt{\sum_{i,j} v_{ij,kk}}} \quad (2)$$

$$168 \quad \gamma \text{ stability} = \frac{\sum_{i,k} \mu_{i,k}}{\sqrt{\sum_{i,j,k,l} v_{ij,kl}}} \quad (3)$$

169 where $\mu_{i,k}$ denotes the mean of species i in local community k , and $v_{ij,kl}$ denotes the covariance
 170 between species i in local community k and species j in local community l .

171 The spatial stability framework clarified that asynchrony among lower-level components
 172 (e.g. species or communities) are key to the scaling of stability (Lamy *et al.* 2019; Wang *et al.*
 173 2019b). In light of this framework, we defined *species asynchrony* as the ratio of α stability to
 174 species stability, which captures the incoherence in population dynamics among species within

175 local communities; we then defined *spatial asynchrony* as the ratio of γ stability to α stability,
176 which captures the incoherence in community dynamics among local communities. We also
177 defined *total asynchrony* as the product of *species asynchrony* and *spatial asynchrony*, which
178 quantifies the total insurance effect provided by species and spatial asynchrony from local
179 species to communities at larger spatial scales. More details about the equations of the stability
180 and synchrony indices can be found in Wang et al. (2019b).

181 *Statistical analysis*

182 To assess the effects of grazing on biodiversity and stability of plant communities at multiple
183 scales, we ran mixed-effects models (MEMs) using the R package ‘*lme4*’ (Bates *et al.* 2015). In
184 these models, the fixed effect was “*grazing intensity*” and random effects were “*month/year*” and
185 “*month*” for examining biodiversity and stability, respectively. We used the Fligner-Killeen Test
186 to test homogeneity of variances among four grazing intensities; if homoscedasticity was not
187 met, we added weights for EMMs using *gls()* function in R (Zuur *et al.* 2010). Considering the
188 autocorrelations among observations through time, we compared models without autocorrelation
189 structure to the mixed-effects models including a first-order autoregressive model (AR (1)); all
190 these MEMs gave similar results; thus, we chose the best fit model according to the lowest
191 Akaike Information Criterion (AIC). To facilitate comparison of effect sizes of grazing intensity
192 gradient on these biodiversity and stability metrics, we used the natural log-transformed response
193 variables. In addition, we ran similar MEMs using “*grazing intensity*” as a factorial variable (GI:
194 no-grazing (NG), low-grazing (LG), medium-grazing (MG), and high-grazing (HG)), and
195 compared grazing effects among different grazing intensities using Tukey’s-range test. We also
196 performed the repeated-measures ANOVA to test whether the main effects of “*grazing intensity*”
197 on these variables vary with “*year*” and “*month*”, or any interactions. “*grazing intensity*”

explained the largest proportion of variance in total of biodiversity (except for plant richness) and stability metrics (Table S1, Table S2, Table S3, Table S4, and Table S5). Here, we used the non-transformed response variables to quantitatively compare their changes under different grazing intensities.

To address our second question of how biodiversity influence stability at different scales, we examined the relationships between biodiversity and stability using MEMs, with “*grazing intensity*” and “*month*” as random factors. In particular, we tested the relationships of species asynchrony with α diversity, of spatial asynchrony with α and β diversity, and of total asynchrony with γ diversity. For the relationship of spatial asynchrony with α and β diversity, we ran partial linear mixed-effects models (p-MEMs). Specifically, we first ran a MEM between spatial asynchrony and β diversity (or Bray-Curtis dissimilarity), we then extracted the residuals of spatial asynchrony and tested their relationship with α diversity using MEMs, with “*grazing intensity*” and “*month*” as random factors. Similarly, we tested the relationship between β diversity (or Bray-Curtis dissimilarity) and the residuals from the MEM of spatial asynchrony with α diversity.

Furthermore, to explore how an increase of grazing intensity directly and indirectly impact the hierarchical stability and asynchrony via regulating biodiversity (Fig. S12), we employed a structural equation modeling (SEM) to quantify the different pathways between grazing intensity (as a continuous variable) and γ stability using the R package ‘*piecewiseSEM*’ (Lefcheck & Freckleton 2016). The piecewise SEM could generate a single structural model by piecing multiple linear mixed-effects models (e.g., “*month*” is a random factor). In light of recent metacommunity theory, we constructed a hypothesized SEM that characterized the effect of grazing on species stability, species asynchrony, and spatial asynchrony via altering α and β

diversity (Wang *et al.* 2019b). We used Shipley's test of d-separation to ensure that we did not miss any potential pathways between variables (natural log-transformed), and chose the final model that had the lowest AIC. All analyses were programmed in R v 3.6.0 (R Development Core Team 2019).

Results

Increasing grazing intensity decreased plant species diversity at both local (α diversity; $F_{1,10} = 22.58$, $P = 0.0008$) and larger (γ diversity; $F_{1,10} = 23.50$, $P = 0.0007$) scales (Fig. 2 and Fig. S2a). Grazing also reduced β diversity (Fig. 2a, $F_{1,10} = 20.12$, $P = 0.0012$), regardless of the metrics used (Fig. S4). Compared to the no-grazing treatment, α , β , and γ diversity were decreased on average by 41 %, 12 %, and 49 %, respectively, in the heavy-grazing treatment (Fig. S2). The reduction of plant species diversity was due to a shift in species composition under grazing, but not to a reduction in the number of species (Fig. S3). Specifically, grazing increased the relative abundance of the dominant species, i.e. *Stipa grandis* across the study area (Fig. S4a, $F_{1,10} = 16.09$, $P = 0.003$), which decreased α diversity and β diversity due to spatial homogenization. We also found that biodiversity metrics exhibited temporal variations that are potentially driven by environmental fluctuations, but overall grazing intensity alone explain the largest proportion of variance in plant diversity (Table S2 and Table S3).

Grazing had context-dependent effects on stability (Fig. 2 and Table S5). At the species level, increasing grazing intensity increased the stability of the dominant species (Table S5, $F_{1,10} = 10.38$, $P < 0.0001$) and thus species stability (Fig. 2c, $F_{1,10} = 16.01$, $P = 0.003$), as grazing did not affect the temporal mean biomass of the dominant species (Fig. S5c, $F_{1,10} = 1.33$, $P = 0.277$) but reduced its temporal standard deviation (Fig. S5d, $F_{1,10} = 11.38$, $P = 0.007$). However,

244 grazing decreased community stability at both local (α stability: $F_{1,10} = 27.50$, $P = 0.0004$) and
 245 larger (γ stability: $F_{1,10} = 10.17$, $P = 0.010$) scales (Fig. 2c). Compared to those in no-grazing
 246 treatment, species stability was on average 55 % higher (i.e., $\frac{\bar{V}_g - \bar{V}_c}{\bar{V}_c} \times 100\%$), α stability was 29
 247 % lower, and γ stability was 24 % lower in heavy-grazing treatment (Fig. S8). Such context-
 248 dependent grazing effect on stability was mediated by its effect on asynchrony (Fig. S9 and
 249 Table S11). Grazing significantly decreased species asynchrony (Fig. 2b, $F_{1,10} = 27.35$, $P =$
 250 0.0004) while increasing spatial asynchrony (Fig. 2b, $F_{1,10} = 12.81$, $P = 0.005$). Specifically,
 251 species asynchrony was 55 % lower and spatial asynchrony was 13 % higher in heavy-grazing
 252 treatment, compared to those in no-grazing. From local species to communities at larger spatial
 253 scales, species and spatial asynchrony together contributed to enhancing stability by 242 % in
 254 no-grazing treatment, but their contribution was only 69 % in heavy-grazing treatment (Fig. S7f).
 255 We found that species asynchrony was positively related to α diversity (Fig. S10a, $R^2_m =$
 256 0.68 , $F_{1,35} = 44.83$, $P < 0.0001$), and spatial asynchrony was associated with both α and β
 257 diversity (Fig. S11). Specifically, spatial asynchrony was negatively related to α diversity after
 258 the effect of β diversity was controlled (Fig. S11a, $R^2_m = 0.11$, $F_{1,35} = 5.51$, $P = 0.025$), and it was
 259 positively related to β diversity after the effect of α diversity was controlled (Fig. S11c, $R^2_m =$
 260 0.06 , $F_{1,35} = 3.18$, $P = 0.083$). These relations were robust to different metrics of β diversity (Fig.
 261 S11). Moreover, total asynchrony was positively related to γ diversity (Fig. 3, $R^2_m = 0.48$, $F_{1,35} =$
 262 22.90 , $P < 0.0001$). Therefore, by decreasing species diversity at different scales, grazing could
 263 impair species and spatial asynchrony and thus insurance effects for community stability at
 264 larger scales.

We used a structural equation model (SEM) to disentangle the direct and indirect pathways through which grazing influenced γ stability. Specifically, we examined how grazing affected the three components of γ stability (= species stability \times species asynchrony \times spatial asynchrony) by altering species diversity (Fig. S12). Our final SEM showed that an increase of grazing intensity increased the abundance of the dominant species, which led to a higher stability of the dominant species and the average species stability (Fig. 4). However, by increasing the dominant species abundance, grazing decreased both α and β diversity. The decreased α diversity in turn decreased species asynchrony but increased spatial asynchrony, whereas the decreased β diversity weakened spatial asynchrony. Combining all these pathways, grazing led to increased species stability (total effect size or TES = 0.462), but decreased community stability at both local (TES = -0.298) and larger (TES = -0.188) spatial scales (Table S7 and Table S8).

Discussion

By quantifying stability across organizational levels and spatial scales in a grassland ecosystem, our study provides, to our knowledge, the first evidence for the context-dependent effects of grazing on stability (Fig. 5). Our results demonstrate that grazing increases species stability but decreases community stability at both local and larger spatial scales. Such context-dependent effects of grazing can be understood from its influence on species and spatial asynchrony (Fig. 5), which are in turn explained by herbivory selective foraging that significantly alters species diversity patterns including the dominant species abundance, species diversity, and spatial heterogeneity.

At the species level, the positive effects of grazing on species stability are mainly attributed to the selective foraging by grazers that increases the relative abundance of one dominant

species, i.e. *S. grandis*. In general, gazers preferentially forage palatable and nutritious plants. In our study system, *S. grandis* is less palatable and nutritious compared to other plants, thanks to a long evolutionary history of plant-herbivore interactions under the nomadic pastoralism on the Mongolia Plateau (Sanjmyatav 2012). As a tussock grass, livestock browsing does not damage the basal meristem of *S. grandis*, which may lead to tiller development via increasing light penetration (Borer *et al.* 2014). Moreover, *S. grandis* produces long-needle seeds (approximately 1.1 cm long) with a barb that can hook on animal fur, and its barb could damage animal's digestion system (e.g., tongue and stomach), particularly for sheep. These morphological and physiological characters make *S. grandis* more resistant to grazing compared to other plants. Consequently, selective foraging on its more palatable competitors eventually led to an increase of abundance of *S. grandis* (Fig. 4). Thus, the grazing-induced dominance by a more resistant species increases stability at the species level. Increasing species stability might in part be attributed to our sampling design. Clipping quadrats with shifting locations through time might accounts for spatial variation in species biomass. More specifically, because grazing decreases spatial turnover (i.e. β diversity; Fig. 2) and thus spatial variation in species biomass, higher species stability may be simply due to the artificial effect of our sampling approach. To evaluate this possibility, we tested the relationships of the stability of *S. grandis* with its abundance and β diversity. Our results showed that whereas both factors are significantly related to dominant species stability, dominant species abundance provided a much better explanation and β diversity became insignificant after the effect of the dominant species abundance was accounted for (Fig. S13, Fig. S14, Table S13, and Table S14). Thus, the increasing species stability with grazing should be mainly explained by the increased dominance of *S. grandis*, rather than the artificial effect of sampling.

By enhancing the dominance of *S. grandis*, grazing decreases plant diversity within local communities and thereby weakens the species insurance effect for community stability (Fig. 4). A large body of experimental and theoretical studies have demonstrated the stabilizing role of species diversity in ecosystem stability (Loreau 2010; Tilman *et al.* 2014). One major mechanism for the stabilizing effect of biodiversity is that different species exhibit asynchronous responses to environmental fluctuations, which compensate for each other and result in a higher stability at the community level (Tilman *et al.* 2006; Gonzalez & Loreau 2009). Our SEM confirmed this hypothesis by showing that grazing decreases species asynchrony indirectly via reducing α diversity. This result is consistent with previous findings that anthropogenic drivers (e.g., nitrogen, carbon dioxide, fire, herbivory, and water) affect ecosystem stability indirectly via biodiversity (Hautier *et al.* 2015). The negative effects of grazing on species asynchrony suppress its positive effect on species stability, leading to a decreasing community stability with grazing (Fig. 5).

The negative effect of grazing on community stability propagate from local (α) to larger (γ) spatial scales, as α stability at the local community scale is the major driver of γ stability (Fig. S8). Yet, the reduction in γ stability was less pronounced than α stability, which was explained by the increase of spatial asynchrony with grazing. Metacommunity theory predicts that spatial asynchrony among communities increases with β diversity and decreases with α diversity (Wang & Loreau 2016). Our data supported these predictions and showed that grazing affected spatial asynchrony through two pathways via changing α and β diversity (Fig. S11). On the one hand, grazing decreases local α diversity and increased spatial asynchrony. On the other hand, due to the selective foraging that increases the dominance of *S. grandis* across the landscapes, grazing decreases β diversity and thus spatial asynchrony. Because the positive pathway via α diversity is

relatively stronger than the negative one via β diversity, grazing eventually leads to higher spatial asynchrony. Such higher spatial asynchrony compensates to some extent for the reduction in α stability and generates a smaller reduction of γ stability.

From local species to communities at larger scales, asynchrony among species and across communities provide insurance effects that enhance ecosystem stability (Wang et al. 2019). In such a context, recent studies provided evidence for a stronger insurance effect by either species asynchrony (Lamy et al. 2019; Wang et al. 2019) or spatial asynchrony (Thorson *et al.* 2018). In our study system, species asynchrony provides a much stronger insurance effect for γ stability, compared to spatial asynchrony, in no-grazing systems (Fig. 5). However, grazing can reverse this pattern by decreasing species diversity, such that spatial asynchrony provides a slightly stronger insurance effect than species asynchrony in high-grazing systems (Fig. 5). This is likely because grazers have a stronger species preference than a patch preference in our study system. These results provide the first evidence that grazing can alter the up-scaling patterns of stability by dampening the overall insurance effect and switching the relative importance of species and spatial insurance.

Conclusion

Our findings contribute to an in-depth understanding of the scaling property of ecological stability and how it may be influenced by disturbances like livestock grazing. In particular, our results demonstrate general destabilizing effects of grazing on grassland ecosystems across scales, but the strengths of these effects depend on the study scale. Our multi-scale approach provides a potential framework to reconcile previous findings of varying grazing effects across scales (Post 2013; Beck *et al.* 2015; Hautier *et al.* 2015; Bluthgen *et al.* 2016; Hallett *et al.* 2017;

Qin *et al.* 2019). Moreover, future studies should extend our approach to even larger scales, particularly those ecosystem management is applied at. Such insights will be valuable for bridging small-scale ecological research with large-scale management, thus providing useful guide for grassland management and decision-making in a changing environment.

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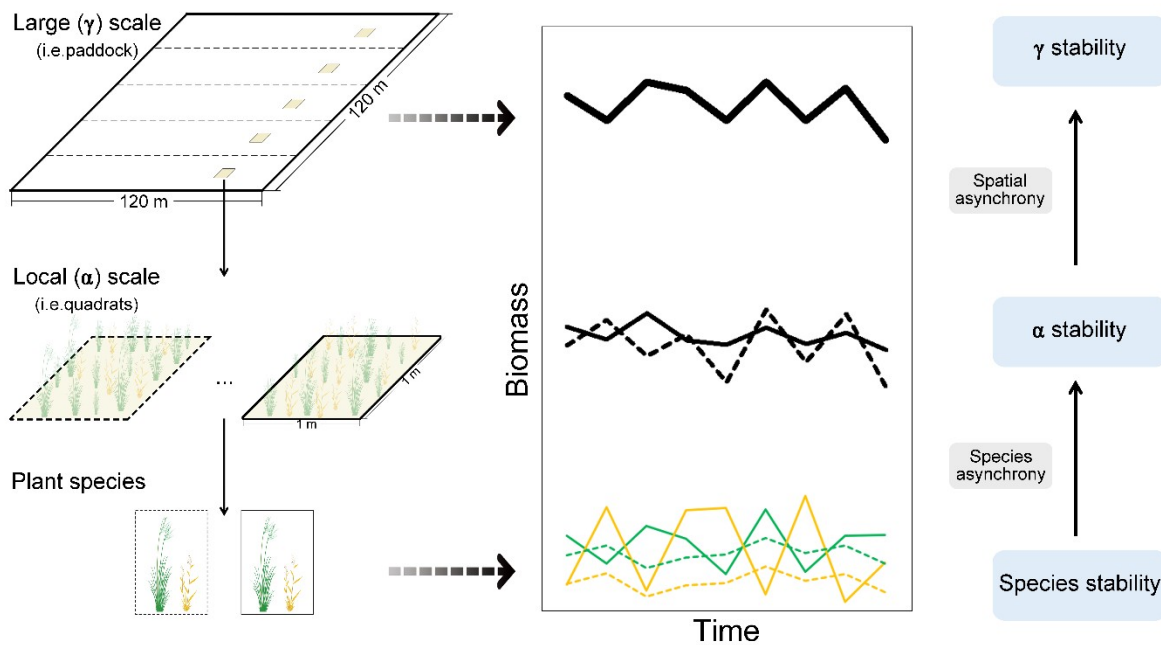


Figure 1 A theoretical framework of ecological stability across scales illustrated with our experiment. Within each paddock ($120 \times 120 \text{ m}^2$), five local communities (1 m^2 quadrats) were sampled. We regard each quadrat as the local (α) scale, and aggregation of the five quadrats as the larger (γ) scale. Ecosystem stability is defined as the mean of an ecosystem function divided by its standard deviation through time across organizational levels (i.e. from species to community) and spatial scales (i.e., from local to larger spatial scales). Theoretically, stability at larger spatial scale (γ stability) is determined by three components, namely *species stability*, *species asynchrony*, and *spatial asynchrony*. At the local scale (i.e. quadrats), asynchronous dynamics among species (solid green and yellow lines) leads to a high α stability (solid black line), despite the low *species stability*; in contrast, synchronous dynamics among species (dashed green and yellow lines) leads to a low α stability (dashed black line), despite the high *species stability*. At the large spatial scale (i.e. paddock), asynchronous fluctuations between local communities (spatial asynchrony) contribute to increasing γ stability.

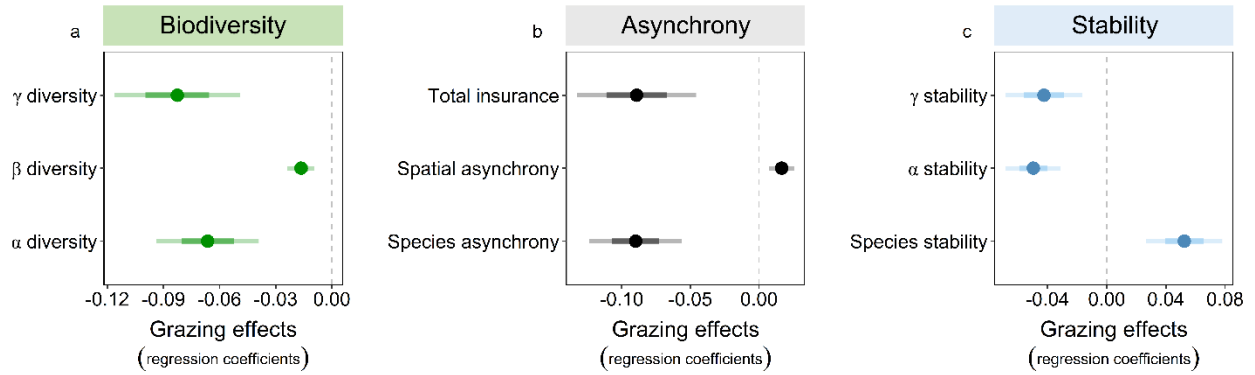
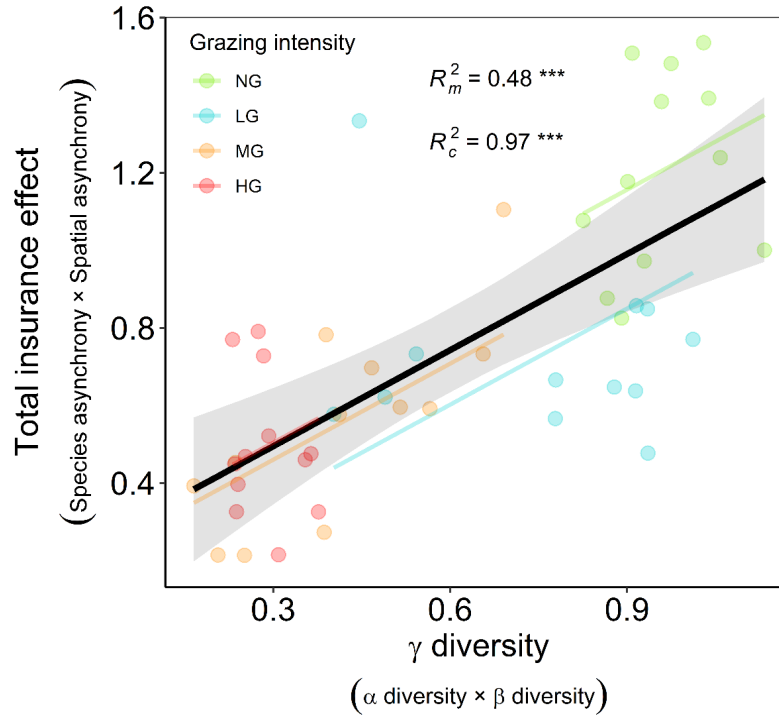
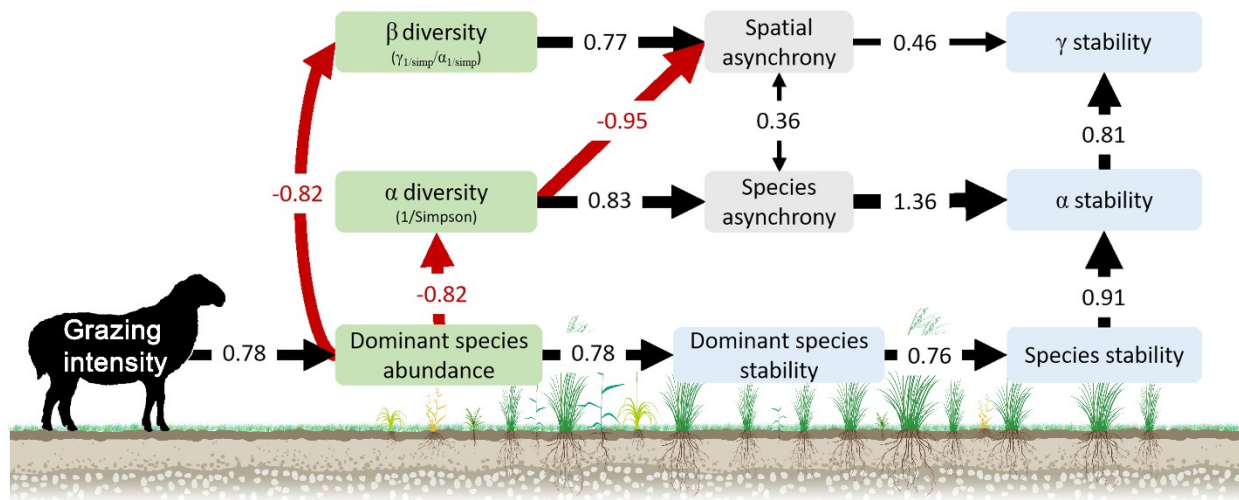


Figure 2 Grazing effects on biodiversity, asynchrony, and stability at multiple scales. Shown are the regression coefficients from linear mixed-effects models, which quantify the effects of increasing grazing intensity on (a) biodiversity (α diversity in 1 m² quadrats, $F_{1,10} = 22.58$, $P = 0.0008$; γ diversity in 120 × 120 m² paddock, $F_{1,10} = 23.50$, $P = 0.0007$; β diversity (γ / α), $F_{1,10} = 20.12$, $P = 0.0012$), (b) asynchrony metrics (species asynchrony, $F_{1,10} = 27.35$, $P = 0.0004$; spatial asynchrony, $F_{1,10} = 12.81$, $P = 0.005$; total insurance = species asynchrony × spatial asynchrony, $F_{1,10} = 16.27$, $P = 0.002$), and (c) stability metrics (species stability, $F_{1,10} = 16.01$, $P = 0.003$; α stability, $F_{1,10} = 27.50$, $P = 0.0004$; γ stability, $F_{1,10} = 10.17$, $P = 0.010$). The darker bar denotes the standard error and the lighter bar represents the 95 % confidential interval. Here, species diversity was defined by the reverse of Simpson index that incorporates both the number and abundance of species (see ‘Methods’). The repeated-measurement ANOVA and Tukey’s-range test for multiple comparisons under four grazing intensities are provided in Table S2, Table S5, Fig. S2, Fig. S7, and Fig. S8, respectively.



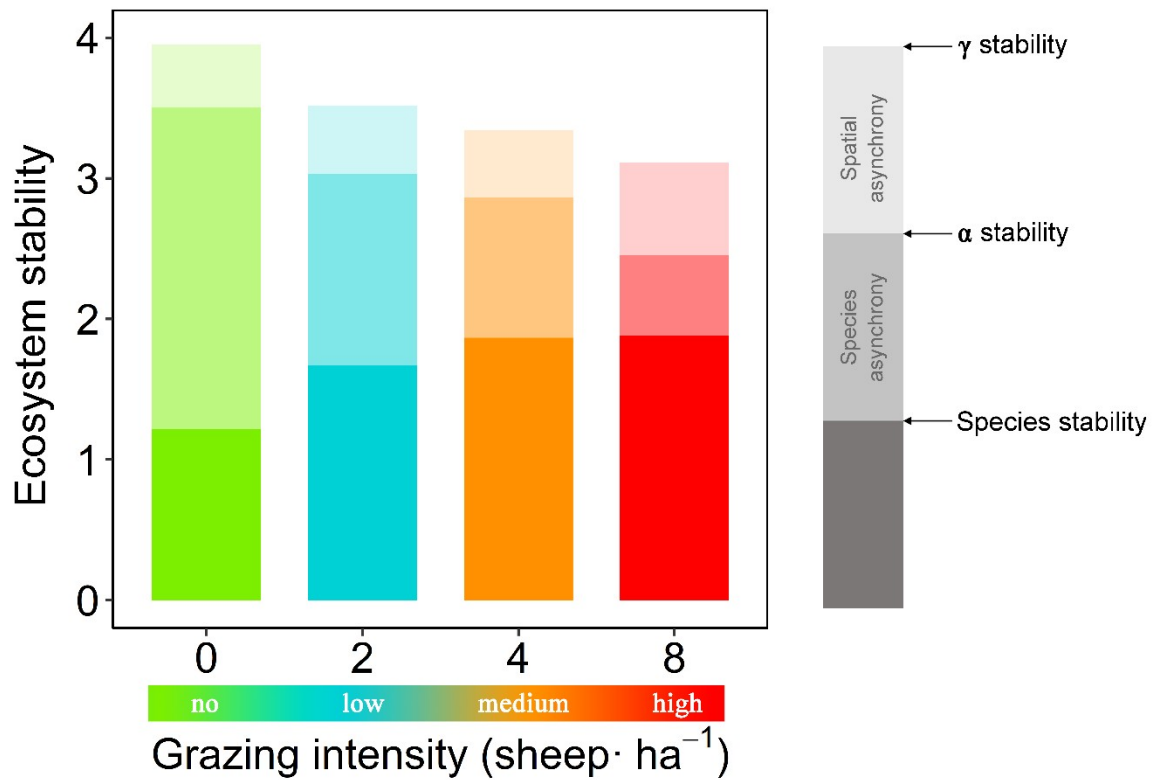
498

499 **Figure 3** Relationship between total insurance effect and γ diversity. Each point represents
500 values for a paddock at a given grazing intensity and particular month. Black line represents the
501 overall relationship ($F_{1,35} = 22.90$, $P < 0.0001$) from a linear mixed-effects model (with the
502 shaded areas denoting the 95 % confidence intervals), and the colored lines indicate random-
503 effect variations for no grazing (NG, green), low grazing (LG, blue), medium grazing (MG,
504 orange), and high grazing (HG, red) intensity. The marginal (R^2_m) and conditional (R^2_c) r-squared
505 represent “fixed effects” and “fixed + random effects” explanations, respectively. The significant
506 level: “*”: $P < 0.05$; “**”: $P < 0.001$; “***”: $P < 0.0001$.



507

508 **Figure 4** The structural equation model (SEM) depicting the direct and indirect effects of
 509 grazing intensity on grassland biodiversity and ecosystem stability at multiple scales. Shown are
 510 the final SEM with the standardized path correlation coefficients. Black and red arrows denote
 511 positive and negative associations, respectively. Fisher's $C = 69.695$; $df = 64$; $p = 0.292$; $AIC =$
 512 157.695 . Information about the priori SEM, the unstandardized direct effects, and the R^2 of
 513 individual response variables are provided in Fig. S12, Table S7, and Table S8, respectively.



514

515 **Figure 5** Visualized effects of grazing on stability across scales. Shown are the mean values of
 516 species stability, α stability, and γ stability under four grazing intensities: no- (0 sheep·ha⁻¹), low-
 517 (2 sheep·ha⁻¹), medium- (4 sheep·ha⁻¹), and high- (8 sheep·ha⁻¹) grazing intensity. As the grazing
 518 intensity increases, species stability increases, but α and γ stability decrease due to the reduced
 519 insurance effect of species asynchrony. In no-grazing treatment, species asynchrony provided a
 520 much stronger insurance effect for γ stability compared to spatial asynchrony. But an increase of
 521 grazing intensity reduces biodiversity and its insurance effects, such that in high-grazing
 522 treatment, spatial asynchrony provides a relatively stronger insurance effect than species
 523 asynchrony.