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# Soil aggregate microbiomes steer plant community overyielding in ungrazed and intensively grazed grassland soils

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## ABSTRACT

Plant and soil microbial community composition play a central role in maintaining ecosystem functioning. Most studies have focused on soil microbes in the bulk soil, the rhizosphere and inside plant roots, however, less is known about the soil community that exists within soil aggregates, and how these soil communities influence plant biomass production. Here, using field-conditioned soil collected from experimental ungrazed and grazed grasslands in Inner Mongolia, China, we examined the composition of microbiomes inside soil aggregates of various size classes, and determined their roles in plant-soil feedbacks (PSFs), diversity-productivity relationships, and diversity-dependent overyielding. We found that grazing induced significantly positive PSF effects, which appeared to be mediated by mycorrhizal fungi, particularly under plant monocultures. Despite this, non-additive effects of microbiomes within different soil aggregates enhanced the strength of PSF under ungrazed grassland, but decreased PSF strength under intensively grazed grassland. Plant mixture-related increases in PSF effects markedly enhanced diversity-dependent overyielding, primarily due to complementary effects. Selection effects played far less of a role. Our work suggests that PSF contributes to diversity-dependent overyielding in grasslands via non-additive effects of microbiomes within different soil aggregates. The implication of our work is that assessing the effectiveness of sustainable grassland restoration and management on soil properties requires inspection of soil aggregate size-specific microbiomes, as these are relevant determinants of the feedback interactions between soil and plant performance.

## Author contributions

X.L. and Y.L. designed the research. X.L., Z.Z. and Y.L. conducted the experiment and microbiome analyzing. X.L., X.T.L., Y. L. and K.J. performed statistical analyses with advice from W.P. X.L., X.T.L., Y.L., K.J. and W.P. wrote the first draft and the revisions of the manuscript.

## 1. Introduction

Biodiversity positively affects community productivity, as well as ecosystem service provisioning, and has been observed across multiple biomes (Polley et al., 2013; Tilman et al., 2006; Zhang et al., 2019). According to numerous empirical and theoretical studies, diversity-dependent overyielding is influenced by certain underlying mechanisms, such as complementary (e.g., resource partitioning) and

selection (i.e., the role of a particular species) effects (Loreau and Hector, 2001; Prieto et al., 2015). Despite this, considerable debate still remains on the context dependency of biodiversity-productivity relationships. For example, studies focused on anthropogenic disturbance, such as grazing in grasslands, have revealed uncertainties in predicting diversity-dependent overyielding (Zhu et al., 2020). Grazing is the most widespread anthropogenic utilization of grasslands (Fetzel et al., 2017), which strongly impacts the composition of plant and soil communities, as well as their interactions (Byrnes et al., 2018; Filazzola et al., 2020). However, major gaps remain in our understanding of how grazing influences diversity-productivity relationships via interactions between plants and soil biota.

Traditionally, diversity-dependent overyielding has been attributed to plant characteristics, particularly the complementarity of resource use, or even selection on plant species for increased niche differentiation

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(van Ruijven and Berendse, 2005; Xiao et al., 2010; Zuppinger-Dingley et al., 2014). Similarly, the feedback effects of soil communities on plant performances regulating diversity effects remain largely unknown (Schnitzer et al., 2011). Regarding this, plant-soil feedback (PSF) is defined as the complex process by which plants modify soil biota and abiotic properties, which in turn influences the growth of the same plants or subsequent plants (Bever et al., 1997; Van der Putten et al., 2013). In Mediterranean-climate scrublands, PSF varies with plant strategy, for example, among nitrogen-fixing and mycorrhizal plants, suggesting that PSF plays a central role in maintaining plant community taxonomic and functional diversity (Teste et al., 2017). While PSF has been proposed to play a central role in plant species coexistence, it may also contribute to diversity-dependent overyielding (Crawford et al., 2019; Wang et al., 2019). However, there is little experimental evidence on whether and how PSF regulates diversity-productivity relationships (Thakur et al., 2021).

Ecologists have long strived to characterize mechanisms underlying PSF based on interactions among different soil microbiome functional groups, such as bacteria and pathogenic, saprotrophic, and/or symbiotic fungi (Semchenko et al., 2018; van der Putten et al., 2016). Both pathogens and symbiotic mutualists are determinants of plant productivity and species coexistence (Van der Putten et al., 1993, 2013; Westover and Bever, 2001). The PSF effects of soil microbes on plant productivity can be affected by plant richness (Jing et al., 2015). Specifically, negative effects of soil pathogens depend on plant species richness (Schnitzer et al., 2011), resulting in less negative PSF effects in high-diversity assemblages due to the dilution effect on host-specific pathogens (Maron et al., 2011). Similarly, Luo et al. (2016) found that plant diversity can largely buffer the inhibitory effects of soil biota on ecosystem productivity. Furthermore, resource partitioning mediated by symbiotic microbiome components may also contribute to diversity-dependent overyielding (Wang et al., 2019).

Overgrazing is one of the most widespread global anthropogenic disturbances and has induced unprecedented impacts on grasslands, resulting in a global loss of grasslands' ecosystem services (Wang et al., 2017b). Grazing can reshape soil microbial characteristics, such as the bacteria/fungi ratio,  $\alpha$ -diversity, and community composition (Bardgett et al., 2001; Xun et al., 2018; Yang et al., 2019b), via trampling, defoliation, and excrements such as dung and urine (Liu et al., 2015; Schrama et al., 2013). Moreover, various size classes of soil aggregates with specific abiotic properties can provide specific growing environments for microbiomes (Tiemann et al., 2015), leading to altered structure of microbial community composition (Bailey et al., 2013). In this context, grazing-induced shifts in soil aggregate composition can substantially alter bacterial and fungal community composition (Wang et al., 2020), with the potential to feed back to grassland plant community productivity (Bergmann et al., 2016). However, little is known about such microbiome effects within different soil aggregates on PSF.

In this study, we performed a comprehensive PSF experiment (live versus sterilized soil; monoculture versus species mixtures) in both ungrazed and grazed grasslands using field-conditioned soil from a well-maintained grazing experiment (2014 – present) in the Inner Mongolian grasslands of China. We characterized the bacterial and fungal communities as potential factors influencing PSF and diversity effects. The aim of this study was to address three questions. First, how does the history of grazing affect PSF, and how does this relate to changes in soil functional groups, such as bacteria and pathogenic, saprotrophic, and/or symbiotic fungi? Second, we examined whether the interaction effects on plant performance and PSF among microbiomes within different soil aggregates are additive or non-additive? Third, we examined how does grazing-induced PSF mediate plant diversity-productivity relationships in grassland?

## 2. Methods

### 2.1. Study area and target species

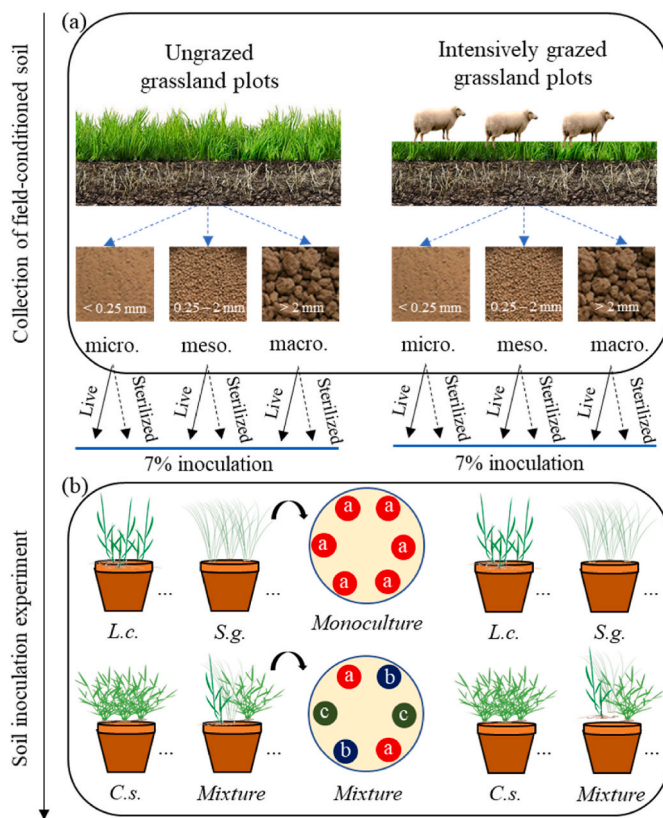
A well-maintained sheep grazing field experiment was conducted at the Xilingol Grassland Research Station (XGRS; 44°15'24" N, 116°32'08" E, approximately 1200 m above sea level) of the Chinese Academy of Agricultural Sciences, located in Xilinhot, Inner Mongolia, China. This region has a temperate continental climate with a mean annual precipitation of 263.5 mm and a mean annual temperature of 3 °C (1981–2010; China Metrological Services, <http://www.cma.gov.cn/>). According to the World Reference Base for Soil Resources (WRB-FAO), our study area soil type is classified as Kastanozem (WRB, 2014). The native vegetation is dominated primarily by the perennial grass *Leymus chinensis* (Trin.) Tzvel. In overgrazed grasslands, the dominant species in middle and later successional stages are the grasses *Stipa grandis* P. A. Smirn. and *Cleistogenes squarrosa* (Trin.) Keng., respectively (Li et al., 2008; Yu et al., 2020). Hence, we selected these three species as the target species to examine PSF, the biodiversity-productivity relationship, and their potential linkages. *L. chinensis* is a perennial rhizomatous grass of significant economic importance due to its high palatability and nutritional value to livestock. In contrast, *S. grandis* and *C. squarrosa* are perennial bunch grasses with a relatively low nutrient content and palatability.

### 2.2. Field-conditioned soil

For our PSF test, soils for inoculation were collected from the field sheep grazing experiment (2014–), including five grazing intensities and each with three replications (Li et al., 2021). We used plots at two grazing intensities, namely control and heavy grazing, for the ungrazed and overgrazed treatments, respectively (Fig. 1a). For more detailed information, see Supplementary Method 1. We collected the field-conditioned soil underneath selected plant species in ungrazed and overgrazed plots and considered those plants to have conditioned the soils. As for the PSF experiment we lumped the replicate soils within each of the grazing intensity treatments, we are not able to compare the effect of grazing intensity on PSF (Reinhart and Rinella, 2016). Therefore, we considered the PSF studies using soils of ungrazed and grazed grasslands as two separate experiments. The aggregates, however, were analyzed for each of the field replicates separately, so that we were able to analyze grazing effects on aggregate properties.

In early August 2018, corresponding to the peak growing season, we randomly set up five 25 cm × 25 cm quadrats along an S-shaped transect in each replicate plot. In total, 30 quadrats (2 grazing treatments × 3 replicate plots × 5 quadrats per plot) were selected for field-conditioned soil collection. The soil sampling depth was about 20 cm, which corresponded to the depth of the main root layer of plant species in the Inner Mongolian grassland. To maintain the primary structures of the samples before aggregate isolation, we placed the fresh soil into hard plastic boxes and transported them to the laboratory immediately after collection.

A modified dry-sieving method was used to acquire soil aggregate samples of different sizes (Garcia-Franco et al., 2015; Wang et al., 2017a). An approximate soil moisture content of 10% was found to be optimal for aggregate size fractionation of the samples; this moisture level minimizes the impact on microbial communities and activity during soil aggregate isolation (Wang et al., 2017a; Yang et al., 2019a). The soil was sieved and divided into macro-aggregates (>2 mm), meso-aggregates (0.25–2 mm), and micro-aggregates (<0.25 mm), respectively. To remove large stones and root residues from the soils, samples were subsequently passed through a 4 cm mesh sieve. In each plot, we randomly collected about 20 g of each soil material type (macro-, meso-, and micro-aggregates) to be stored at –20 °C for high-throughput sequencing of microbial community structure within three weeks. The remaining soils from the three aggregate classes were



**Fig. 1.** Experimental design. (a) The field grazing experiment (see details in [Supplementary Method 1](#)) and the collection of field-conditioned soil. (b) The soil inoculation experiment in the greenhouse. In the greenhouse planting, the six sowing points were uniformly arranged in the center of each pot along its circular perimeter. Here, the letter a, b, and c represent different plant species. Abbreviations: micro., micro-aggregates (<0.25 mm); meso., meso-aggregates (0.25–2 mm); macro., macro-aggregates (>2 mm); L.c., *Leymus chinensis*; S.g., *Stipa grandis*; C.s., *Cleistogenes squarrosa*.

stored at 4 °C prior to the feedback experiment.

The field-conditioned soil aggregates were then divided into two parts. One part was sterilized with an autoclave for 2 h at 121 °C ([Chen et al., 2017, 2018](#)). The other part was maintained as live soil. Soil samples corresponding to similar land use histories were merged uniformly to obtain a composite soil sample. Meanwhile, the bulk soils that were used for the feedback experiment were collected from an adjacent grassland near the grazing experiment. Soil samples were collected from the top 20 cm layer and merged uniformly. All bulk soils were sterilized by an autoclave for 2 h at 121 °C.

### 2.3. Microbial sequencing and data processing

We sequenced the microbial samples before the feedback phase. Soil sample genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instruction manual. Genomic DNA purity and quality were checked by electrophoresis on 0.8% agarose gels. The primers 338F/806R and ITS1/ITS2 targeting the V3–V4 regions of the bacterial 16S rRNA and the fungal ITS gene regions, respectively, were amplified. Amplicons were barcoded and sequenced on the MiSeq platform at Allwegene Company (Beijing, China). After the sequencing run, reads of both bacterial and fungal communities were paired and filtered to extract high-quality sequences using the QIIME package (Quantitative Insights Into Microbial Ecology) (v1.3.1). We then discarded the reads that could not be assembled. Using the UCLUST algorithm, unique sequences were classified into operational taxonomic units (OTUs) at a

threshold of 97%. The taxonomy of each 16S rRNA and ITS gene sequence was analyzed by UCLUST.

### 2.4. Inoculation trial

The design of the feedback phase in the greenhouse involved two land use histories (grazed and ungrazed), four soil aggregates (macro-, meso-, micro-aggregates, and their mixture according to the actual composition), two soil inoculation treatments (live and sterilized soil), four planting treatments (three monocultures and one species mixture), and four replicates, resulting in a total of 256 pots (12 cm diameter × 16 cm height). To minimize possible abiotic differences ([Brinkman et al., 2010](#); [Png et al., 2019](#)), all pots were prepared by inoculating sterilized subsoils with 7% (v/v) field-conditioned soil that had either been sterilized to serve as a control, or not sterilized to serve as the treatment condition ([Fig. 1b](#)).

Prior to the inoculation trial, *S. grandis* and *C. squarrosa* seeds were collected from an adjacent grassland, which was fenced and therefore free from livestock grazing. However, we could not obtain *L. chinensis* seeds from the XGRS grasslands, because no seeds were produced under the climatic conditions of this region. Therefore, *L. chinensis* seeds were collected from a well-watered common garden at another field station of the Chinese Academy of Agricultural Sciences located in Hohhot, Inner Mongolia, China. From both sites, mature seeds were harvested manually from July to September 2018. After collection, the seeds were rapidly air-dried and stored in a refrigerator at 4 °C until later use.

Before sowing, seeds of the three grass species were surface sterilized with a 2% (w/v) sodium hypochlorite solution and rinsed with sterile deionized water. Six sowing points were uniformly arranged in the center of each pot along its circular perimeter. Particularly, the interval planting method was adopted in the mixture treatment ([Fig. 1b](#)). To ensure ample seedling availability, at least three seeds were sown at each sowing point. After three weeks, one random seedling was retained at each sowing point by manual thinning.

The pots were placed in a greenhouse at 55% relative humidity, a day/night cycle of 14/10 h, and temperatures of 25 °C/20 °C, respectively. Plants were grown in the greenhouse and watered so that the soils were maintained at a 40% field water holding capacity. After approximately 2 months, the above- and belowground portions of each plant species were harvested, oven-dried at 70 °C for 72 h, and weighed to determine dry biomass.

### 2.5. Statistical analysis

For fungi, we assigned the OTUs to three fungal functional guilds, namely pathogenic, saprotrophic, and symbiotic (such as ectomycorrhizal fungi and mycorrhizal fungi), according to [Nguyen et al. \(2016\)](#). For the bacterial and functional fungal analysis, the relative abundance, Shannon-Wiener diversity index, and richness of fungi, mycorrhizal fungi, and putative pathogen communities for each sample were calculated using the diversity function in the vegan package in R, after OTU identification. The bacterial and fungal community composition was visualized by principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity matrices, also using the vegan package in R. The first component of the PCoA analysis (PCoA1) was used to represent the variation in total and functional fungal composition in the subsequent analyses. Permutational multivariate analysis with 1000 permutations was used to test whether the soil microbial community was significantly affected by soil aggregates and grazing history.

The PSF effects based on biomass were calculated as the difference between  $\log_e$ -transformed performance of plants in live and sterilized soil ([Png et al., 2019](#)). Values greater or less than zero indicate positive or negative feedback, respectively ([Li et al., 2022](#); [Png et al., 2019](#)). To determine if the roles of microbiomes within different soil aggregates were additive or non-additive in regulating plant performance and PSF, we directly compared the weighted averages of individual effects of



macro-, meso-, and micro-aggregates with the observed effects (i.e., the microbial effect of combined soil for the three aggregate sizes). For the aggregate compositions of ungrazed and grazed soil in our field plots, see Fig. S1. According to previously reported additive partitioning diversity effect equations (Jing et al., 2015; Loreau and Hector, 2001), the net diversity effects (NEs) were partitioned into complementary effects (CEs) and selection effects (SEs) (see Supplementary Method 2). To determine the effects of plant diversity on PSF, we analyzed the changes in PSF, calculated as the mean absolute difference between PSF in monoculture and species mixture conditions. Likewise, we analyzed the changes in net diversity ( $\Delta_{NE}$ ), complementary ( $\Delta_{CE}$ ), and selection effects ( $\Delta_{SE}$ ), calculated as the mean absolute difference in diversity effects after inoculation with live and sterilized soil, to assess the effects of soil microbiome inoculation. Moreover, we performed a Pearson correlation analysis to determine the linkages between changes in PSF ( $\Delta_{PSF}$ ) and changes in diversity effects (i.e.,  $\Delta_{NE}$ ,  $\Delta_{CE}$ , and  $\Delta_{SE}$ ).

Plant performance differences, PSF, and diversity effects were conducted by multivariate analysis of variance (MANOVA). Prior to conducting each MANOVA, data were checked for normality and homogeneity of variance and transformed whenever necessary to meet the assumptions of the analysis. Means are reported with standard errors.

### 3. Results

#### 3.1. Intensive grazing impacts on soil aggregate microbiomes

Grazing had a significant effect on bacterial and fungal communities, as well as on the corresponding functional groups (pathogenic, saprotrophic, and symbiotic microbes) within different soil aggregates (Fig. 2, Figs. S2 and S3). Grazing did not affect the relative bacterial abundance and richness of generalist and specialist communities (Figs. S4 and S5,

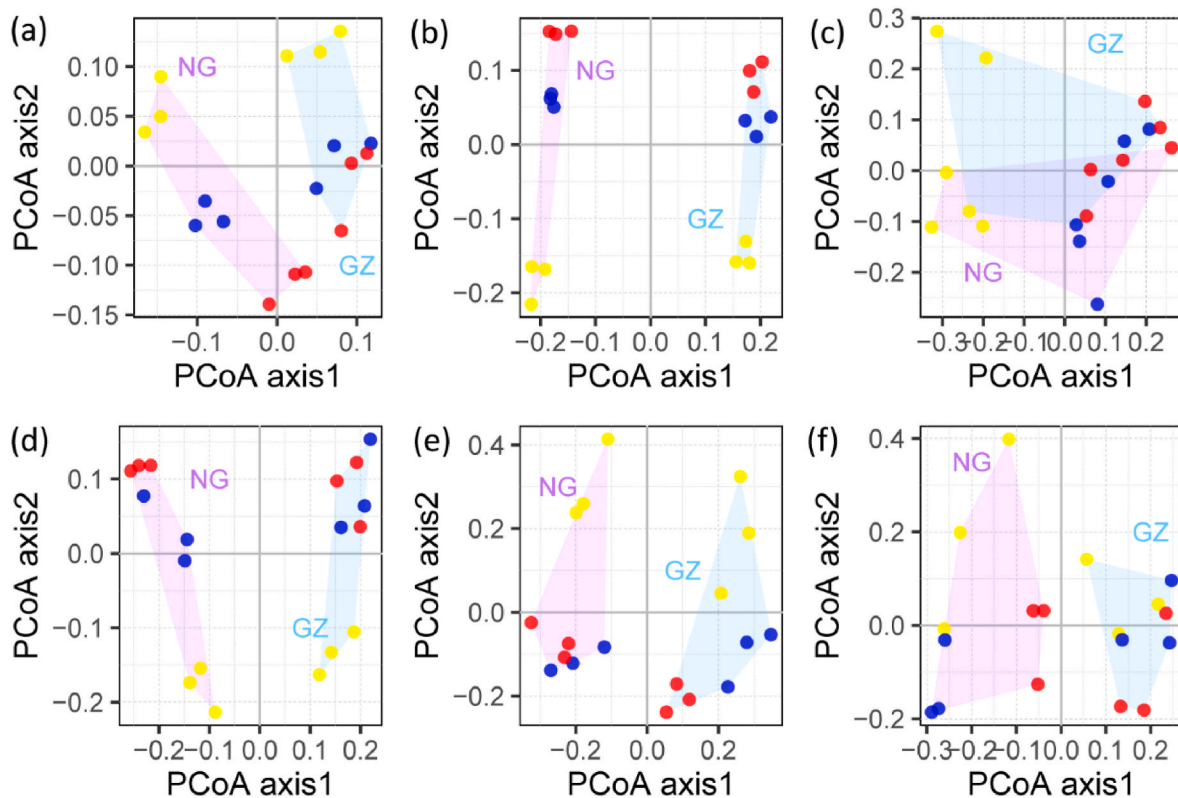
Tables S1 and S2). However, bacterial richness significantly varied with soil aggregates of different sizes, and with the interaction between soil aggregates and historic grazing (Fig. S5 and Table S2). The bacterial Shannon-Wiener diversity index was influenced independently by both soil aggregate size and grazing (Fig. S6 and Table S3). Although the overall fungal communities exhibited weak differences, the abundance, richness, and diversity of the functional groups were profoundly influenced by grazing, soil aggregates, and their interaction (Figs. S4–S6, Tables S1–S3). Notably, grazing decreased the abundance, richness, and diversity of pathogenic fungi, but significantly increased these indices for symbiotic fungi (Figs. S4–S6, Tables S1–S3).

#### 3.2. Effects of soil inoculation on diversity-productivity relationships

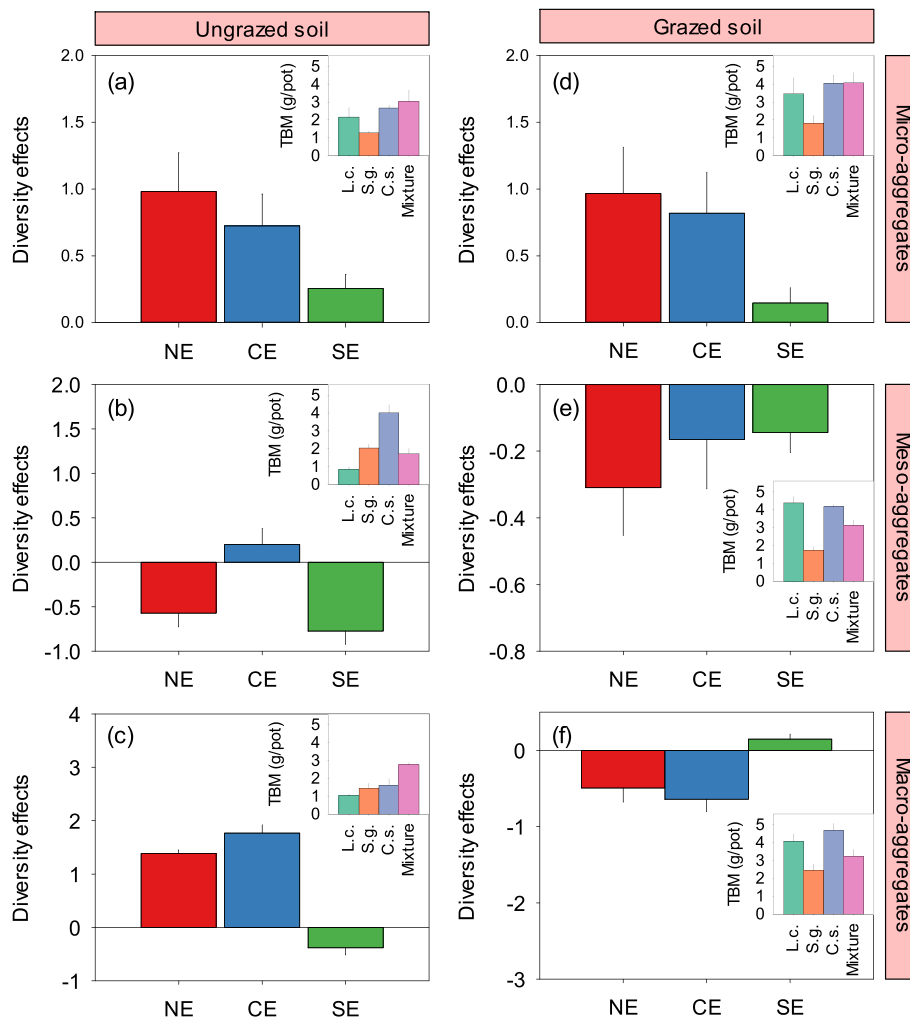
Compared to monoculture, plant species mixture showed a significant increase in total biomass production of plants grown in sterilized soil and soil inoculated with field-conditioned soil (Figs. S7 and S9). For plants grown in soil inoculated with ungrazed soil, the microbiome within different soil aggregates generated negative selection effects (SEs) (excepting the treatment of micro-aggregates) and positive complementarity effects (CEs) (Fig. 3a, 3b and 3c). For plants grown in soil inoculation with grazed soil, micro-aggregate (Fig. 3d) rather than meso- or macro-aggregate (Fig. 3e and f) microbiomes generated the positive CEs. Compared to the predicted values calculated by the treatments of the three soil aggregate size classes, ungrazed soil generated substantially lower observed net diversity effects (NEs) and SEs (Fig. 4). In contrast, predicted NEs and CEs were significantly higher than observed values under the grazed soil treatment (Fig. 4).

#### 3.3. Biotic plant-soil feedbacks under monoculture and mixture

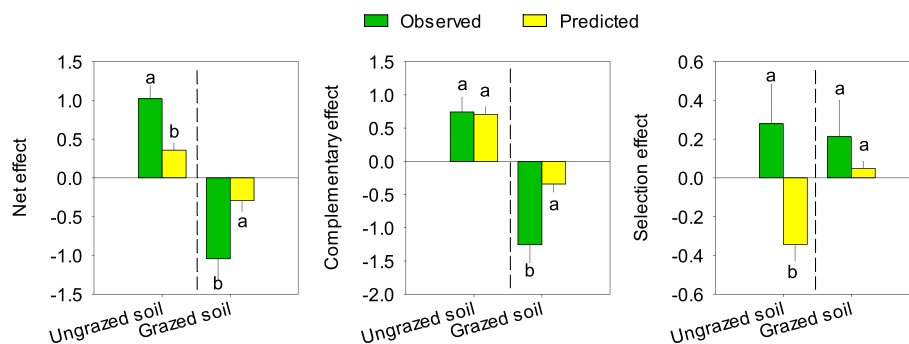
In monoculture, compared to the plants grown in sterilized soil, the



**Fig. 2.** Principal co-ordinate analysis (PCoA) of overall bacteria (a), overall fungi (b), putative pathogenic fungi (c), saprotrophic fungi (d), symbiotic fungi (e), and mycorrhizal fungi (f) based on Bray-Curtis distances. Abbreviations: NG, soil from ungrazed grasslands; GZ, soil from grazed grasslands; ●, micro-aggregates (<0.25 mm); ●, meso-aggregates (0.25–2 mm); ●, macro-aggregates (>2 mm).



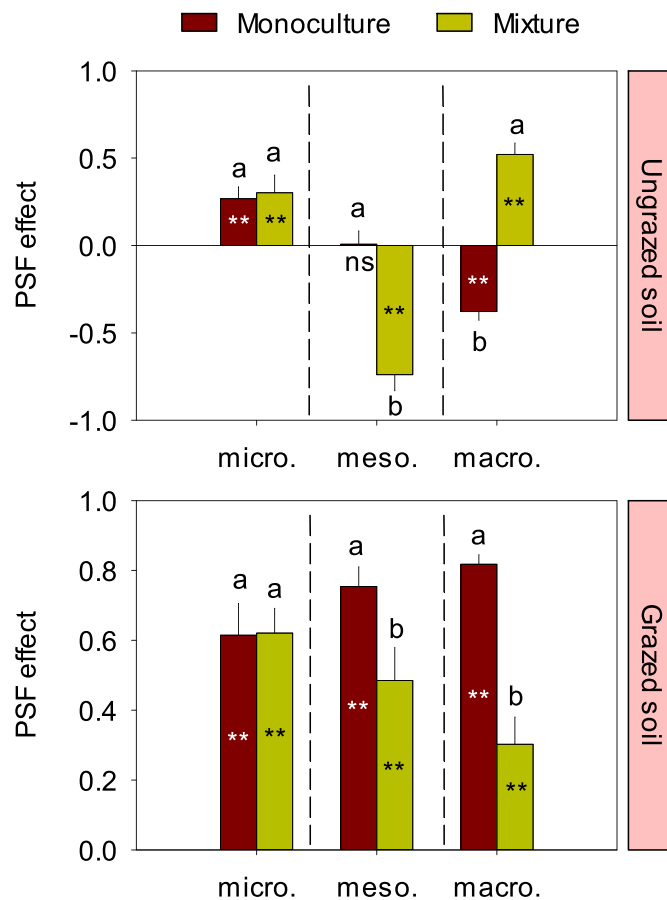
**Fig. 3.** Net diversity effects (NE), complementary effects (CE), and selection effects (SE) following inoculations of different soil aggregates in both ungrazed and intensively grazed grasslands. Abbreviations: micro., micro-aggregates (<0.25 mm); meso., meso-aggregates (0.25–2 mm); macro., macro-aggregates (>2 mm); L.c., *Leymus chinensis*; S.g., *Stipa grandis*; C.s., *Cleistogenes squarrosa*.



**Fig. 4.** Additive or non-additive effects contributed to by soil aggregates of different sizes on observed and predicted net diversity, complementary, and selection effects. The bars in each plot with different lowercase letters were significantly different between observed and predicted values at  $p < 0.05$ .

plants grown in soil inoculated with soil from grazed plots on average had more biomass (positive PSF) (Fig. 5, Figs. S7 and S8). Moreover, the mixture of the three grasses significantly influenced the PSF (Fig. 5, Figs. S7 and S8). Compared to monoculture, PSF in mixture induced an increase and a decrease in the plants grown in soil inoculated with ungrazed and grazed soil, respectively (Fig. 5). Additionally, there were no significant changes under monoculture between predicted and observed PSF for total biomass in both ungrazed and grazed soils

(Fig. 6), leading to additive effects on PSF based on total biomass. However, there were significant non-additive PSF effects in mixtures (Fig. 6). Non-additive effects decreased the observed PSF in the grazed soil, whereas these effects significantly increased the observed PSF in the ungrazed soil (Fig. 6).



**Fig. 5.** Plant-soil feedback (PSF) following inoculations of different soil aggregates in both ungrazed and intensively grazed grasslands in the context of monoculture and species mixture conditions. Abbreviations: micro., micro-aggregates (<0.25 mm); meso., meso-aggregates (0.25–2 mm); macro., macro-aggregates (>2 mm). The bars in each plot with different lowercase letters were significantly different between monoculture and mixture at  $p < 0.05$ . In the middle of each bar, \*\* and ns respectively represented significant ( $p < 0.01$ ) and neutral ( $p > 0.05$ ) PSF effects.

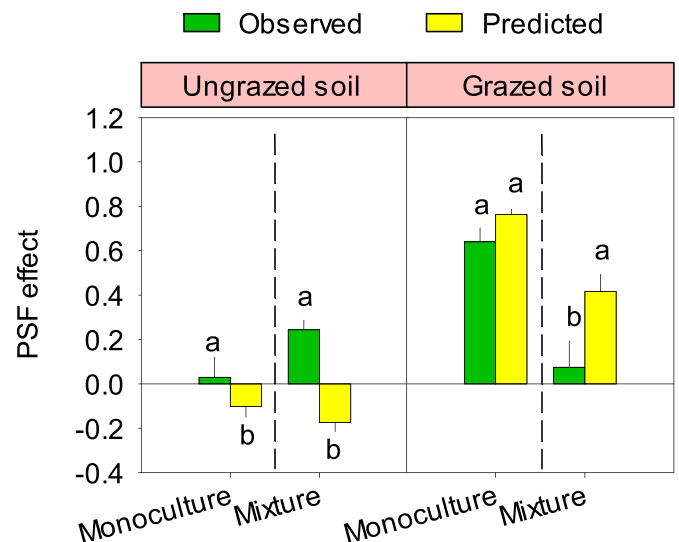
### 3.4. Linkages between plant-soil feedback and diversity-productivity relationships

The inoculation of ungrazed and grazed soil, respectively, generated an increase and a decrease in diversity effects, respectively, compared to the sterilized soil (Fig. S10). Moreover, these microbiome-driven changes in diversity effects were highly correlated with mixture-induced changes in PSFs (Fig. 7a, Fig. S11). These linear relationships were mainly driven by CEs and not by SEs (Fig. 7b and c).

## 4. Discussion

### 4.1. Historic grazing generated positive plant-soil feedbacks

Our results suggest that grazing resulted into positive PSF of the three focal species in monoculture. This is supported by previous studies in different grassland types (Chen et al., 2017; Veen et al., 2014), and the most likely reason is that grazing changes the composition of soil microbial communities (Eldridge and Delgado-Baquerizo, 2018). Soil microbiomes can drive the direction and magnitude of PSF in complex ways via antagonistic and synergistic interactions (Semchenko et al., 2018). Our results suggest that under grazing the pathogenic component of the microbiomes was suppressed, whereas the symbiotic microbes were stimulated. These changes, the diversity and abundance of



**Fig. 6.** Additive or non-additive effects contributed to by microbiomes within different soil aggregate sizes of both ungrazed and intensively grazed grasslands on observed and predicted plant-soil feedback (PSF), both in the context of monoculture and species mixture conditions. The bars in each plot with different lowercase letters were significantly different between observed and predicted values at  $p < 0.05$ .

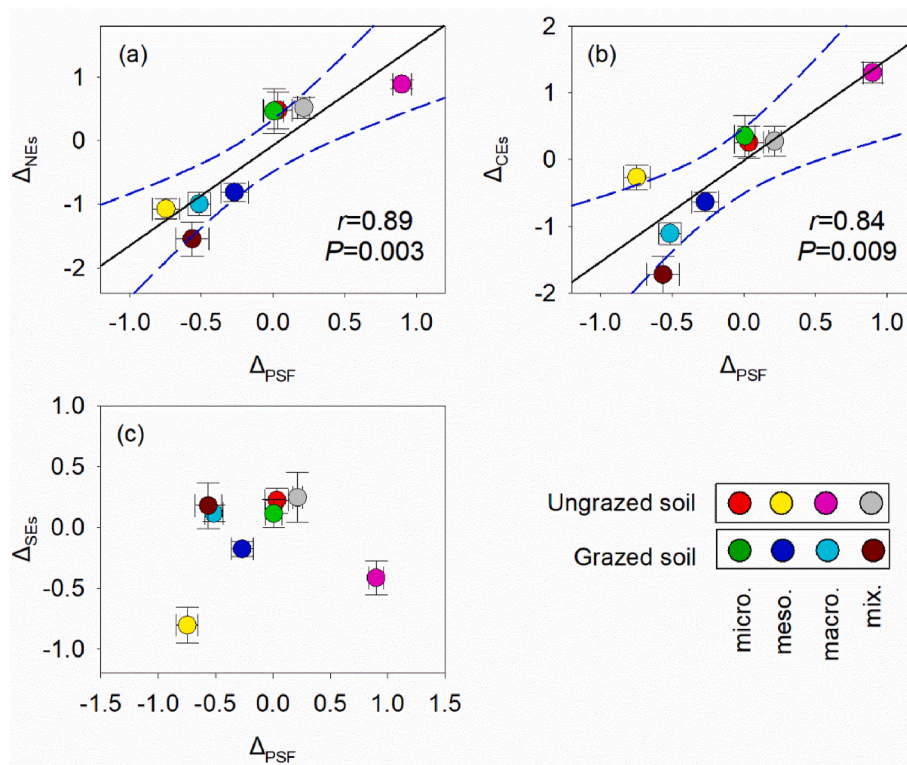
symbiotic communities in particular, were tightly correlated with the PSFs. Overgrazing is the primary cause of grassland ecosystem degradation (Xun et al., 2018), however, little attention has been given to plant-soil feedback interactions during this process. Combining our findings with previous studies, we predict that positive PSFs may potentially buffer grazing-induced declines in productivity (Li et al., 2018; McDonald et al., 2019). Our method of sample processing did not allow us to compare PSFs between ungrazed and grazed field treatments (Reinhart and Rinella, 2016). Our opposing patterns in the two PSF experiments suggest that the decline of vegetation productivity under overgrazing conditions might not be enforced by enhanced negative PSF, but this testing hypothesis requires further studies.

The inoculation of soil collected from ungrazed plots, which can be considered native habitats, generated neutral or negative feedback PSFs. In a meta-analysis, Kulmatiski et al. (2008) reported that terrestrial plants, with grasses in particular, generally produced negative PSF effects in approximately 70% of the cases. In our experiment, *L. chinensis*, the dominant native grassland species, experienced negative PSFs compared to *S. grandis* and *C. squarrosa*, which are the dominant species in degraded habitats. Hence, our results provide evidence that PSF is plant species-specific. According to previous studies on plant traits regulating PSFs via microbiome changes (Crawford et al., 2019; Sweeney et al., 2021), it might be possible that interspecific plant trait variation (rhizome and bunch grasses in our study) may be a key reason for species-specific PSFs.

### 4.2. Interspecific competition dramatically shaped plant-soil feedbacks

The outcomes of PSFs were determined both by microbiome changes at the end of the conditioning phase (Bever, 2003), and by plant interactions in the feedback phase (Chen et al., 2018). For example, Zhao et al. (2018) found interspecific competition to have a substantial impact on plant performance and PSF in grassland species. In our study, the mixture of the three grasses significantly influenced community and species-specific PSF. Since most grazing-induced PSF reports have mainly involved monoculture experiments (Chen et al., 2017; Medina-Roldán et al., 2012; Veen et al., 2014), the present work provides new evidence on the context dependency provided by soil and plant community composition of grazing-induced soil legacy effects.





**Fig. 7.** Linkages between changes in plant-soil feedback ( $\Delta_{PSF}$ ) and changes in diversity effects ( $\Delta_{NEs}$ ,  $\Delta_{CEs}$ , and  $\Delta_{SEs}$ ).  $\Delta_{PSF}$  represents the difference in PSF effects between monoculture and species mixtures.  $\Delta_{NEs}$ ,  $\Delta_{CEs}$ , and  $\Delta_{SEs}$  represent the difference in diversity effects under inoculation with live versus sterilized soil. Abbreviations: NEs, Net diversity effects; CEs, complementary effects; SEs, selection effects. Micro., micro-aggregates (<0.25 mm); meso., meso-aggregates (0.25–2 mm); macro., macro-aggregates (>2 mm); mix., combined soil.

Soil microbiome diversity and compositional changes may be strong drivers of PSF (Ke et al., 2015; Mariotte et al., 2018). However, we found that the influences of grazing-induced changes in microbiomes among different soil aggregates were substantially weaker in plant mixtures compared to monocultures. Consequently, variations in plant performance under mixture conditions were relatively low across treatments. This might be due to plant diversity inhibiting the effectiveness of the pathogenic component in microbial communities (Schnitzer et al., 2011). Thus, considering our results both in mixture and monoculture, it can be concluded that species-specific PSF was influenced not only by grazing and species identity (Bergmann et al., 2016; Thakur et al., 2021), but also by plant-plant interactions in mixtures.

#### 4.3. Non-additive effects acted only when plant grow under mixture conditions

In our field test, grazing significantly decreased the proportions of micro-aggregates, but increased the proportions of meso-aggregates, similar to previous studies in different regions (Liu et al., 2021; Wang et al., 2020). Soil aggregates, which are elementary soil structure components, are key determinants of the abundance, diversity, and composition of the soil microbial community (Wang et al., 2017a; Yang et al., 2019a). Due to the antagonistic and synergistic interactions of different microbiome functional groups (Chen et al., 2020; Leff et al., 2018; Semchenko et al., 2018), the effects of microbiomes among different soil aggregates might be non-additive. That is, the effects of soil with three aggregate types were significantly different from the weighted averages of their independent effects (Kohli et al., 2021; Xu et al., 2018). Consistent with this idea, our results showed that different soil aggregates had significant non-additive effects on microbiomes and on PSFs and diversity effects. Interestingly, these non-additive effects were related to planting treatment, where mixtures experienced a higher possibility than monocultures. A possible explanation for this is the positive interactions between plant and microbial diversity. First, microbial diversity effects, to which different soil aggregates contributed to in the present study, will be more effective in a mixture than in a

monoculture (Yang et al., 2021). Second, multi-species communities have a greater possibility for increased plant trait diversity, thereby benefiting niche complementarity (Tilman et al., 2006). Our results suggest that degraded grassland restoration could be promoted by using a targeted management practice, such as soil inoculation (Wubs et al., 2016) and increased plant diversity (Ladouceur et al., 2020).

#### 4.4. Linkages between plant-soil feedbacks and community overyielding

Understanding the role of PSF in shaping diversity-productivity relationships is critical to account for the effects that persist after grazing has ceased, or so-called grazing legacy effects (Li et al., 2022). Previous studies have illustrated the critical role that pathogen suppression has in driving a positive diversity-productivity relationship (Maron et al., 2011; Schnitzer et al., 2011). In the present study, compared to monoculture, a species mixture induced an increase in community PSF, which was most evident when plants were grown with inocula from ungrazed soil. This implies that a species mixture can benefit community biomass production via PSF, which has been concluded in previous studies (Schnitzer et al., 2011). Moreover, we found a mixture to sharply reduce the negative effects of pathogenic fungi, and the positive effects of symbiotic fungi, on PSF. We hypothesize that this result is most likely related to the rapid microbiome community changes generated by multi-species communities with divergent and complex root traits (Lugo et al., 2015; Sweeney et al., 2021). Additionally, grazing decreased the abundance and diversity of pathogenic soil fungi. This provides indirect evidence for our finding that grazing decreased net diversity effects via soil microbiota changes. Several studies have found that mutualists, such as arbuscular mycorrhizal and ectomycorrhizal fungi, promote plant growth (Chen et al., 2018; Walder et al., 2012), but may play negative roles in shaping positive diversity-productivity relationships (Wang et al., 2019). Our results support this finding by showing that grazing increased symbiotic fungal abundance, but decreased interspecific complementary effects.



## 5. Conclusion

We show that grazing generated an increase in the diversity of mutualistic-symbiotic microbiome components, which induced significant positive plant-soil feedback (PSF) effects when plants were grown in monoculture. In the grazed soil, plant species mixtures had less positive PSF than monocultures. Meanwhile, non-additive effects (i.e., the significant difference between the weighted averages of individual effects and the observed microbial effect of combined soil of three soil aggregate sizes) weaken the positive PSF of grazed soil. Consequently, mixture-induced changes in PSFs correlated with net diversity effects, and appeared to contribute to changes in complementary effects. Therefore, our results suggest that the changes in PSF effects induced by plant competition (mixture vs monoculture) contribute to diversity-dependent overyielding via non-additive effects of microbiomes that are present in the soil aggregates. The implication of these findings is that restoring grassland functioning through reduced grazing pressure involves changes in soil aggregation conditions and aggregate size-specific microbiomes. In practice, managing degraded ecosystem could possibly be promoted by using some targeted measure such as soil microbial inoculation, ultimately influence the PSFs and plant community productivity.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data that has been used is confidential.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2022.115919>.

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