Livestock overgrazing disrupts the positive associations between soil biodiversity and nitrogen availability

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AUTHORS' CONTRIBUTIONS

L.W. developed the original idea of the analyses presented in the manuscript in consultation with M.D-B and D.W. The field experiment was designed by L.W. Together, L. W., D.W., J.L. and H.Z. coordinated sampling and laboratory analyses. Q.C., X.Z., T.C., Y.S., Z.L. and Y.C. conducted samplings. L.W., X.Z. and M.Z conducted statistical analyses. The paper was written by L.W. and M.D-B, and the remaining authors provided editorial inputs.

DATA AVAILABILITY STATEMENT

Data is available in the Dryad Digital Repository.

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Abstract

Livestock overgrazing influences both microbial communities and nutrient cycling in terrestrial ecosystems. However, the role of overgrazing in regulating the relationship between soil biodiversity and nitrogen availability remains largely unexplored. We performed long-term grazing exclusion experiments across eight sites along precipitation gradient covering three major types of grassland in northern China to compare the linkage between soil microbial diversity and N availability in overgrazed versus non-grazed conditions. We found a significantly positive association between fungal diversity and soil available N in non-grazed grasslands. However, the positive association was absent in overgrazed environments. Bacterial diversity is not related to soil available N in either non-grazed or overgrazed grasslands. Moreover, in bacterial community, we found a positive link between the relative abundance of Actinobacteria with soil available N in non-grazed, but not overgrazed, grasslands. Instead we found the links between relative abundance of Bacteroidetes and Acidobacteria with soil available N in overgrazed grasslands, but not non-grazed,

grasslands. Our work provides evidence that the relationships between microbial diversity and ecosystem functions are context-dependent, and so microbial community diversity is likely not the major driver of soil N mineralization in overgrazed grasslands. Our study suggests that high intensity anthropogenic activities in grasslands restrains the capacity of diverse soil microbial communities to sustain ecosystem function, and more broadly the capacity of entire ecosystems to maintain important ecosystem processes such as plant production. Our study also indicates that the fundamental microbial communities associated with N availability change with differing land management strategies (e.g. livestock grazing).

Keywords: Soil microbial diversity, grazing management, grassland N cycling, herbivore grazing, land-use intensification

Introduction

Livestock grazing is one of the most widespread forms of intensive resource management on Earth and plays a fundamental role in food production. However, overgrazing is also one of the most pervasive and significant processes that degrades grassland (Eldridge & Delgado-Baquerizo 2017), especially in northern China, where 90% of grasslands have been overgrazed and thus degraded (Kemp et al. 2013).

Soil nitrogen (N) is one of the most limiting factors and important drivers of ecosystem productivity in terrestrial ecosystems (Schlesinger 1996). Soil microbes support critical processes associated with N cycling and are also among the most abundant and diverse organisms on earth. There is growing evidence that herbivore grazing can alter the community composition and diversity of belowground soil microorganisms (Bardgett et al. 2001; Yang et al. 2013; Peschel et al. 2015; Cline et al. 2017; Eldridge et al. 2017). A growing number of studies also suggest that greater microbial diversity can enhance the rapid breakdown of litter derived from aboveground plant matter, increasing soil organic matter content and nutrient availability (van der Heijden et al. 1998; Wardle et al. 2004; Gessner et al. 2010). Degrading complex and recalcitrant polymers into simpler and more labile monomers requires the cooperation of a large and diverse group of microorganisms (Hooper et al.

2000; Wardle et al. 2004; Schimel et al. 2005; van der Heijden et al. 2008). Much less is known, however, about the potential impacts of livestock overgrazing on the linkage between soil biodiversity and N availability.

Livestock grazing may directly and indirectly affect below-ground properties (Bardgett & Wardle 2003). For example, herbivore trampling can directly change soil structure or permeability (Gass & Binkley 2011; Schrama et al. 2013). Plant consumption by herbivores and dung and urine deposition can affect the quantity and quality of resources that are returned to the soil (Ruess & Seagle 1994; Frank & Evans 1997; Frank et al. 2002; Bakker et al. 2004). All these changes can affect soil nutrient cycling (Augustine et al. 2003; Bakker et al. 2009; Wang et al. 2019) and the activity and abundance of soil organisms (Bardgett & Wardle 2003). Notably, different soil microbial taxa could vary in their response to changes in soil environment such as temperature and pH (Laanbroek & Woldendorp 1995; Stark & Firestone 1996), so those changes resulting from herbivore grazing could also alter the effects of microbial community diversity on soil N mineralization. Assessing the robustness of soil biodiversity-N availability relationships in highly managed grasslands is believed necessary for predicting ecosystem response to the ongoing global land use intensification.

Here, we tested the potential effects of overgrazing by livestock (cattle and sheep, etc.) on the relationship between soil microbial diversity and nitrogen availability by using an experimental approach with multiple grazing exclosures in arid and semiarid grassland ecosystems across northern China, which contains the largest remaining grassland on Earth. We hypothesized that soil biodiversity is positively associated with nitrogen availability for plants, but also that overgrazing by livestock can disrupt relationships between soil microbial diversity and nitrogen cycling.

Materials and Methods

Sampling sites

The study area was located in temperate grasslands of the Inner Mongolian Plateau in Northern China (111.23 E to 120.10 E, 41.25 N to 49.64 N), where the climate is

predominantly arid and semi-arid continental; Mean annual precipitation ranged from 224 mm to 397 mm and mean annual air temperature ranged from -2.1°C to 3.5°C. To generate enough variability to test the link between microbial diversity and function, we selected three different types of grasslands along this transect from east to west: meadow steppe, typical steppe and desert steppe. A total of eight sites with livestock overgrazing and with various dominating plant species were selected along this transect, including three meadow steppes, three typical steppes and two desert steppes (Fig. S1; Supporting Information Table S1). Within each site a long-term grazing exclosure was established in an area with a history of long-term heavy grazing and where more than 90% of the annual aboveground productivity was consumed. Livestock were excluded via the exclosure for over five years at each of the eight sites. The differences between the grassland structure inside and outside the exclosure were great (Fig. S2). For one, plant height was extremely and significantly lower in the overgrazed than in the non-grazed grasslands (inside the exclosure) (Fig. S3; Table S3).

Field sampling and measurements

Plant and soil sampling were carried out during the summer (late July to August) of 2016, corresponding to annual peak-standing biomass. At each site, a 14 m × 14 m plot was selected randomly, and five 0.5 m × 0.5 m quadrats were set at the four corners and the center of the plot. Above-ground biomass was clipped at the ground level and oven dried at 65°C for 48 h. Then it was weighed and ground into a fine powder on a ball mill for plant community nitrogen analyses. Nitrogen content was measured using the CHNOS Elemental Analyzer (vario EL cube).

Soil samples were collected by taking three soil cores (2.5-cm diameter) at 10 cm depth in each of the five 0.5 × 0.5 m quadrats at one site. The three soil cores were mixed in situ to form one composite sample representing each quadrat. After removing the rocks and roots, the soil was passed through a 2-mm-mesh sieve and separated into two parts. One part was air-dried and used to determine soil pH, which was measured in a 1: 2.5 (soil: water) suspension. The other part was kept in a freezer (MOBICOOL CoolFreeze CF-50) to maintain a temperature of -18°C and carried

back to the laboratory as soon as possible for soil microbial community analysis and available nitrogen analysis. Soil NH_4^+ and NO_3^- were analyzed using an Alliance Flow Analyzer (Alliance Flow Analyzer, Futura, frépillon, France). Soil available N was determined as the sum of ammonium and nitrate.

Microbial community analyses

Microbial DNA was extracted from soil samples using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined by the NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). Bacterial communities were assessed with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), targeting the V3–V4 regions of the 16S rRNA gene. Fungal communities were assessed using the forward primer ITS-1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and the reverse primer ITS-2R (5'-GCTGCGTTCTTCATCGATGC-3'). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles (bacterial) or 35 cycles (fungal) and 30 s at 95 °C, 30 s at 55 °C for annealing, 45 s at 72 °C for elongation, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulting PCR products were extracted from a 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. Purified amplicons were pooled in equimolar and paired-end sequences on an Illumina MiSeq platform (Illumina, San Diego, USA).

The MiSeq sequences were demultiplexed and quality-filtered by Trimmomatic on the criteria of having an average quality score higher than 20 over a 50 bp sliding window. Sequences whose overlap was longer than 10 bp were merged according to their overlap sequence. After removing the reads containing ambiguous bases, paired-end reads with at least a 10 bp overlap were joined using FLASH and allowing

for 2 mismatched nucleotides. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (Edgar 2013). Singleton OTUs were removed as well as the chimeric sequences identified by the UCHIME algorithm. The taxonomy of each 16S rRNA gene sequence was analyzed with the RDP Classifier (Wang et al. 2007) against the Silva (SSU123) 16S rRNA database using a confidence threshold of 70%.

For each taxonomic group analysed, samples were rarefied to compare all samples at equivalent sequencing depths corresponding to the lowest sequencing coverage. Rarefied data was used to calculate Shannon diversity for these groups. Nitrification plays a key role in determining how much and which forms of soil inorganic N are available for plants. We also calculated the relative abundance of different bacterial phylum.

Statistical analysis

We firstly run general linear mixed models (GLMMs) including grazing and microbial diversity as predictor variables, and grassland type as random factor to analyze the interactive effects of grazing and microbial diversity on soil available N, which help examine whether grazing significantly affected the relationship between microbial diversity and soil available N. Further, we explored the relationship between soil microbial diversity, as estimated with the Shannon index (Haegeman et al. 2013), and soil N availability using two approaches (regression models and linear mixed models) across eight non-grazed and eight overgrazed grasslands, respectively. First, we fitted OLS regression models to show the relationship between microbial diversity and soil N. Great variation was found among the five sampling replication points within each site/plot, and spatial dependency for the soil variables often disappeared after a few centimeters (e.g., Delgado-Baquerizo et al. 2013). Thus, the five replications within each site were considered as individual observations in the analyses (n=40). Secondly, to further examine whether the relationship between microbial diversity and soil available N was driven by cross-grassland types difference, we fitted linear mixed models to individual site/plot-level data using the site/plot means, i.e. means of the five sampling replication within each site/plot (n=8). Linear mixed models employed

restricted maximum likelihood estimation, and included grassland types as a random factor, and microbial diversity as fixed factor. Moreover, we re-fitted linear mixed models to individual soil sampling level data, i.e the five replications within each site were considered as individual observations in the analyses (n=40). We found the consistent result from the two fitted linear mixed models so, for simplicity, present only the linear mixed models based on individual soil sampling level data. To check the accuracy of soil available N, which was only measured once during the peak growing season to reflect annual N availability to plants, we further analyzed the relationship of soil available N with plant community N.

Soil pH is globally the most important predictor of microbial diversity and N availability (Lauber et al. 2008, 2009; Delgado-Baquerizo et al. 2016a), and therefore, any assessment of the linkages between microbial diversity and function need to control for soil pH. We therefore used SEM to evaluate the direct and indirect effects of soil microbial diversity and soil pH on soil available nitrogen and plant community nitrogen content. We fitted separate SEMs for non-grazed and overgrazed grasslands. The analysis was performed on standardized variables (deviation from mean / standard deviations), and we quantified direct and indirect effects as standardized path coefficients. Our structural equation modeling was carried out using the sem function of the lavaan package (Rosseel 2012) in R (version 3.4.3, R Developmental Core Team 2017). The performances of the SEMs were evaluated using a combination of the chi-square statistic (where $0 \leqslant \chi 2 \leqslant 2$ df and $P > 0.05$ indicate a good fitting model), Bentler's comparative fit index (CFI, where $CFI > 0.95$ indicates a good fitting model) and the standardized root mean square residual (SRMR; where $SRMR \leqslant 0.08$ indicate a good fitting model).

Results

The results from GLMMs showed that there was significant interactive effect of grazing and fungal diversity not bacterial diversity on soil available N, indicating that grazing significantly altered the association between fungal diversity and soil available N (Table 1). Further, the results from regression models showed that soil

fungal diversity was significantly and positively related to soil available nitrogen in non-grazed grasslands, while this relationship was absent in overgrazed grasslands (Fig. 1a, b). There was not any significant correlation between bacterial diversity and soil available N in either non-grazed or grazed grasslands (Fig. 1c, d). However, in the bacterial community, we found a positive link between the relative abundance of Actinobacteria with N availability in non-grazed, but not overgrazed, grasslands (Fig. 2a, b). Instead we found the links between the relative abundance of Bacteroidetes and Acidobacteria with soil N availability in overgrazed grasslands, but not non-grazed, grasslands (Fig. 2c, d, e, f). The results from GLMMs also showed that there were significant interactive effects of grazing and the relative abundance of Actinobacteria Bacteroidetes and Acidobacteria on soil available N (Table 1). The results from linear mixed models including grassland types as a random factor were consistent with that of all the regression models (Table S2), indicating that these relationships did not result from the cross-grassland type difference. Soil available N was significantly and positively related to plant community N in both non-grazed and overgrazed grasslands (Fig. 3), indicating that the soil available N during the peak growing season was a valid proxy for annual N availability in this study.

We adopted SEM to further examine the direct and indirect effects of fungal diversity on soil and plant N content when controlling for soil pH, which is the most widely acknowledged soil factor affecting N mineralization in soil. We ran separate models for overgrazed and non-grazed locations. Our SEMs explained 21% and 35% of the variance found in the soil available nitrogen of non-grazed and overgrazed grassland data sets, respectively (Fig. 4). In non-grazed grasslands, we still found a direct positive effect of fungal diversity on soil nitrogen (Fig. 4a). However, such an association was lost in overgrazed grasslands (Fig. 4b).

Discussion

To our knowledge, this study provides the first empirical evidence that human-initiated overgrazing can disrupt the positive associations between microbial diversity and soil N mineralization, and thus the levels of plant-available N. Such a result suggests that as grazing by livestock continues to increase in order to feed a

growing human population, the important associations between soil biodiversity and N availability could be weakened, or even disappear.

Specifically, we found that fungal, and not bacterial, diversity is strongly related to soil N availability across arid and semi-arid grasslands in northern China, and this relationship was not driven by cross-grassland type difference. Our finding concurs with a global study providing similar results across 78 global drylands (Delgado-Baquerizo et al. 2016b). Fungi are generally more tolerant of desiccation than bacteria, which might explain the importance of these organisms in arid and semiarid ecosystems (Austin et al. 2004). Notably, livestock overgrazing disrupted the positive link between fungal diversity and soil available N, though there was little statistically significant difference in soil available N and microbial diversity between non-grazed and overgrazed grasslands across all the sites (Table S3; Fig. S4). While the relationship between microbial community diversity and soil N cycling has been demonstrated in natural ecosystems in many studies (Schimel et al. 2005; Reed & Martiny 2007; Graham et al. 2014; Wagg et al. 2014; Delgado-Baquerizo et al. 2017), our study indicates that the link between microbial diversity and soil N cycling is context-dependent, and that microbial community diversity is likely not the primary driver of soil N mineralization in widely overgrazed grasslands.

Soil N mineralization could be predominantly controlled by particular microbial taxa in overgrazed grasslands instead of by microbial community diversity. We found, in bacterial communities, the strong relationships between the relative abundance of Actinobacteria and soil N availability which was found in non-grazed grasslands also disappeared in overgrazed grasslands. Actinobacteria was the dominant bacterial phylum here. Actinobacteria are defined as oligotrophs (Bastian et al., 2009; Trivedi et al. 2013), containing a broad array of genes that allow the breakdown and utilization of recalcitrant organic compounds that can be used under low carbon, such as lignin, chitin and cellulose (Maestre et al. 2015; Delgado-Baquerizo et al. 2017). In overgrazed grasslands alone, the relatively high abundance of Bacteroidetes, defined as copiotrophic organisms by Fierer et al (2007), and the low abundance of Acidobacteria was strongly related to soil available N. These findings suggest that the fundamental

301 microbial communities associated with N availability change with differing land
302 management strategies (grazed vs. non-grazed grasslands). Nevertheless, we did not
303 get the actual abundance of these microbial groups. The relationships between soil
304 available N and the actual abundance of the microbial groups could differ from the
305 relative abundance, which need be further explored in future study.

306 We suggest that the grazing-induced improvement in the quality of resources
307 entering the soil could reduce the requirement for the cooperation of a large and
308 diverse group of microorganisms, such as fungi and Actinobacteria in this study.
309 Herbivory can induce an increase in root exudation and hence the amount of labile C
310 entering the soil (Hamilton & Frank 2001). Such physiological responses to herbivory
311 have been suggested to represent an important mechanism for increasing nutrient
312 availability in natural ecosystems (Hamilton & Frank 2001). Moreover, conversion of
313 plant tissue into herbivore dung and urine results in the return of readily available
314 elements to soil pools. Animal excreta are deposited (dung and urine) in the process of
315 grazing and are thought to have major effects on soil N availability (Mikola 2009).
316 Also, herbivory leads to an increase in fast-growing plants of high quality
317 (McNaughton 1979; Bakker et al. 2009), which can also yield decomposed easily
318 litter. Thus, Fungi and Actinobacteria may be less competitive or less necessary in
319 soils with higher labile carbon resulting from herbivore grazing. Bardgett et al. (2001)
320 also found that soil microbial communities of heavily grazed sites are dominated by
321 bacterial-based energy channels of decomposition and that fungi have a proportionally
322 smaller role. Therefore, with fewer recalcitrant organic compounds in the soil, a
323 highly diverse microbial community may not be necessary, though this should be
324 further examined in future studies.

325 Herbivores also have substantial impacts on below-ground processes via changes in
326 key soil properties. High grazing intensity commonly results in harsh soil
327 environment conditions, such as increased soil compaction, reduced aeration
328 (Milchunas & Lauenroth 1993; Eldridge et al. 2016) and increased or decreased soil
329 pH (Smolik et al. 1972; Yong-Zhong et al. 2005) due to trampling and the removal of
330 vegetation. Soil pH was found to be one of most important factors affecting soil N

mineralization on a global scale (Li et al. 2019). Microbial diversity and composition have been shown to be influenced by soil pH (Fierer et al. 2007; Rath & Rousk 2015), and thus changes in pH resulting from livestock grazing may indirectly affect microbial community diversity and thereby soil N mineralization. Moreover, soil pH was found to have considerable direct negative impacts on soil N mineralization by changing soil metabolic and enzymatic activities (Li et al. 2019), which could also alter the way in which soil microbial diversity relates to soil N mineralization. For instance, the activity of urease and some protease decreased as soil pH increased (Singh & Nye, 1984; Kamimura & Hayano, 2000). Our results from the SEMs showed that soil pH had an important direct negative impact on soil available N in overgrazed grasslands, and that soil pH directly explained 58% of soil available N (Fig. 4). Therefore, grazing-induced changes in soil pH could prohibit the direct effects of some microbial taxa on soil N mineralization by changing their enzymatic activities. Consistently, Delgado-Baquerizo et al. (2017) suggests that the positive effects of particular microbial taxa on multifunctionality resistance could potentially be controlled by altering soil pH. Our study also indicated that soil abiotic factors instead of soil organisms may play the predominant role in controlling soil N availability in grasslands that have high intensity grazing disturbances.

In conclusion, our data shows that the biodiversity of Fungi not bacteria was positively correlated with soil nitrogen availability in arid and semiarid grassland ecosystems across northern China, and that overgrazing by livestock can disrupt this important association. Our study also indicates that the link between microbial community diversity and soil N cycling is context-dependent, likely depending on the quality of both the soil substrate and the soil physical environment (e.g. pH). Currently, most of grasslands are facing degradation and desertification worldwide. Thus, understanding the relationship between microbial diversity and nitrogen cycling in terrestrial ecosystems during the Anthropocene has fundamental implications for managing grasslands under global change scenarios. Our study suggests that high intensity grassland disturbance by anthropogenic activity could not only reduce the biodiversity in ecosystems (Bardgett et al. 2001; Allan et al. 2014; Eldridge et al. 2016),

but it may also restrain the capacity of soil microbial diversity to sustain ecosystem function. More broadly, ecosystems may be unable to maintain important ecosystem processes such as plant production.

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AUTHORS' CONTRIBUTIONS

L.W. developed the original idea of the analyses presented in the manuscript in consultation with M.D-B and D.W. The field experiment was designed by L.W. Together, L. W., D.W., J.L. and H.Z. coordinated sampling and laboratory analyses. Q.C., X.Z., T.C., Y.S., Z.L. and Y.C. conducted samplings. L.W., X.Z. and M.Z conducted statistical analyses. The paper was written by L.W. and M.D-B, and the remaining authors provided editorial inputs.

DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.z08kprr96, (Wang et al., 2020). The raw sequencing data were archived at the National Center for Biotechnology information (https://www.ncbi.nlm.nih.gov/), in the Sequence Read Archive (SRA) database.

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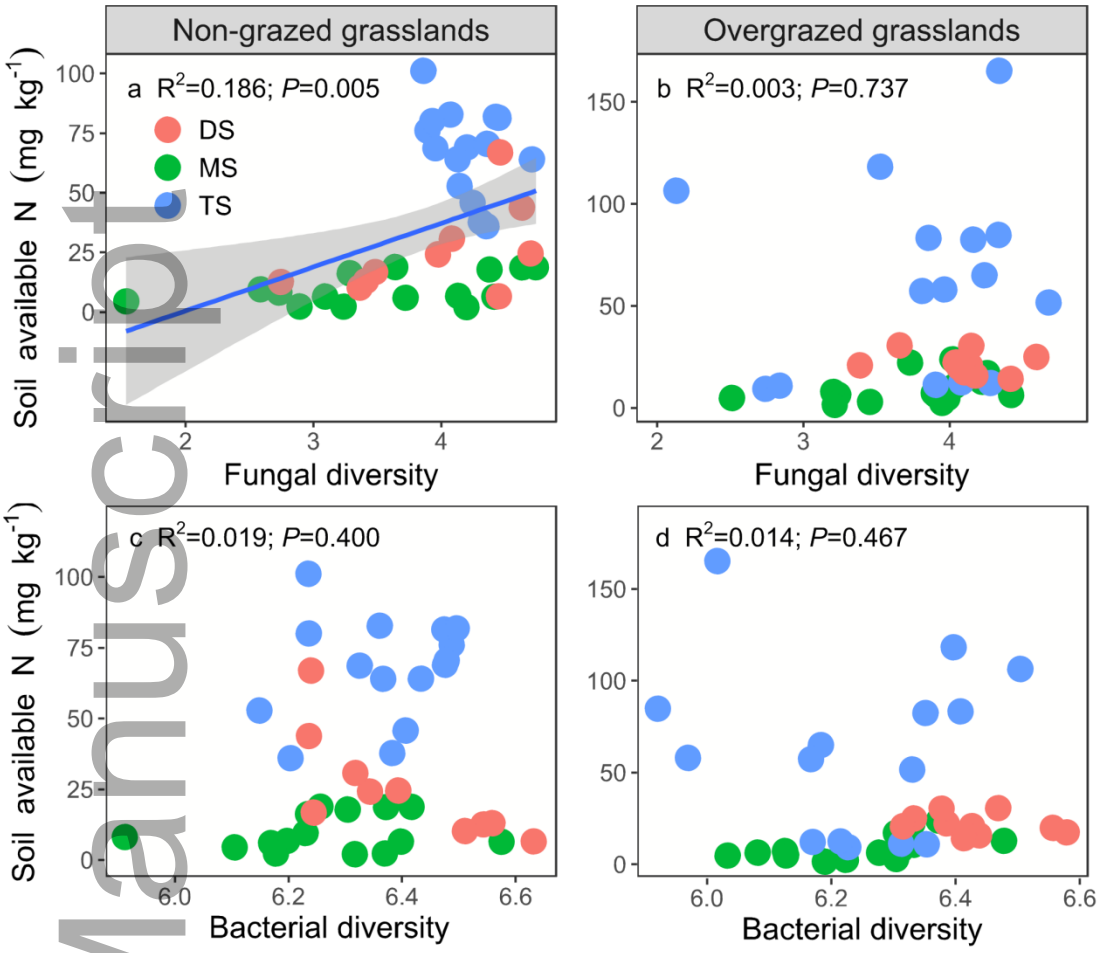
Table 1. Summary of general linear mixed models (GLMMs) analyzing the interactive effects of grazing and microbial diversity on soil available N. Grazing and microbial diversity were taken as predictor variables, and grassland type was taken as random factor. The GLMMs were run for fungal diversity, bacterial diversity, and the relative abundance of Actinobacteria, Bacteroidetes and Acidobacteria, respectively.

Variable	Estimate	Std. Error	z-value	p-value
Fungal diversity: Grazing	-0.167	0.079	-2.098	0.036
Bacterial diversity: Grazing	0.365	0.274	1.333	0.183
Actinobacteria abundance: Grazing	-4.832	1.036	-4.662	<0.001
Bacteroidetes abundance: Grazing	39.525	5.921	6.675	<0.001
Acidobacteria abundance: Grazing	-5.018	1.247	-4.024	<0.001

Figure 1



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591 **Figure 1. Relationships between soil fungal, bacterial diversity and soil available**
592 **N (mg/kg) across non-grazed (a, c) and overgrazed grasslands (b, d).** The fitted
593 lines are from the OLS regression. Shaded areas show the 95% confidence interval of
594 the fit. DS-desert steppe; MS-meadow steppe; TS-typical steppe.

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600 **Figure 2**

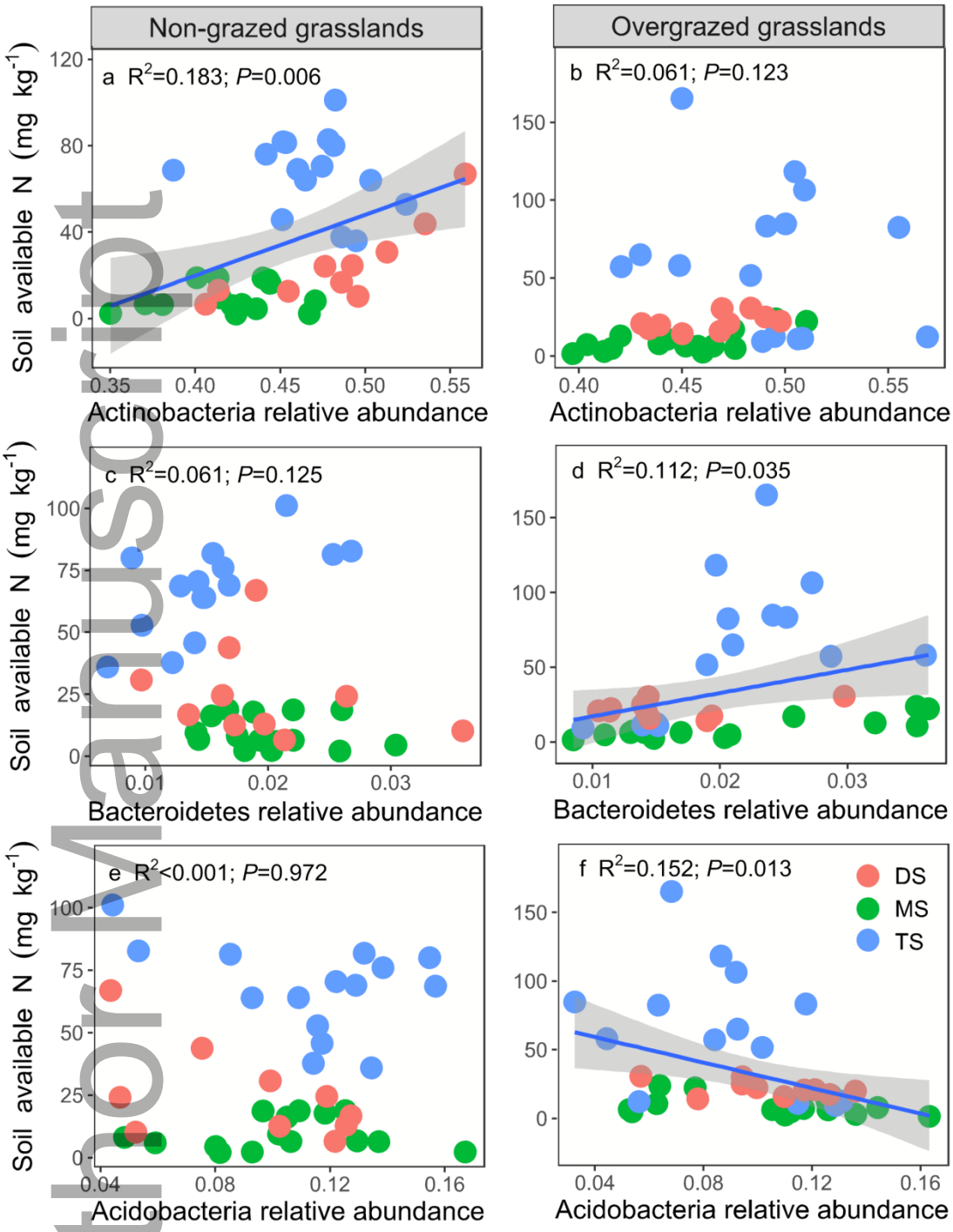


Figure 2. Relationships between the relative abundance of different microbial groups (Actinobacteria, Bacteroidetes, Acidobacteria) and soil available N (mg/kg) across non-grazed (a, c, e) and overgrazed grasslands (b, d, f). The fitted lines are from the OLS regression. DS-desert steppe; MS-meadow steppe; TS-typical steppe.

Figure 3

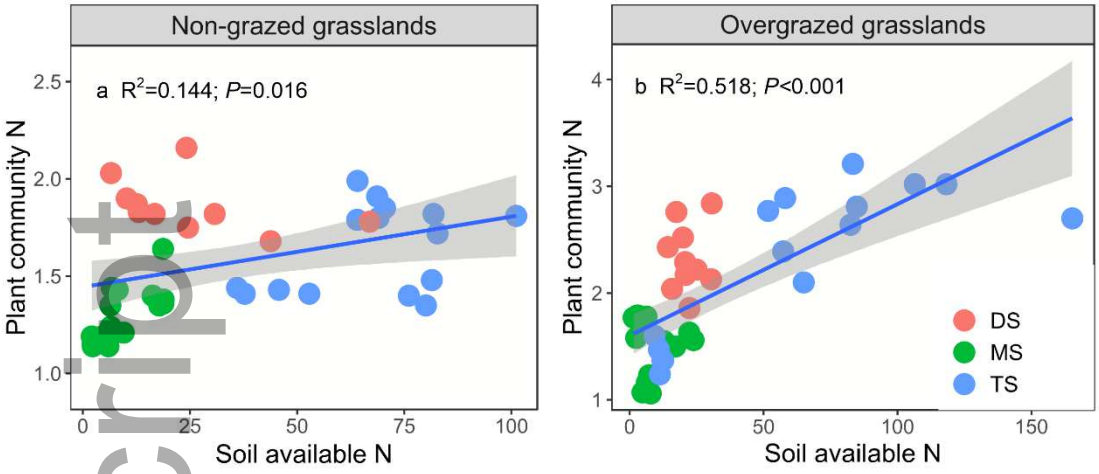
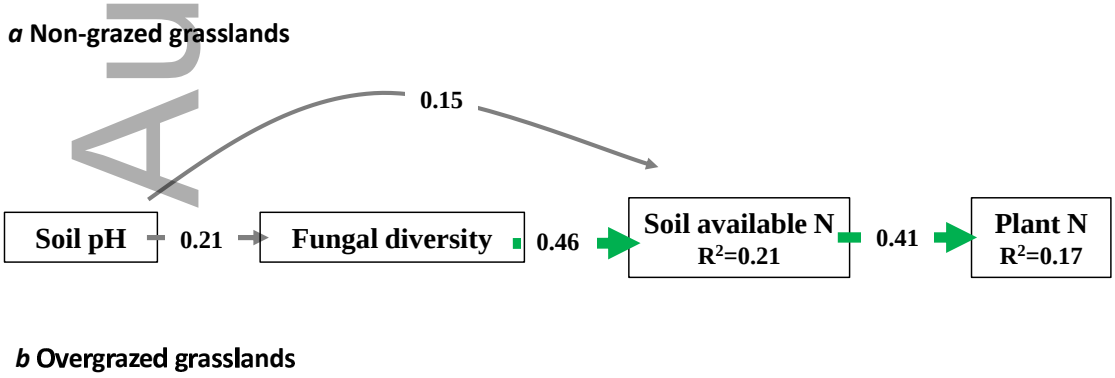


Figure 3. Relationship between soil available N (mg/kg) and plant community N content (%) across non-grazed and overgrazed grasslands. The fitted lines are from the OLS regression. DS-desert steppe; MS-meadow steppe; TS-typical steppe.

Figure 4



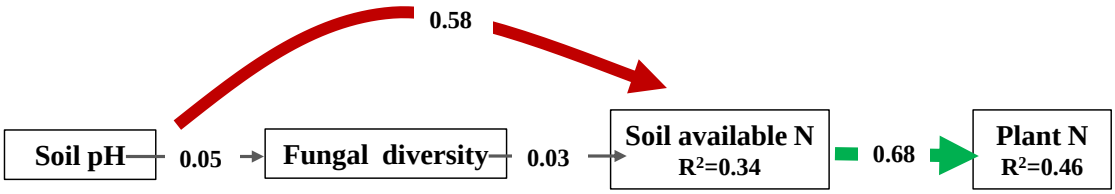


Figure 4. Structural equation models with soil pH and fungal diversity as predictors of soil available N and plant community N content for non-grazed (a) and overgrazed (b) grasslands. Green and red solid arrows indicate positive and negative effects, respectively, and grey arrows indicate nonsignificant paths. The thickness of the arrows reflects the magnitude of the standardized SEM coefficients. There was non-significant deviation of the data from the models (non-grazed: CFI=0.997; P=0.359; overgrazed: CFI=0.985; P = 0.278).

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Table S1. Characteristics of the geographic, climatic and plant variables at the study sites.

Table S2. Summary of linear mixed models analyzing the effects of fungal diversity, bacterial diversity, and the relative abundance of Actinobacteria, Bacteroidetes and Acidobacteria on soil available N in non-grazed grasslands and overgrazed grasslands.

Table S3. Summary of linear mixed effects model analyzing the overall effects of grazing on plant height, soil available N, fungal diversity, and bacterial diversity.

Figure S1. Distribution of sampling sites in northern China.

Figure S2. Vegetation contrast inside and outside exclosures in the three grassland types.

654 Figure S3. Difference in vegetation height inside (non-grazed) and outside
655 (overgrazed) exclosure at eight sites.
656 Figure S4. Difference in soil available N and microbial diversity inside (non-grazed)
657 and outside (overgrazed) exclosure at eight sites in three grassland types.