

Herbivore Assemblages Regulate Soil Multifunctionality through Microbial Diversity and Keystone Taxa in an Alpine Grassland

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Abstract

Grazing effects on soil multifunctionality (SMF) may depend on livestock assemblage. We tested yak-only grazing (YG), Tibetan sheep-only grazing (SG), yak–sheep mixed grazing (MG 1:2, 1:4 and 1:6), and grazing exclusion (NG) in an alpine grassland on the Qinghai–Tibetan Plateau. SMF was quantified using soil physicochemical indicators, and microbial communities were analyzed by sequencing, random forest models and network analysis. YG reduced SMF, whereas SG and MG 1:6 showed the highest SMF. Microbial richness, soil nutrients and fungal keystone taxa were closely linked to SMF enhancement.

Background

- Alpine grasslands are fragile ecosystems that support both ecological functions and livestock production.
- Grazing alters soil resources and microbial communities through feeding, trampling and excreta return.
- It remains unclear whether different livestock assemblages regulate SMF through belowground microbial mechanisms.

Research question:

Do yak, Tibetan sheep and yak–sheep mixed grazing differ in their effects on SMF and soil microbial communities?

Study Design & Methods

Study site:

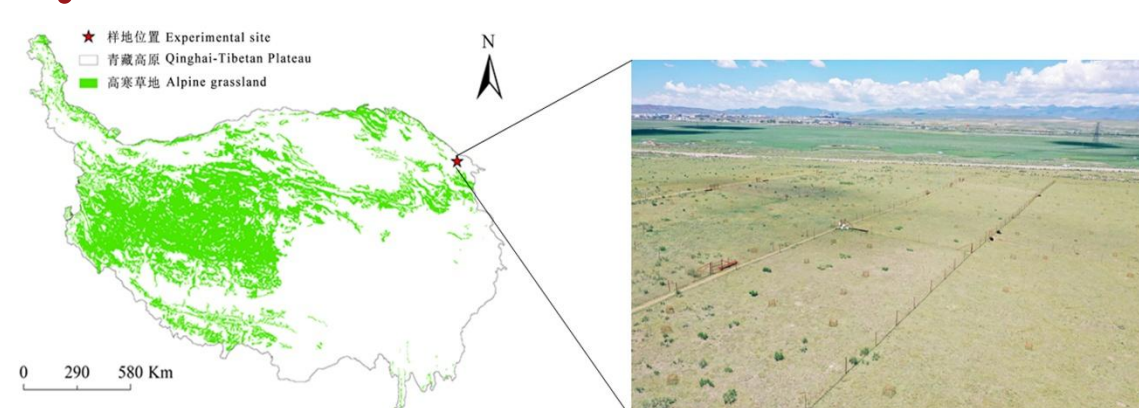


Fig. 1 Geographical location of sampling sites in this study.

Treatments:

yak-only grazing (YG), Tibetan sheep-only grazing (SG), yak–sheep mixed grazing (MG 1:2, 1:4 and 1:6), and grazing exclusion (NG).

Soil indicators:

Total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), available phosphorus (AP), available potassium (AK), soil moisture (SM), bulk density (BD) and pH.

Microbial analyses:

Bacterial and fungal communities were characterized by DNA extraction, PCR amplification and Illumina MiSeq sequencing. Microbial α -diversity was assessed using richness and Shannon index, while β -diversity and phylum-level composition were used to evaluate community shifts.

SMF calculation:

SMF was calculated as the average Z-score of standardized soil indicators. One-way ANOVA, linear regression, NMDS, random forest models, co-occurrence network analysis and Pearson correlation analysis were used to assess treatment effects, key soil drivers, and relationships between microbial keystone taxa and SMF.

Results

Herbivore assemblages changed SMF

SMF varied strongly among herbivore assemblages. YG reduced SMF relative to NG, whereas other grazing treatments increased SMF, with the highest values observed under SG and MG 1:6.

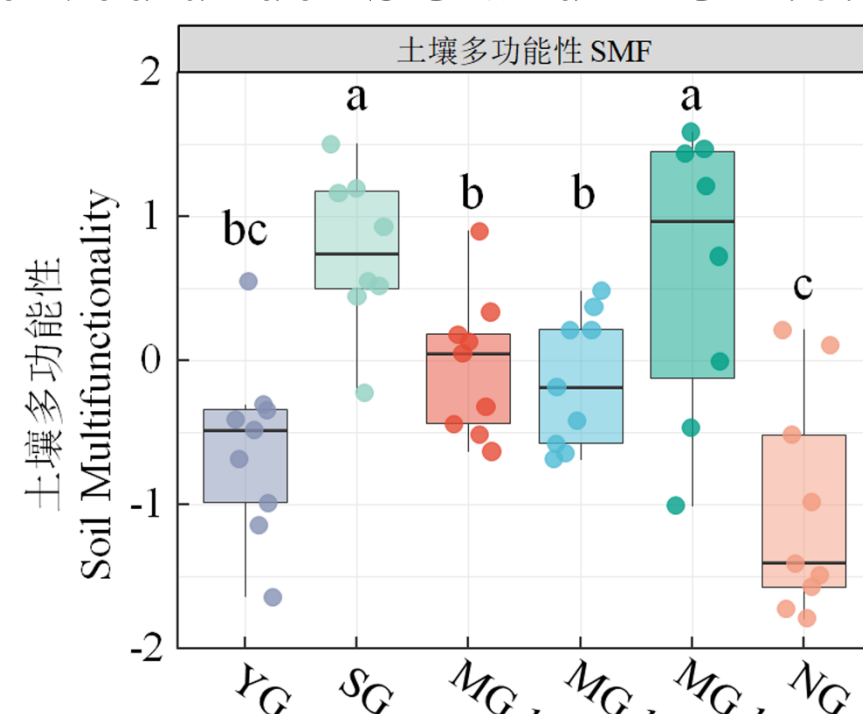


Fig. 2 The effect of different herbivore assemblages on soil multifunctionality. Different lowercase letters in the figure indicate significant differences between treatments for each indicator; $P < 0.05$.

Microbial richness was positively related to SMF

Microbial richness was closely associated with SMF. Bacterial richness was highest under MG 1:4 and MG 1:6, while fungal richness was relatively high under SG; both bacterial and fungal richness were positively correlated with SMF.

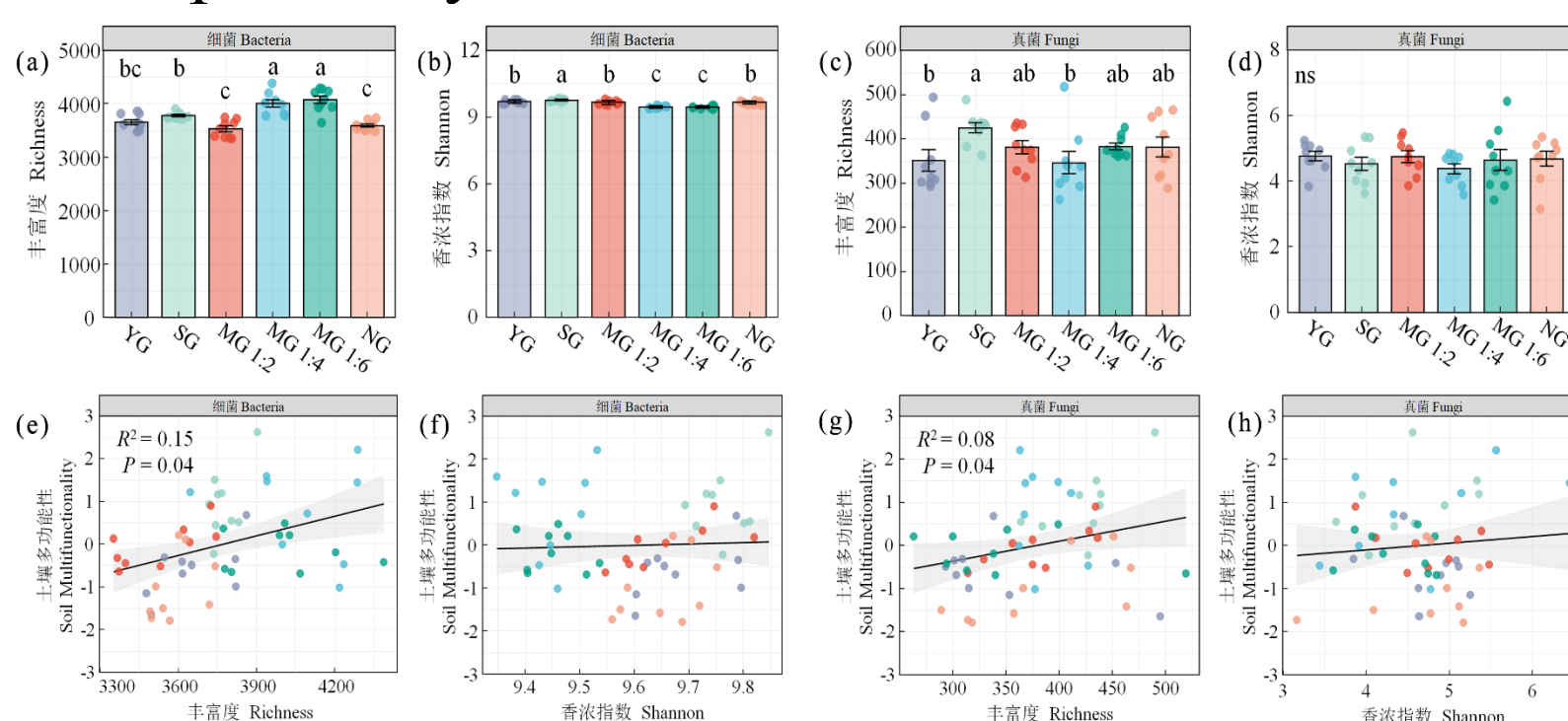


Fig. 3 Changes in bacterial and fungal richness and Shannon index under different herbivore assemblages and their relationship with soil multifunctionality.

Mixed grazing shifted microbial community composition

MG 1:4 and MG 1:6 clearly shifted bacterial and fungal community composition. These treatments reduced Acidobacteria and increased Mortierellomycota and Mucoromycota, suggesting improved soil nutrient conditions under mixed grazing.

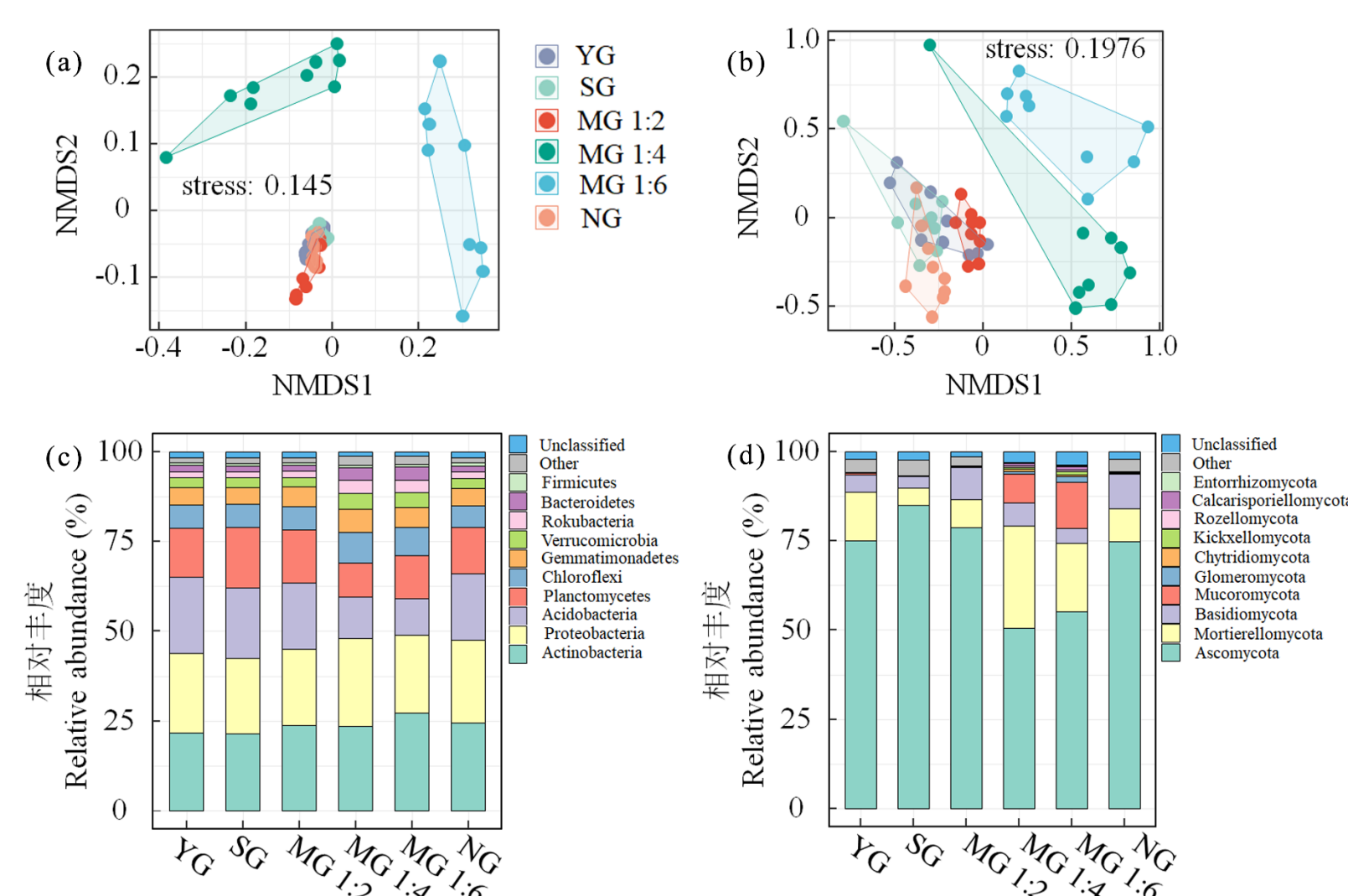


Fig. 4 Changes in β -diversity of bacteria and fungi under different herbivore assemblages (a, b) and changes in the relative abundance of dominant bacteria and fungi phyla (c, d)

Soil nutrients and physical properties drove SMF

Random forest analysis showed that TN, AN, AP, BD and SM were the main predictors of SMF. Bacterial communities were mainly associated with BD, AP, AN and SM, whereas fungal communities were mainly associated with TOC, AN, BD and AP.

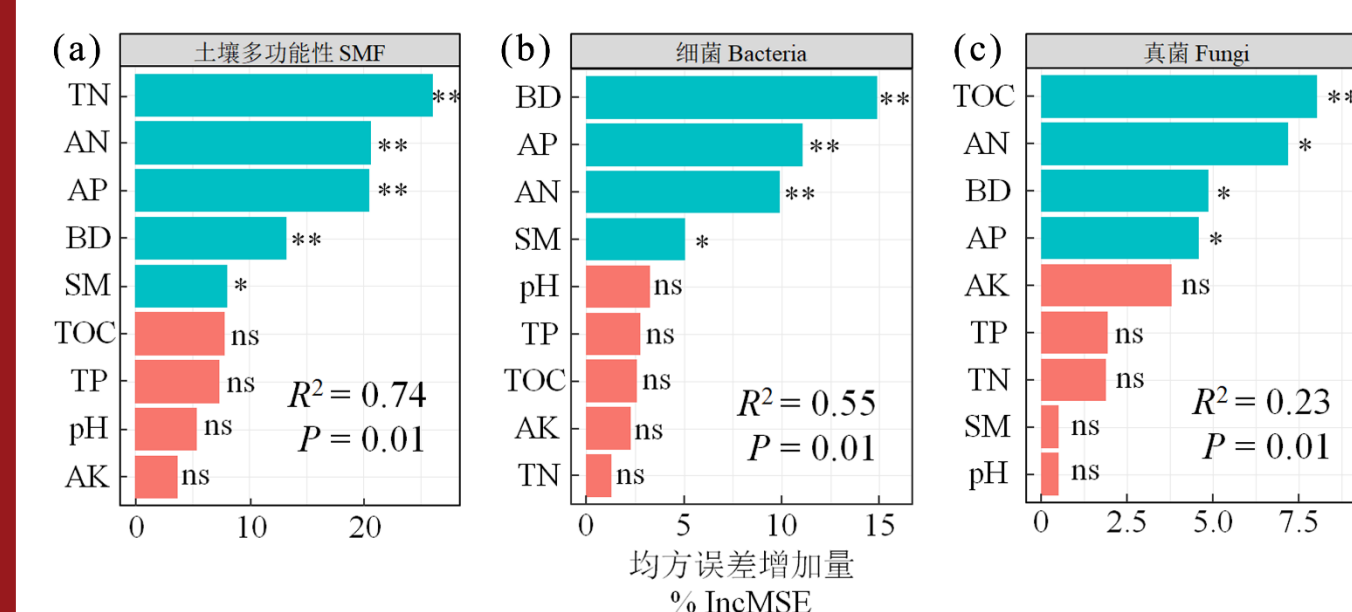


Fig. 5 Key physicochemical factors driving changes in soil multifunctionality (a) and bacterial (b) and fungal (c) communities under different herbivore assemblages; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $p < 0.001$

Keystone taxa showed contrasting relationships with SMF

Network analysis revealed contrasting microbial mechanisms. Bacterial module hubs were negatively related to SMF, whereas fungal keystone taxa were positively related to SMF, indicating a stronger positive role of fungi in maintaining soil functions.

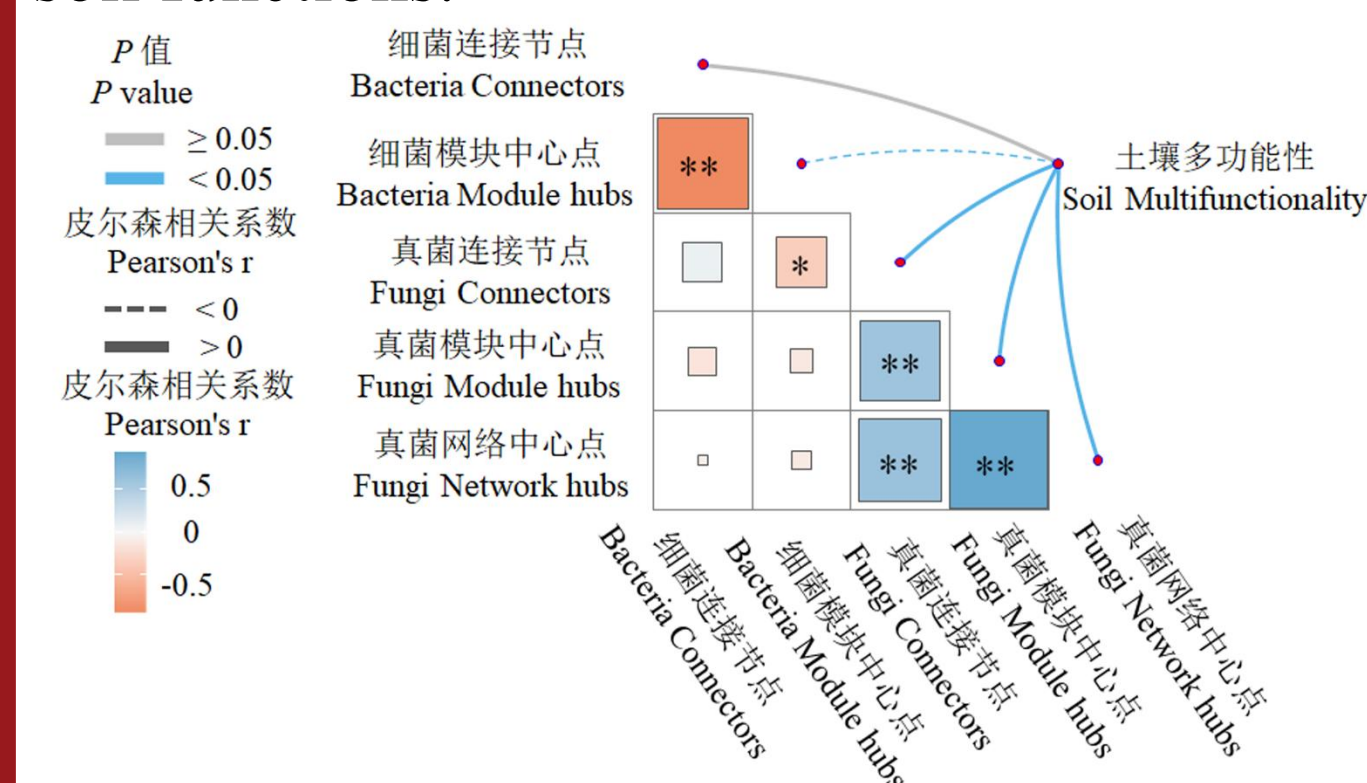


Fig. 6 Correlation between the relative abundance of keystone species of bacterial and fungal communities and soil multifunctionality under different herbivore assemblages

Conclusions

- Livestock assemblage composition strongly regulated SMF.
- YG reduced SMF, whereas SG and MG 1:6 enhanced SMF.
- Microbial richness, rather than Shannon diversity, was positively associated with SMF.
- TN, AN, AP, BD and SM were key drivers of SMF.
- Fungal keystone taxa were positive predictors of SMF.
- MG 1:6 may be a suitable grazing strategy for sustaining soil functions in alpine grasslands.