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Herbivore camping reshapes the taxonomy, functions and network of pasture soil microbial communities

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Despite the effects of herbivore camping on soil physicochemical properties have been studied, whether the effects change soil microbial communities remain unknown, especially below the surface. Our paired subsoil samples from half month-camping and non-camping showed for the first time that camping significantly changed the relative abundance of 21 bacterial phylotypes and five fungal phylotypes with significantly increases in the relative abundance of putative chitinase and Terpenes vanillin-decomposition genes, nitrite reduction function (nirB, nasA), decreases in the relative abundance of putative carbon fixation genes (ackA, PGK, and Pak), starch-decomposition gene (dexB), gene coding nitrogenase (anfG), and tetracycline resistance gene (tetB) for bacterial communities, as well as significantly decreases in the relative abundance of animal endosymbiont and increases in the relative abundance of litter saprotroph and endophyte for fungal communities. However, camping did not change taxonomic and functional diversities. Niche restriction was the main driving force of bacterial and fungal community assembly. Compared with the no camping, camping increased the stability of bacterial networks but decreased the stability of fungal networks. Camping exerted a positive effect on the network through compressing the niche width, and reduced the change in the network through reducing the niche overlap. This study provides a first insight into the effect of animal camping on soil microbial communities for grassland.

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Abstract

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whether the effects change soil microbial communities remain unknown, especially below the surface. Our paired subsoil samples from half month-camping and non-camping showed for the first time that camping significantly changed the relative abundance of 21 bacterial phylotypes and five fungal phylotypes with significantly increases in the relative abundance of putative chitinase and Terpenes vanillin-decomposition genes, nitrite reduction function (nirB, nasA), decreases in the relative abundance of putative carbon fixation genes (ackA, PGK, and Pak), starch-decomposition gene (dexB), gene coding nitrogenase (anfG), and tetracycline resistance gene (tetB) for bacterial communities, as well as significantly decreases in the relative abundance of animal endosymbiont and increases in the relative abundance of litter saprotroph and endophyte for fungal communities. However, camping did not change taxonomic and functional diversities. Niche restriction was the main driving force of bacterial and fungal community assembly. Compared with the no camping, camping increased the stability of bacterial networks but decreased the stability of fungal networks. Camping exerted a positive effect on the network through compressing the niche width, and reduced the change in the network through reducing the niche overlap. This study provides a first insight into the effect of animal camping on soil microbial communities for grassland.

Keywords: fungi; bacteria; grassland; network robustness; niche breadth; niche overlap

Introduction

Camping may involve many fields, such as military, ecotourism recreation, wildlife habitat behavior and grassland management. In the field of grassland management, herbivore camping can be defined as important parts of the technology of improving and restoring degraded grassland by herbivores

night penning that removes the undesired vegetation, and changes the nutrient content and structure of soils through herbivore excretion and trampling during night penning, as well as subsequent sowing (Jiang et al. 1996; Jiang et al. 1999; Yuan et al. 2012; Zhang et al. 2001). It has been proved to be a successful comprehensive technology (Zhang et al. 2001) that can improve natural grassland and rebuild artificial grassland at a very low cost (Yuan et al. 2012; Zhang 2002). The camping has been applied in grassland and pasture management in New Zealand, North Korea, and Inner Mongolia, Guizhou, and Yunnan of China.

The previous in-depth explorations have advanced this field. Camping can change vegetation and soil attributes (Jeffrey & David 1996; Zhang et al. 2001). On the one hand, at the community level, camping is reported to having able to clean weed and shrub, change vegetation composition, increase forage yield (Yuan et al. 2012). For instance, camping (grazing and trampling) can remove almost all of the aboveground original plants, reduce the number of live shrubs, and fast the growth of reseeding grasses (Zhang et al. 2001). Camping significantly increases the coverage, proportion, and yield (Zhang 2002) of grass, but significantly reduces the coverage and proportion of weeds (Yuan et al. 2012) and the sprouted branches number of shrub *Salix inamoena* (Zhang 2002). Furthermore, at the physiological level of plants, sheep manure and urine increase the concentration of the soil solution, which affect the water potential of plants; sheep feces and urine significantly change the cell membrane permeability of leaf and root, however, the feces and urine alone does not work (Zhang et al. 1999a). In terms of plant nutrient content, camping significantly increases the crude protein content in the leaves of shrub *Salix inamoena* (Zhang 2002). Besides, camping impact is found to be varied greatly with topographic positions, trampling intensities and vegetation types (Jeffrey & David 1996).

On the other hand, camping is documented to able to fertilizing grassland (Yuan et al. 2012; Zhang et al. 2001). For example, soil fertility increases with the increase of camping intensity. ~~Camping improves the soil organic matter concentration, the nitrogen (N), phosphorus (P) and potassium availability and the soil pH by 41-63%, 14-33%, 143-460%, 67-330%, and 0.7-1.2 units, respectively (Jiang et al. 1996).~~ Camping increases the concentrations of soil ammonium N and nitrite N which were high enough that potentially toxic to plant roots (Zhang et al. 2001). However, whether and how camping affects the structure, function and the potential interaction of soil microbial communities have not been explored.

~~Predecessors had used the idea of reductionism to decompose the camping into browsing (grazing), trampling and excretion of faeces and urine (Jiang et al. 1999; Zhang 2002; Zhang et al. 1999a; Zhang et al. 1999b).~~ Browsing and trampling completely removes grassy plants (Zhang 2002), whereas some shrub *Salix inamoena* still survives (Jiang et al. 1999). Trampling increases the compactness of 0-20 cm soil and the bulk density of 10-20 cm soil, however, first increases and then decreases the bulk density of 0-10 cm soil (Jiang et al. 1999). Additionally, trampling damages herbage, buries seeds, promotes germination, reduces standing plant litter, increases soil compaction and bulk density, reduces soil porosity, water stable aggregates, water permeability and air permeability, resulting in rain logging and anoxia (Hou et al. 2004). Although treading increases soil total N and P concentrations, and plant N and P contents, but decreases plant C concentrations, C:N and C:P ratios and exacerbates P limitation (Li et al. 2021b). Treading increases the abundance of soil total bacteria, fungi, and arbuscular mycorrhizal fungi (Liu et al. 2015). Inputs of excrement and urine are a crucial pathway by which herbivores change the elements and stoichiometry of plants and soil (Li et al.

2021a), and fertilizing grassland (Yu et al. 2008). Although urine input has a short-term scorch impact on vegetations (Yu et al. 2008), urine increases the root biomass, herbage N, root N, total plant N by 3.03-27.82%, 22.92-88.99%, 21.09-35.51% and 30.90-33.19%, respectively (Williams et al. 1999), and could enhance grass tillering, and elevate the height and weight of grass tillers, which improving in grass biomass (Yu et al. 2008). Moreover, urine increases pH, microbial activity, microbial biomass (Rooney et al. 2006), NH_4^+, and microbial biomass N by 0.1-1.03 units, 63.27%, 7.31%, 535.90-569.05%, and 122.83-185.95% (Williams et al. 1999), respectively. In addition, urine increases the total P, molybdate reactive P, organic P and condensed P contents of soil solution by 470.19%, 431.25%,731.82% and 392.42%, respectively (Williams et al. 1999), and substantially changes bacterial community structure (Rooney et al. 2006), significantly increases ammonia-oxidizing bacteria (AOB) species richness by 18.97-350% (Rooney & Clipson 2008). The studies of dung addition are more in-depth than those of urine addition. Manure input improves nutrient availability, aggregate stability, and enhances soil fertility and yield (Asmita et al. 2021; Shi et al. 2021). Excrement results in more soil (total, microbial and organic) C, N and P accumulations (Arthur & Bruno 2021; Li et al. 2021a; Maillard & Angers 2014), AN, AP, AK, OM, and TK contents (Liu et al. 2021b). For example, excrement increases microbial carbon (C), nitrogen (N), organic C, total N by 88%, 84%, 27%, and 33%, respectively (Liu et al. 2020a). Manure increases the activities of sucrase(Liu et al. 2021b), β-1,4-glucosidase, dehydrogenase, acid and alkaline phosphatase, N-acetyl-β-D-glucosaminidase, urease and sulfatase by 124.1%, 147%, 114%, 39%, 112%, 58%, 104% and 228%, respectively (Liu et al. 2020a), and increases soil bacterial diversity (Liu et al. 2021b), but declines fungal diversity (Guo et al. 2022). Besides, sheep faeces and urine increases soil Fe content and plant Mg, Mn contents,

~~decreases soil Zn content and plant Ca, Fe and Zn contents (Zhang et al. 1999b). However, the influences of urine or faeces input on soil and plant element contents and stoichiometry distinguish from that of co-inputs (Li et al. 2021a).~~ Despite the studies provides credible supports to salubrious effects of excrements on soil and plant (Rayne & Aula 2020), since the microbial community composition in manure to be different and less diverse than in soil (Andrea et al. 2021), animal faeces input into soils results in the introduction of dung-derived microbes (Macedo et al. 2021), and the propagation and proliferation of antibiotic resistance genes in soil, likely constituting serious threat to ecosystem and human health (Zhang et al. 2021), we still need to understand the effects of camping on soil microorganisms and antibiotic resistance genes. Although alone or mix effect of browsing (grazing), trampling and excretion of faeces and urine on soil have been well studied, since complex interactions exist in these effects (Li et al. 2021a), making it difficult to extrapolate to the overall effect of camping, remains unknown about how camping alter soil microbial communities.

Therefore, the aims of this study are to deciphering whether the herbivore camping changes the taxonomic, functional and network patterns of soil microbial communities. Specifically, this study answers for the first time the following questions:(1) whether the camping enhances microbial diversity? (2) What microorganisms are enriched and inhibited by the camping and what are their functional implications? (3) whether and how camping can improve microbial network interaction? This study provides novel insight into the effects of camping on pasture ecosystems that experience herbivore camping, a common to grasslands worldwide, and supplies update knowledges for understanding this technology.

Material & Methods

Sampling sites, design and soil sampling

Sampling sites were located in Weining county (26°52'N, 104°17'E; ca. 2440 m above sea level), Guizhou province, SW China (Zhang et al. 2001). It experiences a subtropical and warm temperate monsoon climate with mean annual rainfall of 926 mm and temperature of 11.6 °C, a frost-free period of 180 d, and average sunshine hours of 1800 (Wu et al. 2020). The yellow brunisolic soil covers this area (Zhang et al. 2001). For surface soil (0-10 cm), the organic carbon content was 61.05 g/kg, total nitrogen content 3.88 g/kg, total phosphorus content 1.01 g/kg, alkali hydrolyzed nitrogen content 354.04 mg/kg, and available phosphorus content 6.43 mg/kg; for subsurface soil (10-20 cm), the organic carbon content 41.30 g/kg, total nitrogen content was 2.78 g/kg, total phosphorus content 0.82 g/kg, alkali hydrolyzed nitrogen content 259.05 mg/kg, and available phosphorus content 3.50 mg/kg. Wumeng semi-fine wool sheep accounts for 62% of sheep in stock of Guizhou (Wu & Shen 2020). Wumeng semi-fine wool sheep is of economic significance in the Wumeng mountain area (Wu et al. 2020) of Guizhou (Wu & Shen 2020). The sheep camping (ca. half sheep/night/m^2) was carried out spontaneously for 15 nights (Jiang et al. 1996) by local herdsmen in October 2018. Sheep camping is a spontaneous grassland management of local herdsmen. The herdsman allowed us to sampling without any official and document permission, and we did not participate in the implementation of the camping. Therefore, this study does not involve animal related ethic matters. At the16th day, we only took subsurface soil (10-20 cm) samples from three paired sites ((camping sites and corresponding no camping sites) × 3) where the topographies, plants, and soil type are nearly identical. Three samples were collected randomly at each site and mix them into a composite sample. This sampling method reduced to the maximum extent the impacts of non-design source variation (e.g., background

148 environmental variation).

149 **DNA extraction, Sequencing of amplicons, Processing of sequence data, and Bioinformatics**
150 **analysis**

151 Soil DNA was isolated by a PowerSoil DNA Isolation Kit, and its quality and quantity were checked by
152 electrophoresis ~~(Ding et al. 2020)~~. The PCR amplification was performed using Applied Biosystems
153 Gene Amp PCR System 9700. The V4 region of the bacterial 16S rRNA was PCR-amplified using the
154 primers 806R and 515F and the fungal ITS2 region was amplified using the primers ITS3_KYO2 and
155 ITS4. The amplified products were extracted using Qubit 2.0 (ThermoFisher). Illumina sequencing was
156 carried out using an Illumina Hiseq platform with PE250 mode (Illumina, Inc.). The original sequencing
157 data are spliced and filtered to obtain high-quality target sequences. Usearch
158 (http://drive5.com/uparse/) was performed to obtain OTU matrixes at a cutoff of 97%. Taxonomy was
159 assigned using Uclust (https://drive5.com/usearch/manual/uclust_algo) based on the Silva database
160 (https://www.arb-silva.de/) for the bacterial community or the Unite database (https://unite.ut.ee/) for
161 the fungal community ~~(Ding et al. 2020)~~. Bacterial communities were rarefied to 10432 sequences and
162 fungal communities were rarefied to 9236 sequences for each sample. To understand the effects of
163 camping on soil microbial biodiversity, Goods Coverage, Observed OTUs, Chao1, ACE, InvSimpson
164 (Inverse Simpson), Fisher's diversity and Faith's Phylogenetic (PD) diversity were calculated. The
165 methods used to retrieve the functional profiles and functional diversity of bacterial and fungal
166 communities follow the methods in our previous study (Ding & Wang 2021) with minor modifications.
167 Briefly, soil bacterial functional profile were predicted using "Tax4Fun" package
168 (http://tax4fun.gobics.de/Tax4Fun/Tax4Fun_0.3.1.zip) in R version 3.0 (https://cloud.r-project.org/),

and FAPROTAX version 1.2.4 (https://pages.uoregon.edu/slouca/LoucaLab/archive/FAPROTAX/SECTION_Download/MODULE_Downloads/CLASS_Latestrelease/UNIT_FAPROTAX_1.2.4/FAPROTAX_1.2.4.zip) in Pyhton version 3.7.4 (https://www.python.org/downloads/release/python-374/). Fungal functional profiles were predicted using FUNGuild (http://www.funguild.org/) in Pyhton version 3.7.4. richness, Shannon, Pielou, InvSimpson of functions were calculated using "vegan" package (https://cloud.r-project.org/) in R version 3.6.

Statistical analysis

Shapiro Wilk test and Levene test were applied to determine the normality and homoscedasticity of data, respectively (Ding & Wang 2021). The ANOVA and t test were used to test the difference between the camping and no camping groups when data could meet the requirements of normality and homoscedasticity. The Kruskal-Wallis test and Wilcoxon test were performed when the data could not meet (Ding et al. 2020).

To figure out the important phylotypes with significant differences between groups, the random forest analysis ("randomForest" package) and Kruskal-Wallis test ("stats" package) were used in R.

Network analysis is a robust and effective method to quantify the microbial interaction (Kong et al. 2019; Zhou et al. 2020). To minimize the impact of data sparsity on the network and strengthen the reliability of the network, only bacterial or fungal OTUs appearing in >= two samples (Banerjee et al. 2019) and had a total relative abundance >= 0.1% in all samples were included in the network analysis (Zhang et al. 2018), resulting in 881 bacterial and 183 fungal OTUs. Microbial interaction networks were constructed using the "WGCNA" and "igraph" packages. The Pearson correlation coefficient was

calculated for each pair of OTUs, Benjamini and Hochberg corrected p value was reported (Fan et al. 2020). Random matrix theory was used to determine the optimal threshold of correlations in constructing microbial interaction networks in both our and previous studies (Zhou et al. 2020). A threshold of strong Pearson's $r > 0.8$ and $p < 0.001$ was set based on the three facts: (a) the microbial phylotypes that shows strong correlations with each other are more probably to interact with each other (Fan et al. 2020); (b) the optimal threshold from random matrix theory and priori knowledges (Fan et al. 2020; Yuan et al. 2021) is about 0.8; (c) network properties were needed to be compared under the same conditions (Zhou et al. 2020). Two sided Kolmogorov-Smirnov test were used by performing "ks.test" function to discriminate the cumulative distribution of 10000 bootstrapping node properties of the camping and no camping networks where the null hypothesis is that the properties under the camping and no camping have same distribution patterns (Banerjee et al. 2019), and Kolmogorov-Smirnov test and Kruskal-Wallis test were employed to determine the difference in robustness (average degree and natural connectivity) between the camping and no camping networks after 50% nodes were stochastically removed (Banerjee et al. 2019; Yuan et al. 2021). Furthermore, network robustness defined as the declines in microbial average degree (and natural connectivity) with the increasing proportion of removing nodes (and edges) were tested (Pan et al. 2021; Shi et al. 2021). The linear regression model was used to describe these relationships. The linear regression model was also used to test the effect of niche width and niche overlap on the network property (degree and difference between degrees (Δdegree)). Bayesian structural equation model (Bürkner 2017) was implemented to examine potential pathways that can account for how camping alter the network property. A pathway was acceptable if the 95%CI (confidence interval) of the coefficient of the pathway

211 does not contain 0 (Bürkner 2017).

212 ## Results

213 **Diversities and composition of soil microbial communities under camping and no camping**

214 ~~> 96% of coverage in all samples suggested that the good sampling and sequencing (Ding & Wang~~

215 ~~2021),~~The normality and homoscedasticity tests showed that ~~Observed~~ OTUs, Chao1, ACE,

216 InvSimpson, Fisher, PD of soil microbial communities followed the premises of normality and

217 homogeneity (Shapiro Wilk's test and Levene's test, p = 0.0610 – 0.9661, Table S1). As expected, the

218 ANOVA and T test showed that all diversity indexes in the camping group were not significantly distinct

219 from the no camping group (p = 0.045 – 0.901, Table S2). Unexpectedly, the PERMANOVA test with

220 9999 permutations showed that there were no evidently difference in soil bacterial or fungal community

221 composition between groups overall community level based on Bray-Curtis (R^2=0.224, p = 0.20 for

222 bacteria; R^2=0.192, p = 0.50 for fungus), jaccard (R^2=0.220, p = 0.20 for bacteria; R^2=0.225, p = 0.30

223 for fungus), unweighted unifrac (R^2=0.214, p = 0.40 for bacteria; R^2=0.204, p = 0.50 for fungus) and

224 weighted unifrac (R^2=0.169, p = 0.60 for bacteria; R^2=0.222, p = 0.50 for fungus) distances (Figure

225 S1). However, Venn plots showed that 385 (1.52% of total sequence number) and 390 (1.46% of total

226 sequence number) bacterial OTUs and 259 (2.96% of total sequence number) and 192 (1.13% of total

227 sequence number) fungal OTUs were unique to the camping and the no camping, respectively (Figure

228 S2). Furthermore, Random Forest analysis found that these two groups could be clearly predicted and

229 significantly separated by 21 bacterial phylotypes and five fungal phylotypes (Kruskal-Wallis test, p =

230 0.0369 – 0.0495, Figure 1). The camping significantly increased the abundance of *Chelatococcus*,

231 *Luteimonas*, *Dyadobacter*, *Ruminococcaceae UCG-005*, *Aquamicrobium*, *Cohnella*, *Limnothrix*,

232 *Pseudanabaena PCC-7429*, *Family XIII AD3011 group*, *Christensenellaceae R-7 group*,
233 *Prevotellaceae UCG-004*, *Olivibacter*, *OLB13*, and *Rummeliibacillus*, whereas significantly reduced
234 the abundance of *Ruminococcus 1*, *LD29*, *Rudaea*, *RB41*, *FukuN18 freshwater group*, *Cellvibrio*, and
235 *Clostridium sensu stricto 18* in bacterial communities. Besides, the camping significantly increased the
236 abundance of *Podospora*, *Rhexocercosporidium*, *Symbiotaphrina*, however, significantly decreased
237 the abundance of *Rhizomucor* and *Candida* in fungal communities (Kruskal-Wallis test p = 0.0369 –
238 0.0495), compared with the no camping.
239
240 **Functional profiles and diversities of soil microbial communities under camping and no**
241 **camping**
242 Contrary to the intuitive, as much as 83 function group levels related to C (Figure S3-16, and 57-
243 59), N (Figure S18-31, and 60-65), P (Figure S66-70), hydrogen (Figure S32), sulfur (Figure
244 S33-37), iron (Figure S38), manganese (Figure S39) cycles, plastic degradation (Figure S17),
245 fermentation (Figure S40), plant pathogens (Figure S41), animal parasites or symbionts (Figure
246 S42), predatory and/or parasites (Figure S43-45), mammal gut bacteria (Figure S46), bacterial
247 nutrition types (Figure S47-55), and antibiotic resistance genes (Figure S71-82) were tested
248 using Wilcoxon rank sum test. These tests suggested that no evident differences were detected in
249 diversity indexes (richness, Shannon, Pielou, InvSimpson) of these bacterial functions between
250 camping and no camping (Figure S3-82). Besides, no significant differences were detected in
251 diversity indexes (richness, Shannon, Pielou, InvSimpson) of growth form, guild, trait, and trophic
252 mode of fungus (Figure S83-86). However, bacterial functional analysis showed that compared

253 with the no camping soils, the camping significantly increased the relative abundance of dark
254 thiosulfate oxidation, and cellulolysis, but significantly decreased the relative abundance of
255 xylanolysis (Welch's t test with two sides p = 0.032 – 0.048, Figure 2). The camping significantly
256 increased the relative abundance of C decomposition genes (chitinase and Terpenes vanillin), nitrite
257 reduction function (nirB, nasA), significantly decreased the relative abundance of C fixation genes
258 (ackA, PGK, Pak), C decomposition gene (dexB), gene coding nitrogenase (anfG), and tetracycline
259 resistance gene (tetB). (Welch's t test with two sides p = 8.95e-4 – 0.046, Figure 2). The camping
260 significantly decreased the relative abundance of animal parasites or symbionts (Welch's t test with
261 two sides p = 0.032). The fungal functional analysis showed that the camping significantly decreased
262 the relative abundance of animal endosymbiont (Welch's t test with two sides p = 0.030).
263 Additionally, the camping significantly increased the relative abundance of litter saprotroph and
264 endophyte (Welch's t test with two sides p = 0.013, 0.018, Figure 2).

265 **Soil microbial networks under camping and no camping**

266 In order to determine the effects of camping on microbiome associations, bacterial and fungal
267 networks under the camping and no camping treatments were established. Results revealed distinct
268 association patterns (Figure 3). Compared to the no camping soils, although the camping decreased
269 the number of nodes (by 3%, 19%), number of clusters (9%, 10%), centralization degree (4%, 9%),
270 negative edges proportion (4%, 39%) of bacterial and fungal networks, elevated the positive edges
271 proportion (3%, 27%) and modularity (0.1%, 37%) of bacterial and fungal networks. The camping
272 decreased central eigen (by 1%) and vulnerability (11%) of bacterial network, however, increased
273 those (15%, 44%) of fungal network. The camping increased number of edges (by 5%), connectance

(11%), average degree (8%), number of positive edges (8%), number of negative edges (1%) and natural connectivity (0.01%) of bacterial network, whereas, increased those (49%, 21%, 36%, 35%, 69%, 38%) of the fungal network (Table S3). Kolmogorov–Smirnov test indicated that node degree, closeness, transitivity, and eigenvector centrality under the camping were statistically distinct from those under no camping (p = 2.2e-16 - 4.62e-8, Table 1). We assessed the difference in network stability (average degree and network connectivity) between the camping and no camping treatments by network bootstrapping after 50% nodes were randomly removed. Kolmogorov–Smirnov test indicated that the camping significantly changed the network stability (average degree and network connectivity, p = 2.2e-16, 2.2e-16), and Kruskal test revealed that average degree and network connectivity of bacterial network were 1.9 – 3.9-fold those of fungal network, regardless of the camping and no camping (Table 2). Interestingly, the camping significantly increased the average degree and network connectivity of the bacterial network by 5% and 1% (p = 2.2e-16, 2.2e-16), respectively. Nevertheless, the camping significantly decreased those of the fungal network by 50% and 40% (p = 2.2e-16, 2.2e-16), respectively. Furthermore, we performed robustness analysis of networks based on removing a proportion of nodes and edges. Results showed that the average degree and network connectivity of bacterial network were higher than those of fungal network, regardless of the camping and no camping. Compared with the no camping soils, the camping increased the average degree and network connectivity of bacterial network, however, decreased those of fungal network, irrespective of the remove of nodes and edges (Figure 4).

Niche breadth, niche overlap, Specialist/Generalist species and assembly mechanism of microbial communities under camping and no camping

295 Wilcoxon rank sum test revealed that the bacterial niche breadth was 1.2 – 1.3-fold those of fungi (p
296 = 2.22e−16 – 1.3e−16), regardless of the camping and no camping. Compared with the no camping
297 soils, the camping decreased the niche breadth index of bacteria and fungi by 3% (p = 0.054) and
298 14% (p = 1.9e−05), respectively (Figure 5a). Wilcoxon rank sum test revealed that fungi niche
299 overlap index was 1.2 – 1.4-fold that of bacteria (p < 2.22e−16), regardless of the camping and no
300 camping. Compared with the no camping soils, the camping increased the niche overlap of bacteria
301 and fungi by 2% (p < 2.22e−16) and 13% (p < 2.22e−16), respectively (Figure 5b). T test
302 suggested that the percentage of bacterial generalist was 4.1 – 4.7-fold that of fungi (p = 0.0022,
303 0.0016), the percentage of fungal specialist was 5.8-fold those of bacteria (p = 0.015, 0.0023),
304 regardless of the camping and no camping (Figure 5c). Compared with the no camping, the
305 camping increased percentage of bacterial generalist and decreased percentage of bacterial
306 specialist by 3% (T test, p = 0.583) and 1% (T test, p = 0.953), respectively. Unexpectedly, the
307 camping decreased the percentage of fungal generalist and percentage of fungal specialist by 10%
308 (T test, p = 0.719) and 1% (T test, p = 0.953), respectively.
309 Understanding how the manage and evolution shape community assembly mainly involves two
310 opposing views: niche and dispersal hypothesis. In order to identify the first-order drivers that drive
311 community assembly, dispersal–niche continuum index (DNCI) was used to quantify the relative
312 importance of niche or dispersal process (Vilmi et al. 2020). Result showed that the E values from
313 niche-controlled model and niche - and dispersed controlled model were lower than that from
314 dispersal-controlled model (Figure 5d-e), indicating that niche restriction was the main driving force
315 of bacterial and fungal community assembly.

316 # Discussion

317 **Herbivore camping changes the structure of subsoil microbial communities with functional**
318 **implications**

319 Few studies found, in the pasture, herbivore camping affects soil physical, and chemical properties
320 (Jiang et al. 1999; Niu et al. 2009; Zhang et al. 1999b; Zhang et al. 2001). Herbivore returns 60-95%
321 of intake nutrients to grassland in the form of urine and feces (Niu et al. 2009). Camping herbivore
322 increases soil compactness by treading and lying and increased soil temperature by lying. In
323 addition, camping also destroys weeds. As far as we know, none researches have ever been
324 reported subsoil microbial communities involved in herbivore camping. Our study firstly
325 demonstrated that the camping significantly enriched and depleted the bacterial and fungal
326 phylotypes, which might result in microbial community function fluctuations, and has important
327 implications for soil carbon and nutrient cycling, and soil and plant health.
328 For increased bacterial genera, the genus *Chelatococcus* is denitrifier with the nirK gene, degrades
329 PAHs, nitrilotriacetate, acesulfame, aminopolycarboxylic acids, and crude-oil (~~Table S5~~), this genus's
330 enrichment in camping could contribute to the increase in the relative abundance of dark thiosulfate
331 oxidation (~~Figure 2~~) since it could employ desulfurization (~~Table S5~~). *Luteimonas* is found in sewage
332 sludges, crude oil, waste compost, rumen, and manure, has a key role during the composting
333 humification, anaerobic digested residue, manure, and the wood composting process. This genus can
334 degrade various carbohydrates such as starch, cellulose, chitin, lignocellulose, aromatic hydrocarbon,
335 has catalytic activities involving in oxidase, catalase, alkaline phosphatase, esterase, and esterase
336 lipase, and organic matter degradation (~~Table S5~~). In addition, *Luteimonas* is aerobic and nitrate

337 reducing bacteria (Mekdimu et al. 2021), has ability in denitrifying (David et al. 2018) by reducing nitrite
338 only to nitrous oxide (N_2O) (Zhang et al. 2017c). It promotes early plant growth and development,
339 increases the acquisition of nitrogen (N) and phosphorous (P) by plants (Claire 2019). *Luteimonas*
340 shows a slight inhibition of pathogen growth, as part of healthy microbiome of healthy plants (Kristina
341 et al. 2020). *Luteimonas* carries some antibiotic resistance genes (Gou et al. 2021). The dissipation
342 of *Luteimonas* in the composts contributed greatly to the reduction in relative abundance of antibiotic
343 resistance gene (Liu et al. 2020b; Wang et al. 2021). It also degrades antibiotic sulfamethoxazole
344 (~~Table S5~~). *Luteimonas* is also considered as an useful indicator for soil amelioration (Guo et al. 2017).
345 *Dyadobacter* utilises organic residue, cellulolytic (~~Table S5~~), is involved in partial nitrification (Zhang
346 et al. 2019) and disease suppression (Fu et al. 2017), and can increases soil Nitrate N, ammoniacal
347 N, and plant growth (Saurabh et al. 2018). *Ruminococcaceae UCG-005* can be found in rumen,
348 intestinal microbiome, faeces of ruminant, and cattle houses; It can digest fiber, and produce short
349 chain fatty acids by fermenting dietary polysaccharides (~~Table S5~~). *Aquamicrobium* genus is found in
350 the activated sludge, sewage, wastewater, and soil (~~Table S5~~). They are ammonia oxidizing bacteria
351 (Su et al. 2021), can largely contribute to the nitrogen removal (Sun et al. 2019), and are able to
352 degrade recalcitrant and complex organic pollutants such as heterocyclic compounds and aromatic
353 compounds (~~Table S5~~). It can also increase the biomass (Qu et al. 2015). *Cohnella* can degrade xylan,
354 cellulose, carboxymethyl cellulose, chitin, sawdust, and litter (~~Table S5~~), and can fix nitrogen (Xiao et
355 al. 2019). *Limnothrix* can metabolise a wide range of organic substrates such as amino acids,
356 carbohydrates, and carboxylic acids (~~Table S5~~). The *Pseudanabaena PCC-7429* genus is small
357 filamentous cyanobacterium, which is often an epiphyte of Microcystis colonies (Christopher & Jennifer

2022) and can be found in microbial mats (Nataliia et al. 2020). *Christensenellaceae R-7 group* degrades organic matter. For instance, it can powerfully degrade carbohydrates, amino acids, and carboxylic acids, and also performs propionate and butyrate methanogenic degradation (~~Table S5~~). The *Prevotellaceae UCG-004* genus can be found in rumen and faeces of ruminant, and utilises starch, protein, peptides, hemicellulose, and pectin (~~Table S5~~). *Olivibacter* can be found in soils, waste and gut, degrade hydrocarbon and polymer, complex and toxic compounds, take part in partial nitrification, and enable fixing nitrogen to promote plant growth (~~Table S5~~). The *OLB13* genus harbors genes that encode key enzymes for respiratory ammonification, N_2O-detoxification and CO_2 fixation pathways, plays key roles in nitrite accumulation, and has anaerobic fermentation function (~~Table S5~~). *Rummeliibacillus* is facultative anaerobic, is found in organic farming soils, and composting processes; It carries carbohydrate-utilizing genes involved in protein and cellulose metabolism; It hydrolyses starch and gelatin, degrades polybrominated diphenyl ethers and fiber, produces medium-chain carboxylic acid can improve the efficiency of carbohydrate uses in the leaf litter and the sludge (~~Table S5~~). Additionally, it has the ability to antagonise the growth of soilborne plant pathogens *Fusarium sambucinum* (Mohamed et al. 2017). Higher abundance of the genera (*Chelatococcus*, *Luteimonas*, *Dyadobacter*, *Ruminococcaceae UCG-005*, *Aquamicrobium*, *Cohnella*, *Limnothrix*, *Pseudanabaena PCC-7429*, *Christensenellaceae R-7 group*, *Prevotellaceae UCG-004*, *Olivibacter*, *OLB13*, and *Rummeliibacillus*) in camping than in no camping (~~Figure 1~~) suggested that camping could be beneficial to soil carbon and nitrogen cycling and health.

For the decreased bacterial genera, the *Ruminococcus 1* genus is the most significant cellulose-degrading genus in the intestine and rumen of herbivores (Zhang et al. 2017a), and produce several

379 types of and most of cellulases and hemicellulases, and a large amount of cellulolytic enzymes, such
380 as exoglucanases, endoglucanases, glucosidases, and hemicellulases; It can degrade plant
381 polysaccharides, hemicellulose, pectin, and cellulose present in the plant cell wall, fiber, xylan and
382 pectin, degrade complex carbohydrates, ferment complex nondigestible polysaccharides (Zhang et al.
383 2022), and break down fibrous plant material ~~(Table S5).~~ Its increases enhance the fiber degradation
384 (Pan et al. 2018). *LD29* can use cellulose, mannan, xylan, chitin, starch, and sulfated polysaccharides
385 ~~(Table S5).~~ This genus can be found in gut (Liao et al. 2021). The higher abundance of *LD29* is found
386 in high oxygen environments than in low oxygen environments (Zhao et al. 2021). The lower
387 abundance of *LD29* genus and higher abundance of the anaerobic genus *OLB13* and
388 *Rummeliibacillus* in camping than that in no camping ~~(Figure 1)~~ likely suggested a hypoxic
389 environment under camping. This speculation may reflect previous findings of impacts of trampling,
390 which showed that camping and trampling reduced soil air permeability and caused soil hypoxia (Hou
391 et al. 2004). *Rudaea* can be found in the activated sludge (Federico et al. 2013), plays key players in
392 nitrification and N assimilation (Meier et al. 2021), can decompose plant residues and organic matter,
393 and convert solid organic waste into useable nutrients for plants; It is involved in denitrification or the
394 biodegradation of some aromatic compounds, cellulose, biphenyl, naphthalene, phenol and antibiotics
395 ~~(Table S5);~~ It is resistant to multiple antibiotics (Zhao et al. 2019) and produce antifungal metabolites
396 (Nasser et al. 2020). In addition, *Rudaea* is a pathogenic species (Li et al. 2021c). *RB41* is commonly
397 found in rhizosphere (Huang et al. 2021b), and petroleum contaminated soil (Alexandria & Rachel
398 2019), accounts for the majority of soil C flux, play key role in nitrification and N assimilation, has
399 strong adaptability to low nutrition, act an crucial role in driving the soil metabolic and biogeochemical

cycling, enhance the biodegradation of polyfluoroalkyl substances and has substantial application value in environmental pollution remediation ~~(Table S5).~~ The genus *FukuN18 freshwater group* is found in pasture soils exposed to urea (urine patches) (Ganasamurthy et al. 2021). Higher abundance of this genus in ecosystems indicate the degradation status of ecosystems (Yang et al. 2019). Lower abundance of this genus in camping than that in no camping (~~Figure 1~~) suggested that camping could improve the soil ecosystem. Most *Cellvibrio* species are saprophytic soil bacteria degrading plant cell wall polysaccharides (Yannick & David 2013), drivers cellulose and hemicellulose fibers hydrolysis (Li et al. 2019), *Cellvibrio* is involved in the degradation of glucans, pectin, mannan, arabinan and chitin, and cell wall polysaccharide, and in the N-cycle, has nitrate-reducing activity (~~Table S5).~~ *Cellvibrio* is also common to plant inhabitants with nitrogen fixation activity (Xiao et al. 2019) that promote the increase of biomass (Akyol et al. 2019; Cristóbal et al. 2022). *Cellvibrio* genus carries some antibiotic resistance genes (Gou et al. 2021), such as tetracycline resistance gene (Zhang et al. 2017b). *Clostridium sensu stricto* 18 is hydrocarbon degrading (Diana et al. 2020) and hydrogen-producing genera (Yang & Wang 2021).

For increased fungal genera, *Podospora* species are saprophytic, obligately coprophilous, cellulose degraders inhabiting the dung of various herbivores such as rabbits, goats or horses and also isolated as endophytes from trees, grass as well as herbaceous plants and soils; It decay recalcitrant lignocellulose and plant biomass due to its lignocellolytic enzymes (~~Table S5~~), consequently result in an increase of soil fertility (He et al. 2019). It could be served as antifungal agent (Liu et al. 2021a), contributes to antagonism relationship with pathogenic microorganisms (Yim et al. 2017), has significant effects on controlling soil-borne diseases (Tao et al. 2020), and also enhance root growth

(Yim et al. 2017). Therefore, *Podospore* is most abundant in healthy soils (Xu et al. 2012). Higher abundance of this genus in camping than that in no camping (~~Figure 1)~~ suggested that camping could improve the soil health. *Rhexocercosporidium* is a phytopathogenic fungus commonly found in soils (Douterelo et al. 2016), and can cause ginseng rusty root rot, rusted root of Ginseng, such as *Rhexocercosporidium panacis* (~~Table S5~~). Higher abundance of this genus in camping than that in no camping (~~Figure 1)~~ suggested that camping might negatively impact on some plants. Members of the *Symbiotaphrina* genus are gut endosymbionts, capable of digesting dried plant and woody substrates, degrading the disaccharide cellobiose, and assisting the host to digest the food and detoxify various plant materials (~~Table S5).~~ It should be noted that no harmful effect of *Symbiotaphrina* is reported (Li et al. 2022).

~~For the decreased fungal genera, *Rhizomucor* is extracellular β-glucosidase producer; *Rhizomucor miehei* in our study contains 110 glycoside hydrolases, 118 glycosyl transferases, 2 polysaccharide lyases, 20 carbohydrate esterases, 155 proteases, 97 lipases and esterases, 15 cellulases, 16 chitinases; It can produce 3-hydroxy-3-methylglutaryl coenzyme A reductase, highly efficient raw starch hydrolyzing r-Amylase, glyceraldehyde-3-phosphate dehydrogenase, aspartic protease, lipases, b-glucosidase, glyceraldehyde-3-phosphate dehydrogenase, L-asparagine amidohydrolase, xylanase, b-1,3-1,4-glucanase and utilize various substrates as a single carbon source (Table S5).~~ However, the *Rhizomucor miehei* is an opportunistic pathogen may cause frequently fatal mycotic diseases (Gyöngyi et al. 2004). ~~some~~ studies suggest that fungi from *Candida* genus are very important human and animal pathogens, and are resistant to drugs (Karpinski et al. 2021). However, some studies show that only a few species from the genus *candida* (near 200 species in this genus)

are human opportunistic pathogens, e.g., *candida albicans*, *Candida parapsilosis*, *Candida tropicalis* and *Candida auris*; *Candida glabrata*, *Candida auris* is multidrug-resistant (~~Table S5~~). ~~Lower abundance of these two genera in camping than that in no camping (Figure 1) suggested possible improvement of soil ecosystem by camping.~~

Interestingly, some genera that transmitted from dung were also predominant among metabolically active phylotypes in dung treated soil (Semenov et al. 2021). *Family XIII AD3011 group*, *Prevotellaceae UCG-004*, *Christensenellaceae R-7 group*, *Ruminococcus 1*, *Ruminococcaceae UCG-005*, *Prevotellaceae UCG 004*, *LD29*, *Olivibacter*, and *Symbiotaphrina* ~~are~~ also detected in rumen, gut and/or dung (~~Table S5~~). This suggested that the subsoil microbial community could be affected by microorganisms from the dung of camping herbivores. More interestingly, a recent study found that most non-native microorganisms from dung did not survive in soils after few months (Semenov et al. 2021). This suggested that the influences of camping on the subsoil microbial communities could be temporary, but this still needs further confirmation.

Since *Dyadobacter*, *Rummeliibacillus*, *Cohnella*, *Luteimonas*, *Podospora*, *Cellvibrio*, *Ruminococcus 1*, *LD29*, and *Rudaea* are cellulose degraders (~~Table S5~~), the enrichment of *Dyadobacter*, *Rummeliibacillus*, *Cohnella*, *Luteimonas*, and *Podospora* (~~Figure 1~~) in camping could contribute to the increase in the relative abundance of cellulolysis (~~Figure 2~~), especially in the case of the depletion of Cellvibrio, Ruminococcus 1, LD29, and Rudaea (~~Figure 1~~). Since Cellvibrio, LD29, Ruminococcus 1, Rhizomucor, and Cohnella are xylanolytic (~~Table S5~~), the depletion of Cellvibrio, LD29, Ruminococcus 1, and Rhizomucor in camping (~~Figure 1~~) could contribute to the decrease in the relative abundance of xylanolysis (~~Figure 2~~), especially in the case of the enrichment of Cohnella

(Figure 1). Cohnella, Luteimonas, Cellvibrio, LD29, and Rhizomucor have chitinase (Table S5), the enrichment of Cohnella and Luteimonas in camping (Figure 1) could contribute to the increase in the relative abundance of C decomposition genes (chitinase, Figure 2), especially in the case of the depletion of *Cellvibrio*, *LD29*, and *Rhizomucor* (Figure 1). The enrichment of *Luteimonas* and *OLB13* in camping (Figure 1) could contribute to the increase in the relative abundance of nitrite reduction function (Figure 2) due to their denitrification (Table S5). *OLB13* harbors genes that encode CO_2 fixation pathways (Table S5), however, the *OLB13* is enriched (Figure 1) but the relative abundance of C fixation genes is depleted (Figure 2) in camping, indicating that there might be other microbes that contain C fixation genes and remain to be explored. Our study partially supports the finding that manure addition increases the relative abundances of diazotrophs (Ye et al. 2021) (e.g. *Cohnella*, *Olivibacter*, Figure 1 and Table S5 in our study). *Cellvibrio*, *Cohnella*, *Olivibacter* can fix N (Table S5), the depletion of *Cellvibrio* (Figure 1) in camping could contribute to the decrease in the relative abundance of gene coding nitrogenase (Figure 2), especially in the case of the enrichment of *Cohnella* and *Olivibacter* (Figure 1). Besides, our results sustained that bacterial nitrifiers (e.g., *Dyadobacter*, *Olivibacter*, Figure 1 and Table S5 in our study) were enhanced (Shi et al. 2021; Ye et al. 2021).

Cellvibrio carries tetracycline resistance gene (Table S5), the depletion of *Cellvibrio* (Figure 1) could contribute to the decrease in the relative abundance of tetracycline resistance gene (tetB, Figure 2). Tetracyclines is the best-selling veterinary antibiotic, its content is also the highest in animal manures (Yue et al. 2021). Furthermore, inputs of manure from antibiotic-free cattle led to increases in abundances of some abiotic resistance genes in comparison to no manure inputted soils

(Shawver et al. 2021). A recent study found that the increase of tetracycline resistance gene abundance dominated the abundance increase of resistance genes in soil treated with manure (Huang et al. 2021a). Our results showed the only significant change occurred in tetracycline resistance gene (tetB, Figure 2). Intriguing, the abundance of tetracycline resistance gene (tetB) was depleted by camping, suggesting that camping could be a potential method for antibiotic elimination of animal faeces. However, the impact of antibiotic use on camping impact still needs to be confirmed. *Cellvibrio* and *Podospora* are saprophytic (Table S5), the enrichment of *Podospora* in camping (Figure 1) could contribute to the increase in the relative abundance of litter saprotroph (Figure 2), especially in the case of the depletion of *Cellvibrio* (Figure 1). The enrichment of litter saprotroph in camping is consistent with the finding that manure addition increases the relative abundance of saprotrophic fungi (Ye et al. 2021). The enrichment of *Podospora* in camping (Figure 2) could contribute to the increase in the relative abundance of litter endophyte because *Podospora* is also as an endophyte from grass (Josphat et al. 2011).

An 18-year experiment showed that manure significantly enhanced soil bacterial diversity (Ye et al. 2021). A meta-analysis of total of 2303 studies found that manure increased bacterial diversity and reduced fungal diversity (Guo et al. 2022). On the contrary, the taxonomic and functional diversities of subsoils in this study showed resistance to camping, which suggesting that soil ecosystems can act as buffers (Semenov et al. 2021) against disturbance. Whether this stability is characterized by short-term time scale still needs further study.

Herbivore camping affects the networks of subsoil bacterial and fungal communities

In this study, camping increased the bacterial and fungal network complexity, particularly the number

of positive edges. The results are consistent with the previous impact of manure on the microbial network (Ye et al. 2021). Four potential mechanisms may explain this phenomenon: (a) the stress-gradient hypothesis. the stress-gradient hypothesis and our previous study about microbial interactions showed that stressed habitats result in facilitations (positive links) higher frequency than competitions (negative links) (Ding & Wang 2021). As discussed above, camping and trampling could reduce soil air permeability and cause soil hypoxia. In addition, camping and dung and urine input resulted in high soil ammonia concentration (up to 377 mg/kg) (Zhang 2002) that could be toxic to microbes. Both hypoxia and high ammonia can stress the soil microbes, therefore, leading to more often positive links. (b) exogenous microorganism. Many studies (Kong et al. 2019; Torres et al. 2021) have shown that microbial inoculation could increase the positive interaction of microbial network. Microorganisms from manure addition could also enhance the association of microorganisms (Yang et al. 2022). Some potential dung-derived phylotypes including *Ruminococcaceae UCG-005*, *Christensenellaceae R-7 group*, *Prevotellaceae UCG-004*, *Ruminococcus 1* ~~(Table S5)~~ elevated their degree of networks by 50 - 2300% (~~Table S4~~). (c) Microorganisms that could cooperate with the original soil microorganisms were more likely to successfully colonize and proliferate in soils. This scenario could also enhance positive microbial interactions. (d) Nutrient input from faeces and urine made some microorganisms proliferate which were reflected by the increased microbial carbon and nitrogen (88% and 84%, respectively) under manure addition (Liu et al. 2020a). These synergic increases could also lead to more positive links. Besides, we found that some low abundance bacteria and fungi taxa had highest degree of the network, suggested that low abundant rather than high abundant bacteria and fungi were keystone

taxa that affected the stability of the network ~~(Figure 5f),~~ supporting previous findings (Pan et al. 2021). To our knowledge, this is first study to show that herbivore camping could affect the stability of the network via shifting the properties of high and low degree taxa. Four types of changes that could explain the change in network stability were found.

(1) more than 62.45 - 72.26% of high degree OTUs and low degree OTUs were specific to the no camping or camping as indicated by Venn plots ~~(Figure 6a, b, e, f)~~ suggested that the camping shifted the node identity of networks, compared with the no camping.

(2) 17.86 - 22.36% of OTUs were shared in the no camping and camping, implied that camping shifted the high degree OTUs and low degree OTUs under the no camping from the states of high and low degree to the states of low and high degree, respectively (~~Figure 6 c, d, g, h).~~ Furthermore, compared with the no camping, the camping decreased the node degree of *Luteimonas*, (OTU_287 and OTU_1365, from 77 and 77 to 1 and 0), *Dyadobacter* (OTU_1216 and OTU_3484, from 77 and 71 to 72 and 1), *Ruminococcaceae UCG-005* (OTU_1880, OTU_399, OTU_4129 from 71,71,5 to1,1,2), *Pseudanabaena PCC-7429* (OTU_3596, from 77 to 71), *Family XIII AD3011 group* (OTU_1230, OTU_594 from 3 and 77 to 2 and 0), *Christensenellaceae R-7 group* (OTU_2495, OTU_2549, OTU_4118, from 2, 3, 66 to 0, 0, 2), *Prevotellaceae UCG-004* (OTU_2810, OTU_378, OTU_529, OTU_1946, OTU_3132 from 71,77,3,4,66 to 8,4,2,1,0), *Olivibacter* (OTU_446 from 77 to 72), *OLB13* (OTU_2259 from 77 to 0), *Ruminococcus 1* (OTU_1411 from 66 to 0). Whereas, the camping increased the node degree of Aquamicrobium (OTU_262, from 2 to 77), *Limnothrix* (OTU_99 from 0 to 72), *Cellvibrio* (OTU_219 0 to 72), *FukuN18 freshwater group* (OTU_392 from 0 to 77), OLB13 (OTU_446 from 1 to 71), *Rudaea* (OTU_2905 from 0 to 1), and *RB41* (OTU_53 from

66 to 72) and some potential dung-derived phylotypes including *Ruminococcaceae UCG-005* (OTU_4330, OTU_1005, OTU_836 from 3,1,0 to 72,4,2), *Christensenellaceae R-7 group* (OTU_490 from 0 to 1), *Prevotellaceae UCG-004* (OTU_2172, OTU_2408 from 2, 1 to 3, 10), *Ruminococcus 1* (OTU_449, OTU_539 from 0,6 to 10,10) (~~Table S4~~), partially reflecting that some dung-derived phylotypes enhanced the network interaction (Yang et al. 2022).

(3) for the bacterial network, the camping decreased the total relative abundance of high degree OTUs (6.54% under camping vs.7.27% under no camping) by 10.02% (~~Figure 6a).~~ Camping significantly decreased the niche overlap of high degree OTUs and specific high degree OTUs (Wilcoxon test, p = 6.5e-13, 7.0e-04), and significantly increased the relative abundance of high degree generalist species and specific high degree generalist species (Wilcoxon test, p = 1.1e-04, 1.1e-04) (~~Figure S87a,c,e,h).~~ The camping decreased the total relative abundance of low degree OTUs (6.09% under camping vs. 6.28% under no camping) by 2.95% (~~Figure 6b).~~ Camping significantly increased the niche overlap of low degree OTUs and specific low degree OTUs (Wilcoxon test, p = 8.4e-4, 0.027) and decreased the niche breadth of low degree OTUs and specific low degree OTUs (Wilcoxon test, p = 1.1e-10, 8.2e-06) (~~Figure 88a-d).~~ However, Camping increased the total relative abundance of the shared OTUs that shifted the states of high degree under no camping (1.87%) to the states of low degree under Camping (2.08%) by 11.26% (~~Figure 6 c~~). Camping significantly decreased the niche breadth of these share degree OTUs (Wilcoxon test, p = 3.0e-05) (~~Figure 89b~~). Camping decreased the total relative abundance of the shared OTUs that shifted the states of low degree under no camping (2.92%) to the states of high degree under camping (2.37%) by 18.84% (~~Figure 6 d).~~ Camping significantly decreased the niche breadth of

these share degree OTUs (Wilcoxon test, p = 1.9e-03) (~~Figure 89d).~~ For the fungal network, the camping decreased the total relative abundance of high degree OTUs (1.24% under camping vs 9.85% under no camping) by 87.43% (~~Figure 6 e~~). Camping significantly increased the niche overlap of high degree OTUs and specific high degree OTUs (Wilcoxon test, p = 0.032, 0.011), and significantly decreased the niche width of high degree OTUs and specific high degree OTUs (Wilcoxon test, p = 2.8e-08, 1.9e-05), significantly decreased the relative abundance of no significant species in high degree OTUs and specific high degree OTUs (Wilcoxon test, p = 0.0048, 0.0018) (Figure S90a-d,e,h). The camping decreased the total relative abundance of low degree OTUs (0.69% under camping vs.1.49% under no camping) by 53.40% (Figure 6 f). Camping significantly increased the niche overlap and niche width of low degree OTUs (Wilcoxon test, p = 0.018, 0.0002) and specific high degree OTUs (Wilcoxon test, p = 0.19, 0.0005), decreased the relative abundance of no significant species in low degree OTUs and specific low degree OTUs (Wilcoxon test, p = 0.045, 0.091) (Figure S91a-d,f,i). However, Camping decreased the total relative abundance of the shared OTUs that shifted the states of high degree under no camping (0.40%) to the states of low degree under Camping (0.18%) by 54.95% (Figure 6 g). Camping increased the niche overlap of these shared OTUs (Wilcoxon test, p = 0.9), and decreased the niche width of these shared OTUs (Wilcoxon test, p = 0.0056) (Figure S92a-b). Camping increased the total relative abundance of the shared OTUs that shifted the states of low degree under no camping (0.16%) to the states of high degree under camping (1.03%) by 535.56% (Figure 6 h). Camping increased the niche overlap of these shared OTUs (Wilcoxon test, p = 0.041), and decreased the niche width of these shared OTUs (Wilcoxon test, p = 9.0e-04) (Figure 92c-d).

589 (4) for the bacterial communities, compared with no camping, the camping significantly changed the
590 relative abundance of functions of the low degree OTUs, high degree OTUs, specific low degree OTUs
591 and specific high degree OTUs (Welch's t test with two sides p = 7.73e-5 - 0.048, Figure S93a-e).
592 Compared with no camping, the camping significantly suppressed the relative abundance of most C-,
593 N-, and P-cycle functions and antibiotic resistance gene of the low degree OTUs and specific low
594 degree OTUs, but improved those of high degree OTUs and specific high degree OTUs (Welch's t test
595 with two sides p = 2.28e-7 - 0.050, Figure S94-99). The camping significantly suppressed the relative
596 abundance of most C-, N-, and P-cycle functions and antibiotic resistance gene of the shared OTUs
597 that shift the states of high degree under no camping to the states of low degree under camping,
598 however, the camping significantly improved the relative abundance of most C-, and P-cycle functions
599 and antibiotic resistance gene of the shared OTUs that shift the states of low degree no camping under
600 to the states of high degree under camping (Welch's t test with two sides p = 9.84e-8 - 0.050, Figure
601 S100-101). The relative abundance of C-, N-, and P-cycle functions and antibiotic resistance gene of
602 high degree OTUs and specific high degree OTUs were significantly higher than those of low degree
603 OTUs and specific low degree OTUs under camping, whereas, the opposite trend was found under
604 no camping (Welch's t test with two sides p = 7.72e-7 - 0.049, Figure S102-110).
605 For the fungal communities, compared with no camping, the camping significantly declined the relative
606 abundance of Microfungus (Welch's t test with two sides p = 0.047, 0.019, Figure S111-112 a,d) of
607 the low degree OTUs and specific low degree OTUs, improved the relative abundance of Undefined
608 Saprotroph of the low degree OTUs (Welch's t test with two sides p = 1.43e-3, Figure S111b) but
609 declined the relative abundance of Undefined Saprotroph of the specific low degree OTUs (Welch's t

test with two sides p = 7.34e-3, Figure S112e), declined the relative abundance of Saprotroph (Welch's t test with two sides p = 3.0e-03, 0.012, Figure S111-112c,f) but improved the relative abundance of Symbiotroph (Welch's t test with two sides p = 0.022, 0.016, Figure S111-112c,f) of both low degree OTUs and specific low degree OTUs, and declined the relative abundance in the white rot of both high degree OTUs and specific high degree OTUs (Welch's t test with two sides p < 1.0e-15, <1.0e-15, Figure S111-112g,h). The camping did not significantly change the relative abundance of functions of the shared OTUs that shifted the states of high degree under no camping to the states of low degree under camping (Welch's t test with two sides p > 0.05, not show), however, the camping significantly suppressed the relative abundance of Facultative Yeast and Yeast of the shared OTUs that shifted the states of low degree no camping under to the states of high degree under camping (Welch's t test with two sides p = 1.0e-15 - 0.048, Figure S111-112i).

As niche restriction was the main driving force of bacterial and fungal community assembly, the niche width decreased the degree of networks (p = 0.0015 and p = 0.3652 for camping and no camping, respectively, Figure 7a) and niche overlap increased the changes in degree of networks (p < 2.2e-16 and p < 2.2e-16 for both camping and no camping, respectively, Figure 7b). Furthermore, the first evidence was provided by our Bayesian structural equation model suggested that camping exerted a positive effect on the network degree through compressing the niche width (Estimate = -0.02 - -0.10, -0.14 - -0.75, Figure 7c), and camping reduced the change in the network degree through reducing the niche overlap (Estimate = -0.04 - -0.05, 0.26 - 0.29, Figure 7d). These results provide useful enlightenments for the first time for the management of camping soil microorganisms in pasture and for insights to the impact of wild camping behaviour on soil that we have generally ignored.

Conclusion

Our study firstly suggested that camping significantly changed the relative abundance of 21 bacterial phylotypes and five fungal phylotypes with implications for soil carbon and nutrient cycling, and soil and plant health, while did not change taxonomic and functional diversities. Compared with the no camping, the camping increased the stability of bacterial network, whereas, decreased the stability of fungal network. Camping exerted a positive effect on the network through compressing the niche width, and reduced the change in the network through reducing the niche overlap. However, the effects of this change on the soil viruses, protozoa, and the plants that are later established remains unknown. This study provides a stepping-stone insight to the effect of herbivore camping on soil microbial communities.

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Zhou H, Gao Y, Jia X, Wang M, Ding J, Cheng L, Bao F, and Wu B. 2020. Network analysis reveals the strengthening of microbial interaction in biological soil crust development in the Mu Us Sandy Land, northwestern China. *Soil Biology and Biochemistry* 144. 10.1016/j.soilbio.2020.107782

Figure 1

Figure 1

Figure 1 Random Forest analysis (left) and Kruskal-Wallis test (right) of bacterial (a) and fungal communities (b). Mean decrease Gini: mean decrease in Gini index. The larger the value, the more important the phylotypes is in distinguishing groups. *, $p < 0.05$.

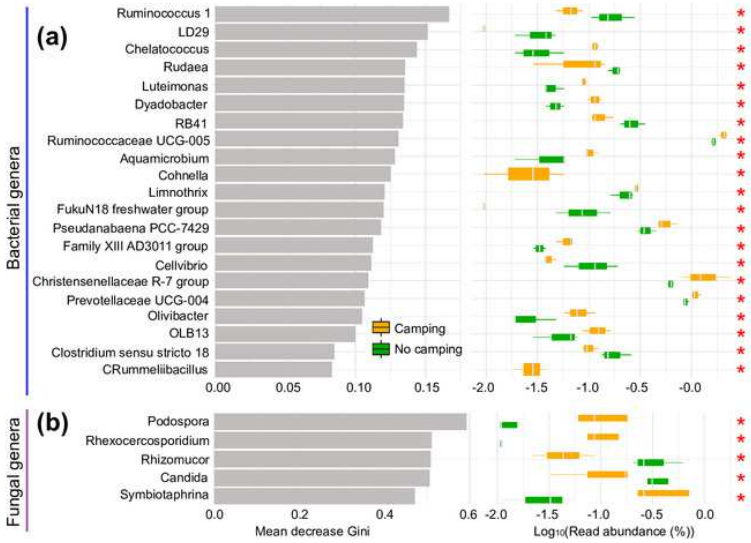

(a)
Bacterial genera
Ruminococcus 1
LD29
Chelatococcus
Rudaea
Luteimonas
Dyadobacter
RB41
Ruminococcaceae UCG-005
Aquamicrobium
Cohnella
Limnothrix
FukuN18 freshwater group
Pseudanabaena PCC-7429
Family XIII AD3011 group
Cellvibrio
Christensenellaceae R-7 group
Prevotellaceae UCG-004
Olivibacter
OLB13
Clostridium sensu stricto 18
CRummeliibacillus
Camping
No camping
0.00
0.05
0.10
0.15
-2.0
-1.5
-1.0
-0.5
-0.0
(b)
Fungal genera
Podospora
Rhexocercosporidium
Rhizomucor
Candida
Symbiotaphrina
0.0
0.2
0.4
0.6
-2.0
-1.5
-1.0
-0.5
0.0
Mean decrease Gini
Log10(Read abundance (%))

Figure 2

Figure 2

Figure 2 Functional differences of soil microbial communities (a-e: bacteria, f: fungi) between camping and no camping

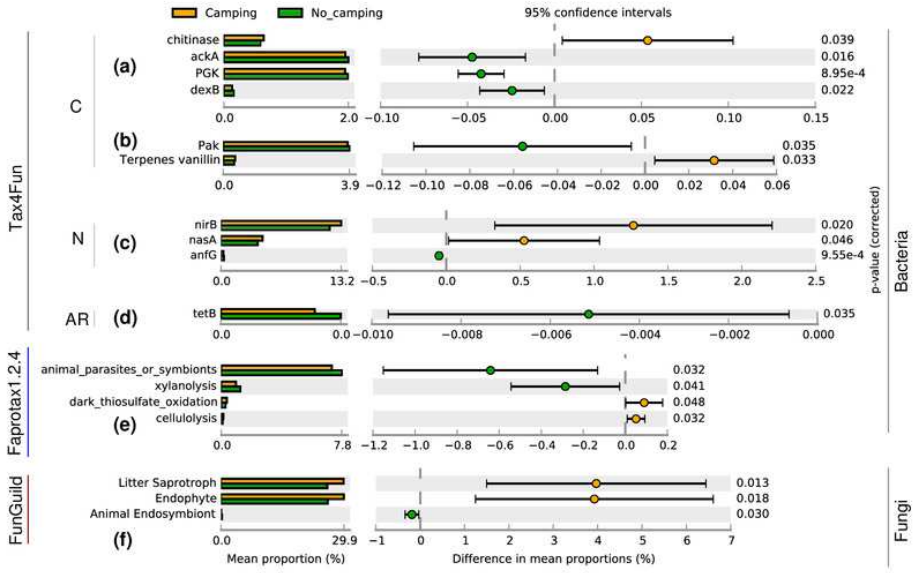

Camping
No_camping
95% confidence intervals
Tax4Fun
C
N
AR
(a)
(b)
(c)
(d)
chitinase
ackA
PGK
dexB
Pak
Terpenes vanillin
nirB
nasA
anfG
tetB
0.039
0.016
8.95e-4
0.022
0.035
0.033
0.020
0.046
9.55e-4
0.035
p-value (corrected)
Bacteria
Faprotax1.2.4
animal_parasites_or_symbionts
xylanolysis
dark_thiosulfate_oxidation
cellulolysis
(e)
0.032
0.041
0.048
0.032
FunGuild
Litter Saprotroph
Endophyte
Animal Endosymbiont
(f)
0.013
0.018
0.030
Fungi
Mean proportion (%)
Difference in mean proportions (%)

Figure 3

Figure 3

Figure 3 Co-occurrence networks for microbial communities between camping and no camping. A node suggests an OUT, its colour and size are proportional to its degree; a link represents the significant Pearson correlations with $r > 0.8$ and the Benjamini and Hochberg corrected $p < 0.001$. A red link indicates a positive relationship, but a blue link indicates a negative relationship.

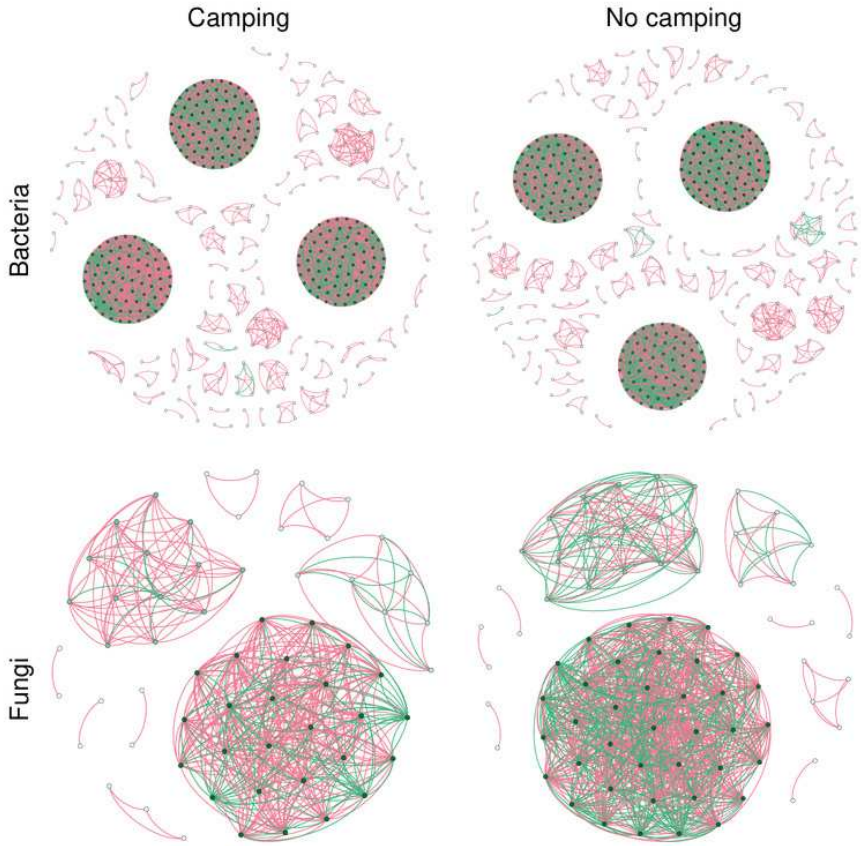

Camping
No camping
Bacteria
Fungi

Figure 4

Figure 4

Figure 4 Network robustness analysis for microbial communities between the camping and no camping. Smaller decline at the same proportion indicates more stability within networks. B, bacteria; F, fungi.

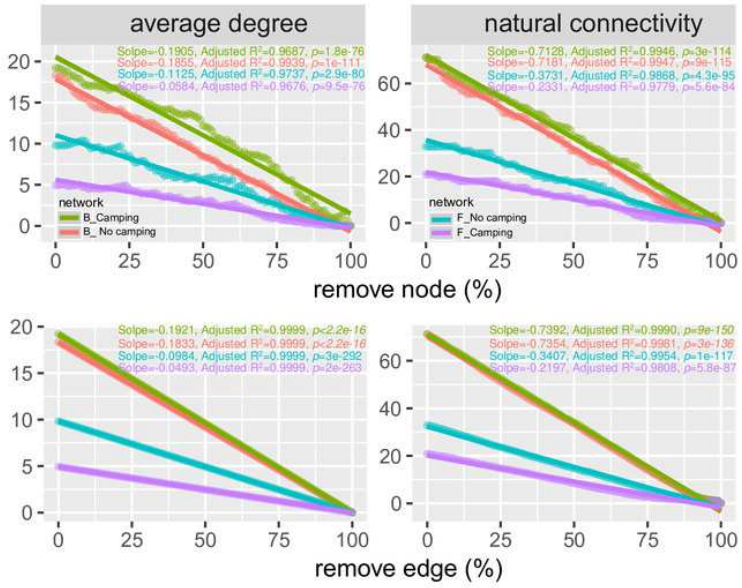

average degree
natural connectivity
Solpe=-0.1905, Adjusted R²=0.9687, p=1.8e-76
Solpe=-0.1855, Adjusted R²=0.9939, p=1e-111
Solpe=-0.1125, Adjusted R²=0.9737, p=2.9e-80
Solpe=-0.0584, Adjusted R²=0.9676, p=9.5e-76
Solpe=-0.7128, Adjusted R²=0.9946, p=3e-114
Solpe=-0.7181, Adjusted R²=0.9947, p=9e-115
Solpe=-0.3731, Adjusted R²=0.9868, p=4.3e-95
Solpe=-0.2331, Adjusted R²=0.9779, p=5.6e-84
network
B_Camping
B_ No camping
F_No camping
F_Camping
remove node (%)
Solpe=-0.1921, Adjusted R²=0.9999, p<2.2e-16
Solpe=-0.1833, Adjusted R²=0.9999, p<2.2e-16
Solpe=-0.0984, Adjusted R²=0.9999, p=3e-292
Solpe=-0.0493, Adjusted R²=0.9999, p=2e-263
Solpe=-0.7392, Adjusted R²=0.9990, p=9e-150
Solpe=-0.7354, Adjusted R²=0.9981, p=3e-136
Solpe=-0.3407, Adjusted R²=0.9954, p=1e-117
Solpe=-0.2197, Adjusted R²=0.9808, p=5.8e-87
remove edge (%)

Figure 5

Figure 5

Figure 5 Differences in niche breadth (a), niche overlap (b), specialist/generalist species (c), assembly mechanism (d, e), and the node degree distribution with relative abundance ranks (f) of bacterial and fungal communities between camping and no camping.

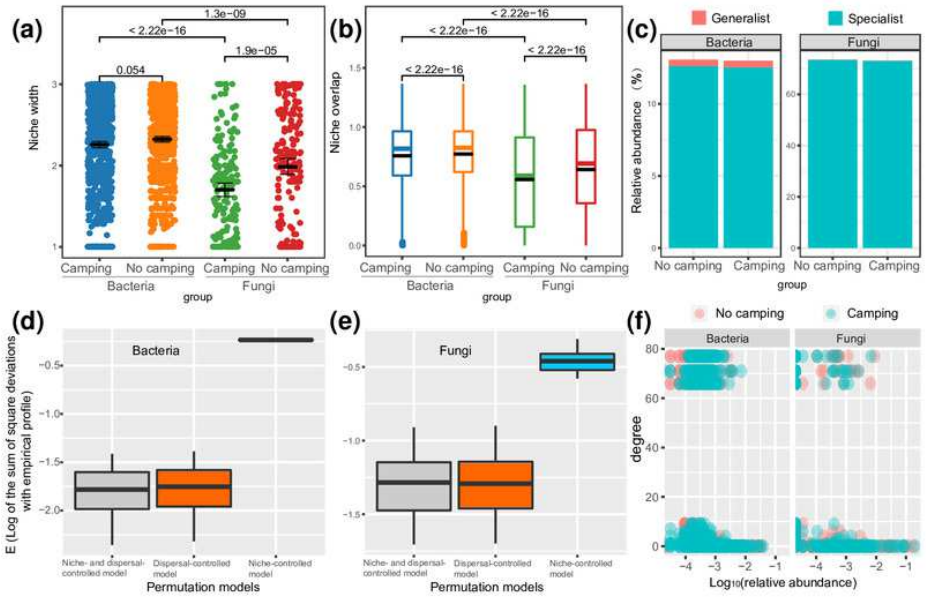

(a)
Niche width
1.3e−09
< 2.22e−16
1.9e−05
0.054
Camping
No camping
Camping
No camping
Bacteria
Fungi
group
(b)
Niche overlap
< 2.22e−16
< 2.22e−16
< 2.22e−16
< 2.22e−16
Camping
No camping
Camping
No camping
Bacteria
Fungi
group
(c)
Generalist
Specialist
Bacteria
Fungi
Relative abundance (%)
No camping
Camping
No camping
Camping
group
(d)
Bacteria
E (Log of the sum of square deviations with empirical profile)
Niche- and dispersal-controlled model
Dispersal-controlled model
Niche-controlled model
Permutation models
(e)
Fungi
Niche- and dispersal-controlled model
Dispersal-controlled model
Niche-controlled model
Permutation models
(f)
No camping
Camping
Bacteria
Fungi
degree
Log10(relative abundance)

Figure 6

Figure 6

Figure 6 Venn diagram showing the number of specific and shared nodes between different degrees and different groups (camping and no camping).

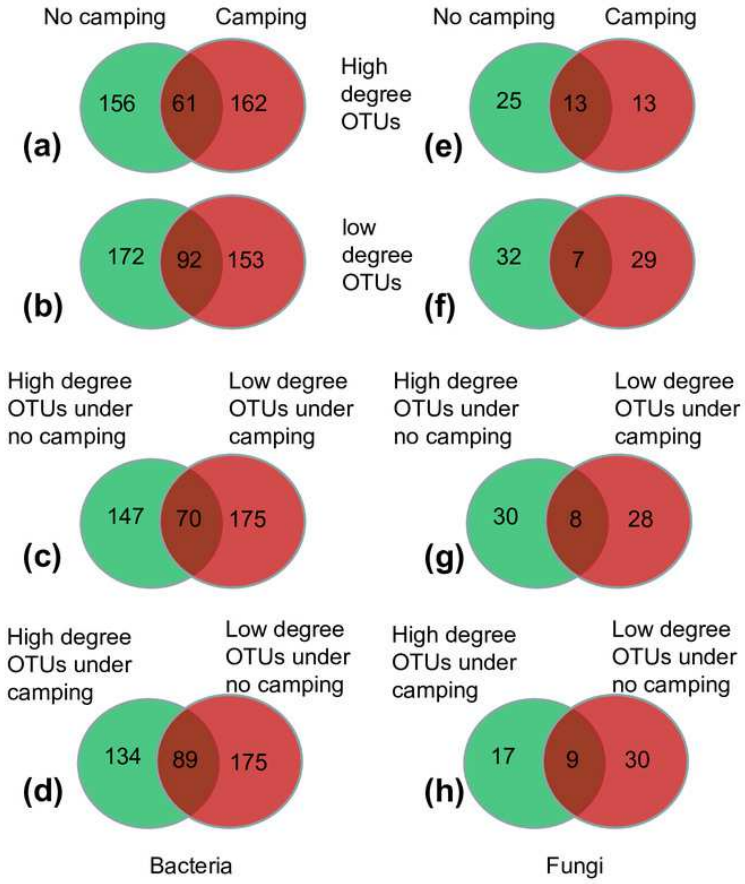
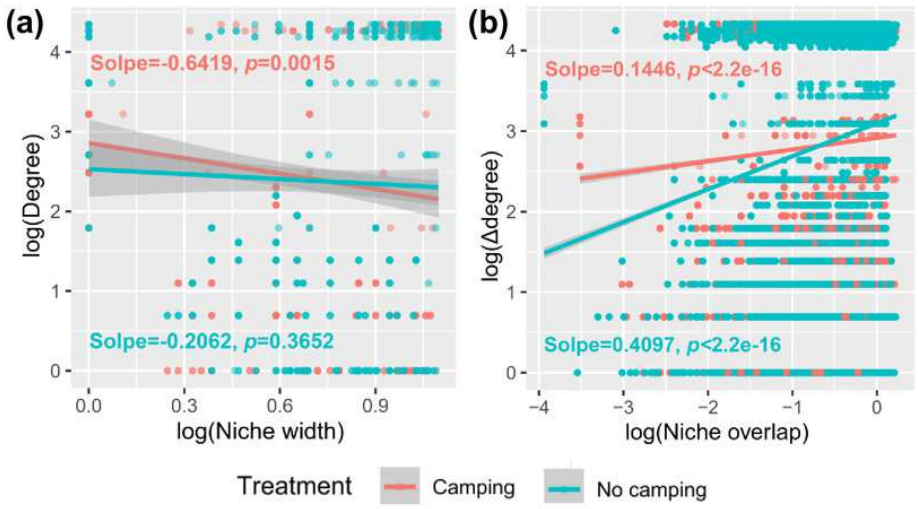
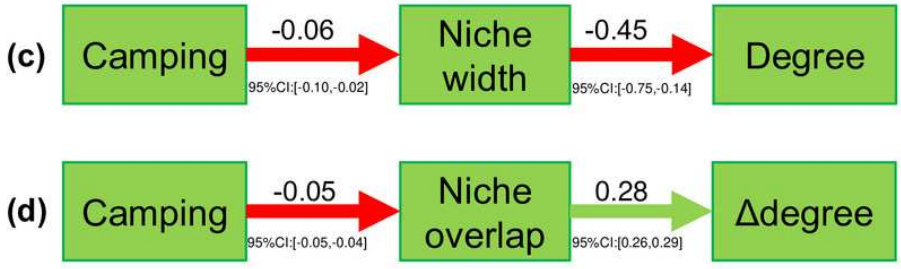

No camping
Camping
156
61
162
(a)
High degree OTUs
172
92
153
(b)
low degree OTUs
No camping
Camping
25
13
13
(e)
32
7
29
(f)
High degree OTUs under no camping
Low degree OTUs under camping
147
70
175
(c)
High degree OTUs under no camping
Low degree OTUs under camping
30
8
28
(g)
High degree OTUs under camping
Low degree OTUs under no camping
134
89
175
(d)
High degree OTUs under camping
Low degree OTUs under no camping
17
9
30
(h)
Bacteria
Fungi

Figure 7

Figure 7

Figure 7 Linear regression model showing the effect of niche width (a) and niche overlap (b) on the network property (degree and difference between degrees (Δdegree)). Bayesian structural equation model showing how camping alter the network property via niche width (c) and niche overlap (d).


(a)
Solpe=-0.6419, p=0.0015
Solpe=-0.2062, p=0.3652
log(Degree)
log(Niche width)
(b)
Solpe=0.1446, p<2.2e-16
Solpe=0.4097, p<2.2e-16
log(Δdegree)
log(Niche overlap)
Treatment
Camping
No camping


(c)
Camping
-0.06
95%CI:[-0.10,-0.02]
Niche width
-0.45
95%CI:[-0.75,-0.14]
Degree

(d)
Camping
-0.05
95%CI:[-0.05,-0.04]
Niche overlap
0.28
95%CI:[0.26,0.29]
Δdegree

Table 1(on next page)

Table 1

Table 1 Results of the Kolmogorov-Smirnov test comparing bootstrapped node attributes of networks under No camping and Camping. For each network, node attributes were computed by bootstrapping 10 0000 times. Kolmogorov-Smirnov test compares the cumulative distribution of two properties where the null hypothesis is that the properties have same distribution patterns.

1

Comparison	Degree	Closeness	transitivity	Eigenvector centrality
No camping B vs Camping B	0.1665****	0.5276****	0.0133****	0.2984****
No camping F vs Camping F	0.4941****	0.5066****	0.0595****	0.2462****
No camping B vs No camping F	0.4693****	1****	0.0967****	0.3248****
Camping B vs Camping F	0.4913****	1****	0.1430****	0.2604****

2 The values in each cell represents the maximum difference in the absolute cumulative
3 distribution function. **** indicate statistical significance at $p < 0.0001$, respectively. B,
4 bacteria; F, fungi.

5

Table 2(on next page)

Table 2

Table 2 Results of the Kolmogorov-Smirnov test and Kruskal-Wallis test comparing network stability (average degree and network connectivity) of networks under No camping and Camping after 50% nodes were randomly removed. For each network, node properties were computed by bootstrapping 100000 times.

1

Method	Comparison	Average degree	Natural connectivity
Kolmogorov-Smirnov test	No camping B vs Camping B	0.2021****	0.0987****
	No camping F vs Camping F	0.9043****	0.8718****
	No camping B vs No camping F	0.9811****	1****
	Camping B vs Camping F	1****	1****
Kruskal-Wallis test	No camping B vs Camping B	11.1137 vs 11.6460****	42.3549 vs 42.9296****
	No camping F vs Camping F	5.9364 vs 2.9788****	18.3614 vs 11.0958****
	No camping B vs No camping F	11.1137 vs 5.9364****	42.3549 vs 18.3614****
	Camping B vs Camping F	11.6460 vs 2.9788****	42.9296 vs 11.0958****

2 The values in top four cells represent difference for Kolmogorov-Smirnov test, which the
3 maximum difference in the absolute cumulative distribution function; The values in bottom four
4 cells represent mean value for Kruskal-Wallis test. **** indicate statistical significance at $p <$
5 0.0001. B, bacteria; F, fungi.

6

7

8