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Dominant woody plants alter soil microbial community composition during succession

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ABSTRACT

Belowground assemblages are closely related to the aboveground vegetation and edaphic properties, which are also driven by dominant plants due to direct and indirect influences. However, the effects of dominant woody plants on the belowground organisms along successional gradients remain poorly understood. Plant and soil samples were collected from an initial herbaceous stage (i.e. alpine meadows) and four stages dominated by woody species, beneath and between patches of the dominant woody plants, to assess the effects of dominant woody plants on the succession of microbial communities along a secondary successional gradient. We quantified herbaceous, edaphic, bacterial, and fungal dissimilarities between stages to explore how dominant woody plants affect bacterial and fungal dissimilarities between stages using structural equation modeling. We found that dominant woody plants generally increase the succession of microbial communities in early stages, but decrease it in late stages. Our results further suggest that the herbaceous dissimilarity between stages plays more important roles than the edaphic one in mediating the effect of dominant woody plants on both bacterial and fungal dissimilarities between stages. Our results provide insight into the relative role of direct and indirect influences on microbial dissimilarity between stages and highlight the importance of dominant woody plants in driving microbial succession. As woody encroachment increases in alpine meadows, the dominant woody plants may have strong consequences on the dynamic of microbial communities, thereby affecting ecosystem functioning.

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1. Introduction

The importance of the linkage between aboveground and belowground communities has been stressed because of its important role in governing ecosystem functions (De Deyn et al., 2004; Wardle et al., 2004; Teng et al., 2021). For example, Eisenhauer et al. (2013) showed that plant diversity increased the biomass and activity of soil microorganisms, which may influence carbon and nitrogen cycling. Moreover, it has been recognized that dominant plants should have more important roles than others in an ecosystem (i.e., mass ratio hypothesis, Grime, 1998), indicating that the presence of dominant plants may impact vegetation structure and edaphic conditions with potential belowground consequences (Eldridge et al., 2011; Leitner et al., 2018). However, few studies have explicitly assessed the effects of dominant woody plants on the succession of microbial communities along secondary successional gradients, through changes in aboveground-belowground linkages. Comparing initial microbial communities to the soil microbial communities under and between dominant woody plants along secondary successional gradients (hereafter “microbial dissimilarity between stages”; e.g., differences between microbes in meadows and microbes under shrubs/trees v.s. differences between microbes in meadows and microbes from gaps of shrubs/trees; Fig. 1) is therefore important for understanding the role of dominant plants in driving microbial processes and succession, and for predicting changes in ecosystem functioning under global change (Jangid et al., 2011; Schmidt et al., 2014; Saintilan and Rogers, 2015; Ding and Eldridge, 2019; Eldridge and Ding, 2021).

Soil microbial communities are highly variable along successional gradients and influenced by the variability in vegetation composition (Jangid et al., 2013; Cutler et al., 2014; Miralles et al., 2020). For example, Lozano et al. (2014) showed that changes in soil bacterial and fungal communities were highly influenced by vegetation structure and productivity along a secondary succession in a dry environment, although increasingly decoupling relationships between plant and microbial communities were also reported during long-term ecosystem development (Chang and Turner, 2019). Indeed, some studies have highlighted the importance of edaphic properties in driving soil microbial community along successional gradients (Chabrerie et al., 2003; Kaneda et al., 2019; Qiang et al., 2021). Furthermore, Sui et al. (2021) have found, along a forest successional gradient, that soil microbial communities were more driven by edaphic properties than plant communities.

It has been well established that vegetation compositions could be driven by the presence of dominant woody plants (Michalet et al., 2015; Wang et al., 2021). In particular, woody plants could protect more or different herbaceous species from harsh conditions. So that woody plants host-specific communities that largely differ from gaps (e.g., forest gaps), indicating the presence of woody plants may increase the herbaceous dissimilarity between stages. Similarly, woody plants could strongly impact edaphic properties, particularly by mitigating water and nutrient stress in harsh environments, thereby increasing the edaphic dissimilarity between stages (Filazzola and Lortie, 2014; Mihoč et al., 2016). Within this framework, woody plants are expected to have strong effects on microbial dissimilarity between stages, thus increasing herbaceous and edaphic dissimilarity between stages along successional gradients (Fig. 1).

Additionally, the dominant woody plant could serve as direct determinants of soil microbial communities, because root-associated organisms (e.g., root pathogens and mycorrhizal fungi) are determined by the characteristics and resources of living roots (Korthals et al., 2001; De Deyn et al., 2011). Furthermore, leaf litters of dominant woody plants may also serve as drivers of microbial community structure by selecting specific decomposers (Ayles et al., 2009; Chomel et al., 2016). Additionally, root characteristics and litter quality

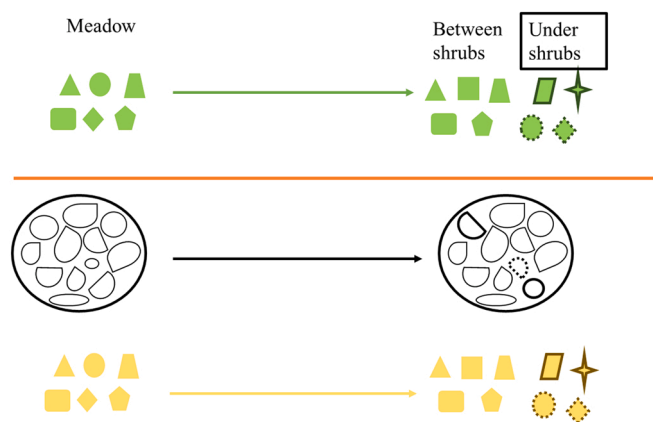


Fig. 1. Schematic diagram of dominant woody plants modifying herbaceous, edaphic, and microbial dissimilarity between stages along successional gradients. Green symbols: herbaceous species; yellow symbols: microbial species; symbols in black circles: edaphic properties. Herbaceous species from the initial stage (e.g., meadows in our case) may be lost and be replaced by other species along secondary successional gradients (e.g., herbaceous species between shrubs). However, the presence of dominant woody plants (e.g., herbaceous species under shrubs) may avoid losing species (symbols in dashed frames) or help to recruit new species that cannot live without woody plants (symbols in solid frames). Thus, the presence of dominant woody plants may either increase or decrease herbaceous dissimilarity, respectively, between the given stage and the initial stage (i.e., the dissimilarity between stages). Similarly, the presence of dominant woody plants can also modify the development of soils thereby resulting in an increase or decrease in edaphic dissimilarity between stages. Thus, the microbial dissimilarities between stages are expected to be modified by the presence of dominant woody plants directly and indirectly because of aboveground-belowground linkages.

would be changed when dominant woody plant species increased in size or were replaced by other woody species along successional gradients (Perez et al., 2013; Veen et al., 2018), indicating that the presence of dominant woody plants could alter changes in microbial communities along successional gradients. Taken together, microbial community composition varies along successional gradients and can be modified by the presence of a dominant woody plant. However, we do not know whether and to which extent the direct and indirect effects of dominant woody plants on microbial dissimilarity between stages varies along successional gradients.

To have a better understanding of the role of dominant woody plants in driving microbial community succession, we selected an initial successional stage (i.e., meadow) and four later stages dominated by woody plants (i.e., small and large shrubs of *Dasiphora fruticosa* and then trees of *Picea crassifolia*) along a secondary successional gradient in Zhangye (Northwestern China). In this study, we collected plant and soil samples in the meadow, and under and between patches of the dominant woody plants at each successional stage. We quantified changes between stages in edaphic property and composition and diversity of herbaceous plants and microbial communities, to answer the following questions: (1) do dominant woody plants affect edaphic properties and the composition and diversity of herbaceous plants and microbial communities along the successional gradient? (2) whether and to which extent a dominant woody plant accelerates or decelerates microbial community succession via changes in herbaceous species and edaphic properties along the successional gradient? (3) do community succession rules differ between bacterial and fungal communities?

2. Methods

2.1. Study area, experimental design, and field sampling

The study area is located at Pailugou Basin (38.55E, 100.29N) in the Qilian Mountains, near Zhangye City, in northwestern China. According to 1951–2012 records from the nearest meteorological station (Zhangye Station, 100.37E, 38.56N, 1480 m AMSL), the mean annual air temperature in the study area was between 5 and 6 °C with the mean monthly maximum temperature in July. The mean annual total precipitation was between 480 and 500 mm which mostly happened between May and September. The mean annual soil temperature of the study site was 2.5 °C. In the study area, spruce forests are distributed within the elevation range of 2680–3200 m (Yang et al., 2017).

A chronosequence of woody encroachment due to grazing abandonment (i.e., secondary succession) was selected in a north-facing slope at the elevation between 2900 and 3000 m, with an alpine meadow at the bottom, shrublands (*Dasiphora fruticosa*) at a lower position, and spruces (*Picea crassifolia*) forest at a higher position. The government had planned to build a conservation area since the 1960s and the Gansu Province Academy of Qilian Water Resource Conservation Forests Research Institute was established in 1978, which extensively reduced grazing activities. Moreover, Tibetan Plateau has experienced significant warming since the 1950s. These combined effects resulted in a shift from grass-dominated ecosystems to shrub- and small tree-dominated ecosystems. Five stages were selected along the chronosequence for the succession treatment, namely meadow, small shrubs (~5 years), big shrubs (~20 years), small trees (~50 years), and big trees (forest; ~150 years). The distance between succession treatment was 10 m, 40 m, 100 m, and 400 m, respectively. The neighbor effect was examined by comparing samples below natural patches of the dominant woody plants and in small gaps between patches of woody plants (less than five meters).

Field sampling was performed on July 28, 2016. Quadrat survey (40 cm × 40 cm) was performed to investigate herbaceous plant composition under dominant woody plants and in gaps. Each individual of herbaceous species was identified at the species level and the aboveground parts of each herbaceous species were collected separately in each plot and oven-dried for three days at 80 °C before weighting. During harvest, the height and canopy dimensions were recorded for each shrub and tree individuals. Within each quadrat, five soil cores (0–15 cm) were collected and pooled into a single sample. Five replicates (the distance between two close replicates was at least 10 m) were applied for both plants investigation and soil sampling. In total, 45 quadrats (5 in meadow and 10 in each succession stages) were investigated and 45 soil samples were collected.

2.2. Measurement of soil characteristics

The pH values were analyzed in 1:5 soil: water mixtures with a pH meter (PT-10, Sartorius, Göttingen, Germany). Electric conductivity (EC) was measured with a conductivity/TDS meter. Total organic carbon (TOC) and total nitrogen (TN) contents were quantified with an automatic element analyzer (Elementar Vario-EL, Germany). The soil water content of each sample was determined gravimetrically after drying the soil in an oven at 105 °C for 12 h.

2.3. DNA extraction, PCR amplification, and high-throughput sequencing

Genomic DNA of soil microbes was extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The quality and quantity of extracted DNA were assessed by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). The PCR amplification followed the protocol described on the website of the Earth Microbiome Project (<http://www.earthmicrobiome.org>). Briefly, primers 515F-806R and ITS1f-ITS2 were used to target the 16S rRNA gene and the fungal ITS gene, respectively. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, U.S.). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform according to the standard protocols.

2.4. Sequencing and data processing

Raw FASTQ files were de-multiplexed and quality-filtered using QIIME (version 1.9.1) (Caporaso et al., 2010) with the following criteria: (i) The 300-bp reads were truncated at any site that obtained an average quality score of < 20 over a 10-bp sliding window, and the truncated reads shorter than 50 bp were discarded; (ii) exact barcode matching, two nucleotide mismatch in primer matching, and reads containing ambiguous characters were removed; (iii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. Reads that could not be assembled were discarded. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE (version 8.0.1517) (Edgar, 2010), and chimeric sequences were removed using UCHIME. The taxonomy of each 16S rRNA gene and ITS sequence was analyzed against the Silva and Unite database at a confidence threshold of 70%, respectively. The rarefaction analysis based on Mothur v.1.21.1 was conducted to reveal the diversity indices, including the Chao, Shannon, and coverage indices. The raw sequence reads data have been deposited in the National Genomics Data Center under the GSA accession number CRA004278 that is publicly accessible at <https://ngdc.cnpc.ac.cn/gsub/submit/gsa/subCRA005845/finishedOverview>.

2.5. Quantification of diversity and dissimilarity

Species richness and Shannon diversity index were used to quantify the diversity of herbaceous, bacterial, and fungal communities. To characterize the dissimilarity of herbaceous plant, bacterial and fungal community composition between the meadow and a given stage, Bray-Curtis dissimilarity index based on species relative abundance per plot was used. The Euclidean dissimilarity index was used to characterize the dissimilarity of edaphic properties between the meadow and a given stage.

2.6. Statistical analyses

To meet the assumptions of normal distribution and variance homogeneity, log-transformation and Tukey's Ladder of Powers transformation were used when necessary. However, residuals of herbaceous, bacterial, fungal, and edaphic dissimilarity did not meet the assumptions of parametric analysis after transformation. Thus, two-way permutation tests were used to assess the effects of the stage, neighbor, and their interaction on the dissimilarity indices. Two-way ANOVAs were used to assess the effects of the stage, neighbor, and their interaction, on remaining indices. Two-way ANOVAs with Tukey tests and two-way permutation tests with pairwise permutation tests were used to test for differences in each index among treatments.

Structural equation modeling (SEM) was used to explore the effects of the neighbor on bacterial and fungal dissimilarity between stages through herbaceous and edaphic dissimilarity between stages along the successional gradient. We conducted SEM according to a priori model (Fig. S1) with the following premises in each stage: (1) the presence of dominant woody plants could modify microenvironmental conditions thereby affecting herbaceous, bacterial, and fungal communities and edaphic properties. Thus, the presence of dominant woody plants may either increase or decrease herbaceous, bacterial, fungal, and edaphic dissimilarity between stages; (2) changes in herbaceous and edaphic dissimilarity between stages can also impact bacterial, and fungal dissimilarity between stages. To account for potential unmeasured effects of the distance and avoid fit full models, we added the variable "Distance" (Fig. S1) to the model. This variable describes the distance between each paired plot, therefore serving as an indicator for relative plot position on the gradient.

Although many indices could be used to test the goodness of SEMs, there is no universally accepted single index. Hence, we used a combination of χ^2 test, root mean square error of approximation (RMSEA) test, and comparative fit index (CFI) to assess the goodness of SEMs. A nonsignificant χ^2 and RMSEA test, and CFI > 0.9 indicate a good fit of the model to the data (Kline, 2011).

Finally, the effects of herbaceous and edaphic dissimilarity between stages on bacterial and fungal dissimilarity between stages were extracted from each SEM, and the relationship between the effects and the successional gradient were fitted with linear regression.

All data were analyzed using R software, version 3.5.3 (R Core Team, 2019). Shannon diversity, dissimilarity indices, and the permutation test were conducted with the 'lmPerm' package (Wheeler and Torchiano, 2016) and 'rcompanion' package (Mangiafico and Mangiafico, 2017), while the SEMs were conducted using the 'lavaan' package (Rosseel, 2012).

3. Results

3.1. The effects of successional stages and dominant woody plants on herbaceous and microbial communities and edaphic properties

The richness and Shannon diversity of herbaceous species were strongly affected by the successional stage, with a regular decrease in diversity from the meadow to the big trees (Fig. S2a, b). Additionally, the Shannon diversity was significantly affected by the presence of dominant woody plants, with a lower diversity below woody plants (Fig. S2b). The richness and Shannon diversity of bacteria and fungi were only significantly affected by the successional stage, due to lower diversity in the stage of 'big trees' (Fig. S2c-f).

Differences in edaphic properties among successional stages were only observed for conductivity, organic carbon, and C:N ratio, mainly due to differences between the meadow and the stage of 'big trees' (Table S1).

3.2. The effects of successional stages and dominant woody plants on bacterial and fungal dissimilarity

Stage and neighbor affected microbial dissimilarity between stages, with an increase in dissimilarity along succession, in particular at the last stage, and with the presence of dominant woody plants (Fig. 2a). Fungal dissimilarity between stages also increased along succession and with the presence of dominant woody plants, except in the ‘big trees’ stage, where the presence of dominant woody plants inversely decreased dissimilarity (Fig. 2b, highly significant stage by neighbor interaction).

Similar results were observed for plant and soil dissimilarity, with even stronger differences among treatments than for microbial communities, in particular for the neighbor treatment (Fig. S3).

3.3. The main driver of dissimilarity between stages along the successional gradient

The SEM explained 19.9%, 23.6%, 46.1%, and 80.5% of the variations in dissimilarity between stages along succession for bacteria and 35.6%, 14.7%, 43.1%, and 50.4% for fungi (Fig. 3). Between the two first stages (meadow to small shrubs), the bacterial and fungal dissimilarity between stages both directly increased with the presence of small shrubs. Additionally, the fungal dissimilarity between stages was negatively affected by the small shrubs via herbaceous dissimilarity between stages (Fig. 3a). Between the second and third stages (small to big shrubs), the bacterial dissimilarity between stages was only indirectly decreased by big shrubs via an increase in herbaceous dissimilarity between stages, while neither direct nor indirect effects were observed for the fungal dissimilarity between stages (Fig. 3b). Between the third and fourth stages (big shrubs to small trees), the presence of small trees directly decreased the bacterial dissimilarity between stages, but indirectly increased the bacterial dissimilarity between stages through increasing herbaceous dissimilarity. Moreover, the fungal dissimilarity between stages was only indirectly decreased by the small tree through increasing edaphic dissimilarity between stages (Fig. 3c). Between the fourth and last stages (small to big trees), both bacterial and fungal dissimilarity between stages were directly decreased by the presence of big trees, but indirectly increased via increasing herbaceous dissimilarity between stages (Fig. 3d).

Overall, the effects of the herbaceous dissimilarity between stages on both bacterial and fungal dissimilarity between stages increased along the successional gradient (Fig. 4). However, effects of the edaphic dissimilarity between stages did not affect bacterial dissimilarity between stages and decreased fungal dissimilarity along the successional gradient (Fig. 4).

4. Discussion

Our study showed that the presence of dominant woody plants had a stronger impact on the composition than the diversity of microbial communities. Additionally, there were different pathways implementing the impact of the dominant woody plants on dissimilarity in the composition of bacterial versus fungal communities. The bacterial dissimilarity between stages was only associated with changes in herbaceous dissimilarity, due to the presence of dominant woody plants, with increasing importance along the

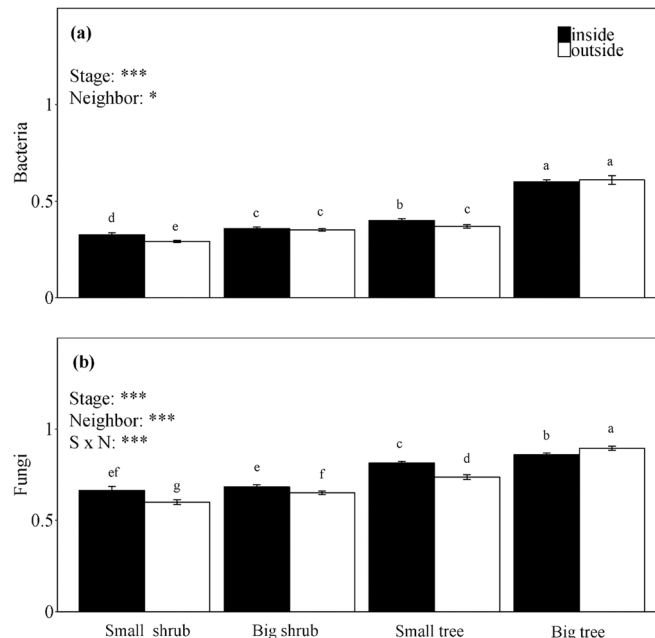


Fig. 2. Bacterial (a) and fungal (b) dissimilarity (mean \pm SE) between successional stages. Results of two-way ANOVAs on the effect of stage, neighbor, and their interaction are shown in each graphic. ***: $P < 0.001$, *: $P < 0.05$. For each graphic, different lower case letters among treatments indicate significant differences ($P < 0.05$, Tukey's HSD tests).

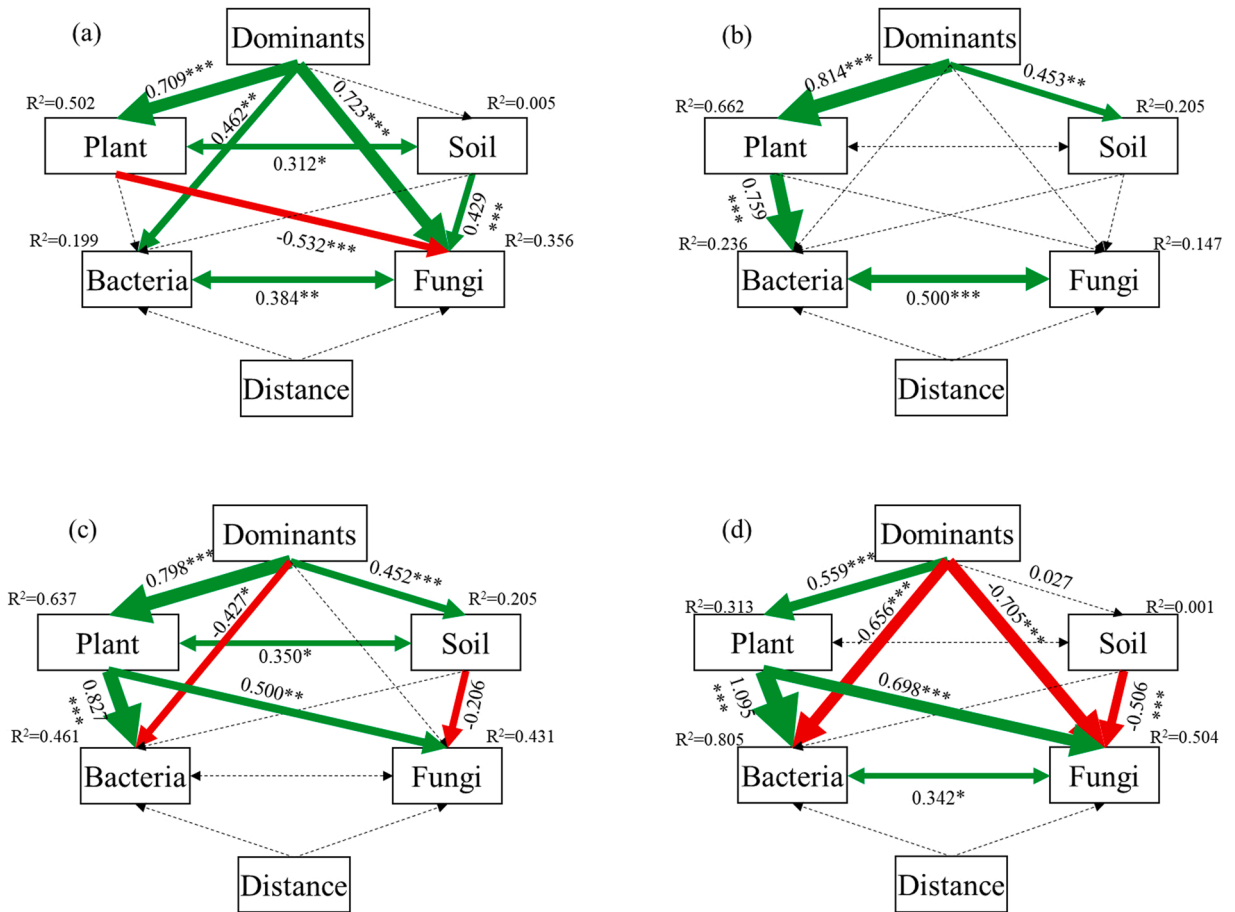


Fig. 3. Structural equation modeling of direct and indirect neighbor effects on bacteria and fungal dissimilarity between successional stages, through the mediation of plant and soil dissimilarity. $df = 1$, $P_{\text{Chi-square}} = 0.612$, $CFI = 1.000$ and $P(\text{RMSEA}) = 0.612$ for (a, between meadow and small shrubs); $df = 1$, $P_{\text{Chi-square}} = 0.258$, $CFI = 0.993$ and $P(\text{RMSEA}) = 0.298$ for (b, between small and big shrubs); $df = 1$, $P_{\text{Chi-square}} = 0.989$, $CFI = 1.000$ and $P(\text{RMSEA}) = 0.986$ for (c, between big shrubs and small trees); $df = 1$, $P_{\text{Chi-square}} = 0.713$, $CFI = 1.000$ and $P(\text{RMSEA}) = 0.708$ for (d, between small and big trees). Green and red solid arrows indicate significantly positive and negative effects (at the level $P < 0.05$), respectively. Black dashed arrows indicate non-significant effect (at the level $P > 0.05$). Values associated with solid arrows represent standardized path coefficients which are also indicated by arrow width. R^2 values associated with response variables indicate the proportion of explained variation by relationship with other variables. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. Plant: herbaceous dissimilarity between stages; soil: edaphic dissimilarity between stages; bacteria: bacterial dissimilarity between stages; fungi: fungal dissimilarity between stages.

successional gradient. By contrast, the fungal dissimilarity between stages was mediated by both herbaceous (positively) and edaphic (negatively) dissimilarity, themselves induced by the presence of dominant woody plants and with also increasing importance along the successional gradient. Our study highlighted that the presence of dominant woody plants has important consequences on the succession of microbial communities through changes in aboveground-belowground linkages.

Both herbaceous and microbial diversity decreased along the successional gradient, indicating that soil microbial community structure paralleled changes in plant communities along the successional gradient, consistent with previous studies (Frouz et al., 2008; Williams et al., 2013; Zhang et al., 2016; Shao et al., 2019). For example, Harantová et al. (2017) have shown that there was a close association between fungal communities and vegetation during primary succession. Although some studies have found that neighbor plants tend to facilitate the establishment of different soil microbial communities with higher microbial abundance and activity compared to gaps in arid and semi-arid ecosystems (Hortal et al., 2013; Rodríguez-Echeverría et al., 2016), microbial α -diversity was not affected by the presence of neighbor plants in our study.

In contrast to the microbial α -diversity, significant changes in microbial dissimilarity in composition were observed as a result of the presence of dominant woody plants along the successional gradient. Specifically, increased and decreased microbial dissimilarity between stages were found in early and late stages, respectively. Although microbial communities are known to vary along successional gradients (Chabrierie et al., 2003; Zhang et al., 2016; Zhou et al., 2017; Chang and Turner, 2019) and can be changed by the presence of dominant woody plants (Hortal et al., 2013), our study provides evidence of the role of dominant woody plants in driving the succession of microbial communities at the earliest successional stages. The effects of dominant woody plants on microbial dissimilarity between stages can be attributed to the turnover of microbial communities because there were no significant changes in

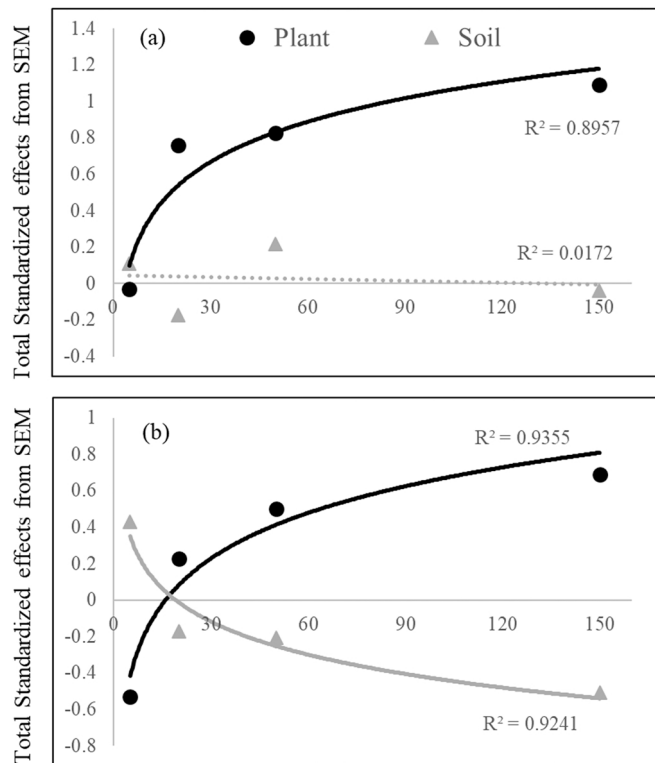


Fig. 4. Linear regressions of total effects of herbaceous and soil on bacterial (a) and fungal (b) dissimilarity between successional stages. Solid lines indicate significant relationships (at the level $P < 0.05$), while dashed lines indicate non-significant relationships (at the level $P > 0.05$).

species diversity.

Our SEMs provide insight into the pathways through which the presence of dominant woody plants resulted in changes in microbial dissimilarity between stages along the successional gradient. The direct effects of dominant woody plants on microbial (for both bacteria and fungi) dissimilarity between stages switched from positive to negative along the successional gradient. Previous studies have shown that the dissimilarity of microbial communities generally increased along the successional gradients (Cui et al., 2019; Sui et al., 2021), but few of them have compared the microbial dissimilarity beneath woody species to that outside woody species like ours. Our result showed that the dominant woody plants accelerated the succession of microbial communities in early stages but decelerated it in late stages, which is likely due to the varying effect of dominant woody plant cover on the homogeneity of the environment along the successional gradient. In the early stages, dominant woody plants generally increase the heterogeneity of habitats, whereas in late stages they decrease heterogeneity because of their increasing cover with important roles in ecosystems. For instance, De Frenne et al. (2019) showed that tree canopies could buffer environmental changes (Frey et al., 2016; Latimer and Zuckerberg, 2017; Wang et al., 2020).

By contrast, the indirect effects of dominant woody plants on the dissimilarity of bacteria and fungi were different. Specifically, the bacterial dissimilarity between stages was only related to the herbaceous dissimilarity between stages, whereas the fungal dissimilarity between stages was related to both the herbaceous and edaphic dissimilarities between stages. Moreover, we found an increasingly positive relationship between ‘the herbaceous dissimilarity between stages’ and ‘the microbial dissimilarity between stages’ along the successional gradient (Figs. 2 and 3), which is consistent with previous studies. For example, Prober et al. (2015) found significant positive relationships between soil microbial dissimilarities and plant dissimilarities, i.e., the more distinct plant communities the more distinct soil microbial communities. Furthermore, our study also indicated that the herbaceous dissimilarity between stages was more important than the edaphic one in driving microbial succession along the secondary successional gradient. This is likely due to that the plant species have a strong ability to select microbes, either via root symbionts or litters (Ayres et al., 2009; Bonito et al., 2014; Uroz et al., 2019). However, relationships between ‘the herbaceous dissimilarity between stages’ and ‘the fungal dissimilarity between stages’ were generally weaker than that between ‘the herbaceous dissimilarity between stages’ and ‘the bacterial dissimilarity between stages’, which is inconsistent with previous studies that fungi are often more directly dependent on plant species (Broeckling et al., 2008; Millard and Singh, 2010; Harantová et al., 2017; Wang et al., 2020).

5. Conclusion

Our study allows us to disentangle the direct and indirect effects of dominant woody plants, on changes in composition and

diversity of microbial communities along successional gradients. Although the presence of dominant woody plants has weak effects on the α -diversity of microbial communities, the dominant woody plants have strong effects on the microbial dissimilarity in composition between stages. Moreover, our results stress that herbaceous communities are more important than edaphic properties in driving the effect of dominant woody species on microbial communities during succession. In the background of woody encroachment in alpine meadows, our findings, therefore, are crucial for predicting the consequences of increasing woody encroachment for microbial communities, as well as ecosystem functioning.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2021.e01852](https://doi.org/10.1016/j.gecco.2021.e01852).

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