# Plant-microbial feedback in secondary succession of semiarid grasslands

Jiaoyang Zhang [a,c], Zemin Ai [b], Hongwei Xu [a,c], Hongfei Liu [a,c], Guoliang Wang [a,c], Lei Deng [a,c], Guobin Liu [a,c], Sha Xue [a,c,*]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China

[b] College of Geomatics, Xi'an University of Science and Technology, Xi'an, Shaanxi 710054, People's Republic of China

[c] Institute of Soil and Water Conservation, Chinese Academy of Sciences & Ministry of Water Resources, Yangling, Shaanxi 712100, People's Republic of China

## HIGHLIGHTS

- Soil conditioned by pre-successional species had a positive feedback on the future plant species.
- The feedback of different successional species to soil microbial communities was mainly positive.
- The main microbial groups affecting the replacement of species during succession varied across taxonomic levels.

## GRAPHICAL ABSTRACT
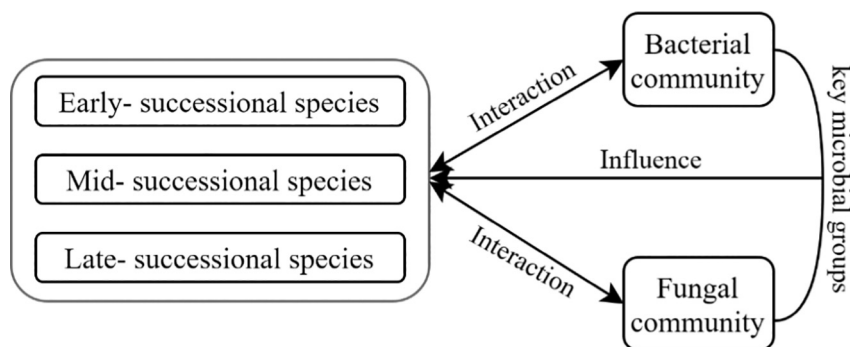


## ARTICLE INFO

## ABSTRACT

Plant–soil feedback (PSF) is an important driver of plant community dynamics. The role of plant species in PSF has been emphasized for secondary succession processes; however, microbial responses to PSF and the underlying mechanisms responsible for their effects on plant succession remain poorly understood, particularly in semiarid grassland ecosystems. Here, we conducted a greenhouse experiment using soil collected from early-, mid-, and late-successional plant communities to measure net pairwise PSF for species grown under monoculture. Soils conditioned by pre-successional species had a positive feedback effect on subsequent plant species, whereas soil conditioned by subsequent plant species had a negative feedback effect on pre-successional species. The feedback effect of plants from different successional stages on soil bacterial and fungal communities was mainly positive. However, the bacterial genera in the soil conditioned by early- and mid-successional species and fungal classes in the soil conditioned by early- successional species had a negative feedback effect on late-successional species. Thus, the effects of soil fungal and bacterial communities on species in other successional stages varied with taxonomic level. Our results provide insight into the manner in which soil microbial communities influence PSF responses during secondary succession processes.

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## 1. Introduction

Ecological succession, which refers to the sequential replacement of species over time or after disturbance (i.e., any relatively abrupt loss of biomass or structure), has become increasingly important due to increasing human disturbance, making it a core issue in ecological research (Prach and Walker, 2011). As an important part of ecological

* Corresponding author at: Xinong Rd. 26, Institute of Soil and Water Conservation, Yangling, Shaanxi 712100, China.
   E-mail address: xuesha100@163.com (S. Xue).

succession, secondary succession occurs when the original plant community in a given ecosystem is gradually reconstituted through the sequential replacement of species after the original vegetation has been disturbed. This phenomenon is a major object of attention in natural-resources management (Kardol et al., 2006; Zhou et al., 2017). However, the mechanisms underlying species replacement during secondary succession remain controversial in ecology (Legendre, 2014; Koziol and Bever, 2016).

Plant-soil feedback is an effective approach to the evaluation of secondary succession processes. Plant-soil feedback (PSF) comprises plant-induced changes in soil biotic and abiotic characteristics that affect the performance of other plants that grow later in the soil (Bever et al., 1997; van der Putten et al., 2013). Feedback is defined as positive (or negative) when plants change the soil environment, then enhancing (or inhibiting) the growth of individual plants in intraspecific-cultivated soils compared to interspecific-cultured soils (Bever et al., 1997; Brinkman et al., 2010). Variation in PSF has been widely demonstrated during vegetation succession (Kardol et al., 2006; Kardol et al., 2007; Jing et al., 2015). Early-successional species tend to experience strongly negative PSF, which accelerates the replacement of plant species, although the advantages are temporary. Neutral PSF occurs in mid-successional species, while positive PSF occurs in late-successional species, with succession decelerating, thereby leading to the stability and persistence of certain species (Klironomos, 2002; Bever, 2003; Kardol et al., 2006; van de Voorde et al., 2011; Kardol et al., 2013; Jing et al., 2015). Positive PSF in mid-successional plant species and neutral or negative PSF in late-successional plant species have also been documented (Jing et al., 2015). It is noteworthy that positive or negative PSF experienced by species at the same succession stage has led to controversy among studies. Pairwise-feedback trials might be used to study this issue. For instance, when two species at different succession stages grow in soil cultured by their own and each other's species, net pair PSF could be obtained. This information may be used to predict the competitive exclusion or coexistence of certain plant species, thus allowing the direction of species succession to be elucidated (Bever, 2003; Kulmatiski et al., 2010).

The interaction between soil microbial communities and plants (i.e., plant-soil microbial feedback) is increasingly recognized as a key factor in promoting and maintaining ecosystem functions (De Deyn et al., 2003; Kardol et al., 2013; Lozano et al., 2019). Previous studies showed that the legacy of PSF effects is largely driven by changes to soil microbial communities, which have both short- and long-term impacts on subsequent plant communities (Kardol et al., 2013; Bailey and Schweitzer, 2016). Examples include plant growth promotion, changes in community composition, and in succession dynamics (Klironomos, 2002; Bever et al., 2015), which ultimately affect plant phenotypes and genotypes. These parameters represent important driving forces of changes in vegetation (Bever, 2003; De Deyn et al., 2003). Consequently, understanding how soil microbial communities contribute to plant communities during succession and development has become a subject of broad and current interest in ecology (Kardol et al., 2006; Bever et al., 2010; Kardol and Wardle, 2010).

The direction of PSF effects in controlling the soil microbial community seems to vary among plant functional groups (Kulmatiski et al., 2010), thereby leading to either positive or negative effects on plant performance (Klironomos, 2002; Bever, 2003; Rodríguez-Echeverría et al., 2013). Plant-microbial feedback might occur at a local scale and tends to be measured at the single plant scale (Bever et al., 2010). In combination with species succession, a negative PSF experienced by early-successional plant species might be related to the accumulation of host-specific plant pathogens (Kardol et al., 2006; Kulmatiski et al., 2010). In comparison, arbuscular mycorrhizal fungi might lead to the positive PSF experienced by late succession species (Koziol and Bever, 2016; Koziol and Bever, 2019). However, our current knowledge of the effects of microbial communities on plant community succession (Pendergast et al., 2013; Koziol and Bever, 2019), and how the microbial communities

change under plant feedback is limited (Laurent et al., 2013; Zhou et al., 2017). In particular, secondary succession in arid environments is driven by the interactions between plant and soil microbial communities, but greater research focus on these interactions and associated outcomes is required (Lozano et al., 2014). In addition, how various microorganisms in the soil affect (drive and alter) or respond to feedback needs further investigation. Assessing key bio-communities and the interactions that promote ecosystem development during secondary succession could help practitioners allocate limited resources for ecosystem restoration more effectively (Kardol and Wardle, 2010).

Here, we conducted a PSF experiment using rhizosphere soils conditioned by early-, mid- and late-successional species to test how plant productivity is influenced by the soil community from different successional stages. Previous studies showed that early-successional species usually experience negative PSF, while late-successional species usually experience positive PSF (Klironomos, 2002; Kardol et al., 2006; Kardol et al., 2013; Jing et al., 2015). Thus, we first hypothesized that a soil conditioned by pre-successional species has a positive feedback on subsequent successional species, and consequently, the feedback is negative. Our second hypothesis was that pre-successional species (early- or mid-successional species) have a negative feedback on soil bacterial and fungal communities of subsequent successional species (mid- or late-successional species). Conversely, they have a positive feedback because it is generally assumed that early-successional species are less dependent on the soil microbial community, whereas late-successional species are more dependent on a positive relationship with this community (Kardol et al., 2006; Zhang et al., 2016b; Koziol and Bever, 2019). Additionally, we explored whether the response of bacterial and fungal communities is the same across different taxonomic levels (phylum, class, order, family, and genus of the microbial community). Our results will be used to elucidate the key microbial groups that are closely related to species succession and will demonstrate that the soil microbial community is one of the main driving factors of PSF.

## 2. Materials and methods

The plant species used for the PSF experiments reported herein are constituents of the typical vegetation growing in the Loess Hilly Region of China after croplands are abandoned for natural recovery. *Setaria viridis*, *Artemisia sacrorum*, and *Bothriochloa ischaemum* were selected as early-, mid-, and late-successional species representative of plant species replacement along secondary successional gradients, respectively. The sampling sites (minimum 20 m × 20 m, long-term monitored grassland plots) for these three plant species (croplands with the same farming history had been abandoned at different periods) were located at the Ansai Research Station (ARS) of the Chinese Academy of Sciences (36°51′N, 109°19′E; 1068–1309 m a.s.l.), Shaanxi Province, China, in April 2015. The precise choice of sites was based on a literature review and an inquiry among the staff at the station. Average annual temperature in the area is 8.8 °C (the extreme maximum and minimum temperatures are 36.8 and − 23.6 °C, respectively); average annual precipitation is 505.3 mm, and the annual frost-free period is 157 days. After investigating each site in detail, rhizosphere soils of the three plant communities were collected at 5–20 cm depth (van de Voorde et al., 2011; Jing et al., 2015). The collected soil was transported to the Ansai Research Station, and the ice bags were replaced in a timely fashion to keep the soil at 4 °C. Finally, the soils from the three plant communities were transported to the Institute of Soil and Water Conservation for the greenhouse experiment.

### 2.1. Greenhouse experiment

To test our hypotheses, we conducted a greenhouse PSF experiment at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau in Yangling, Shaanxi Province, China (34°12′N, 108°7′E, 530 m a.s.l.). The average annual temperature at this laboratory is 12.9 °C, with a

frost-free period of 211 days and average annual precipitation of 637.6 mm.

The sampled soil was sieved (<0.5 cm) to remove coarse fragments and then homogenized. The soils from the same plant species were thoroughly mixed and pots (20 cm × 15 cm) (Pendergast et al., 2013) filled with them, each with 5 kg soil weight per pot (on a dry weight basis). Thirty-five pots were filled with the soil from each plant species; 10 pots were planted with *S. viridis*, *A. sacrorum*, and *B. ischaemum*, respectively, and five pots were used as blank controls with no plants. Overall, the experiment included 3 soil treatments × 3 plant species × 10 replicates +3 soil treatments (blank control) × 5 replicates. Seeds of the three plant species were collected from the ARS the preceding year and were sown in the corresponding pots in early May. Based on a field investigation, plants were removed from each pot to keep the same number for the same plants during the first 2 weeks after planting. Then, from June until September, all pots were randomly repositioned monthly and watered regularly without fertilizer application. The soil water content in each pot was checked (80% of field capacity) twice a week by weighing the pots.

Four pots containing plants were randomly selected from 10 replicates of each treatment for sampling in early September. Aboveground plant biomass was clipped at the soil surface, and all roots were extracted from the soil of each pot and rinsed with distilled water. Plant biomass was measured separately, as leaf, stem, and root biomass for each pot. The soil samples were sieved through a 2 mm mesh and then divided into three subsamples. The subsample used for genetic analyses were immediately stored at −80 °C. One subsample was stored at 4 °C and used for the determination of soil water-soluble nutrients, while the third subsample was air-dried and used for the determination of soil organic carbon and other nutrients. Plant biomass was determined by oven-drying at 70 °C to a constant weight.

### 2.2. Soil DNA extraction, amplification, and sequencing

To determine the diversity and composition of the bacterial and fungal communities in each sampled soil type, a TIANamp Soil DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) was used to extract total soil DNA according to the manufacturer instructions. After a 10-fold dilution, the quality and quantity of the extracted DNA were confirmed by 1% agarose gel electrophoresis and checked using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). For bacteria, the V3–V4 region of the 16S rRNA gene was amplified using the PCR-based protocol described in Caporaso et al. (2012). EMP primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) were used for PCR amplification. For fungi, the internal transcribed spacer (ITS) region of DNA was amplified using the primers ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (Lu et al., 2013). The PCR reaction contained 2 μl of sterile ultrapure water, 15 μl of Phusion Master Mix (2×), 3 μl of 6 μM primers, and 10 μl of DNA template (5–10 ng). The thermal cycling conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 5 min. Triplicate PCR amplicons were pooled and then mixed with the same volume of 1 × loading buffer. The PCR amplification was verified by 2% agarose gel electrophoresis. Amplification products were purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific) and then pooled in equal molar quantities for library construction. The 16S rRNA and ITS amplicons were sequenced using the Illumina HiSeq2500 (Illumina, USA) platform at Novogene Bioinformatics Technology Co. Ltd. (Beijing, China), and 250 bp paired-end reads were generated.

High-quality clean reads were generated under specific filtering conditions according to QIIME (Quantitative Insights into Microbial Ecology) (Caporaso et al., 2010; Bokulich et al., 2012). The effective reads were obtained by using the UCHIME algorithm after removing the chimeric sequences (Edgar et al., 2011; Haas et al., 2011). Effective

reads were assigned to the same operational taxonomic units (OTUs) at ≥97% sequence similarity by the UPARSE software (UPARSE v7.0.1001) (Edgar, 2013). Bacterial and fungal diversity was estimated by calculating the Shannon and Simpson indices of OTUs per sample. Richness was estimated from Chao1 and ACE indices estimated using the ESTIMATES software package (version 8.00, R. K. Colwell) (Hill et al., 2003; Tedersoo et al., 2010).

### 2.3. Calculations

The PSF index was calculated in paired comparisons (Brinkman et al., 2010; Baxendale et al., 2015) according to the following equation:

$$PSF_B \text{ index} = (Biomass_1 - Biomass_2)/Biomass_2 \qquad (1)$$

where, $Biomass_1$ is the total (leaf, stem, or root) plant biomass in its own soil conditions, $Biomass_2$ is the total (leaf, stem, or root) plant biomass of the same species in foreign soil conditions. A positive $PSF_B$ index value indicated that the foreign soil conditions inhibited the growth of the target plant, and a negative $PSF_B$ index value indicated that the foreign soil conditions promoted the growth of target plants.

To test the responses of soil microbial communities to PSF (hypothesis 2), according to Eq. (1), the PSF index of soil microbial communities was calculated as:

$$PSF_M \text{ index} = (M_1 - M_2)/M_2 \qquad (2)$$

where, $M_1$ and $M_2$ represent the two plant species planted in different pots with same soil conditions, respectively. $M_1$ is the bacterial (fungal) diversity index (Shannon, Simpson, Chao1, and ACE) or relative abundance (phylum, class, order, family, and genus) of the soil community upon the growth of its own plants, while $M_2$ is the bacterial (fungal) diversity index (Shannon, Simpson, Chao1, and ACE) or relative abundance (phylum, class, order, family, and genus) of the soil community upon the growth of plant species at different succession stages. A positive $PSF_M$ index value indicated that the plant species at different succession stages reduced the abundance or diversity of the soil microbial communities, while a negative $PSF_M$ index value indicated that the plant species at different succession stages increased the abundance or diversity of the soil microbial communities.

### 2.4. Data analysis

One-way analysis of variance followed by Duncan's multiple-range test ($P < 0.05$) was used to test the effect of PSF on plant biomass and the soil microbial community using the statistical software SPSS 20 (IBM, New York, NY, USA). Spearman correlations and constrained redundancy analysis (RDA) were performed to test the relationships of microbial community characteristics and plant biomass using the statistical software SPSS 20 and CANOCO 5.0 (Microcomputer Power, Ithaca, NY, USA). All graphs were plotted using SigmaPlot 12.5 (Systat Software, San Jose, USA).

## 3. Results

### 3.1. PSF index of plant biomass

Soil feedback effects were significant in the pairwise feedback trials, except for the effect of soils conditioned by late-successional species on mid- successional species (Fig. 1). The PSF index value calculated from the plant biomass of early-successional species was positive in both the soils conditioned by mid- and late-successional species. In comparison, the PSF index calculated using the plant biomass of mid-successional species in soil conditioned by the early-successional species was negative, along with the PSF index value calculated using the plant biomass of late-successional species in soil conditioned by early- and mid-successional species. The responses of the leaf, stem, and root
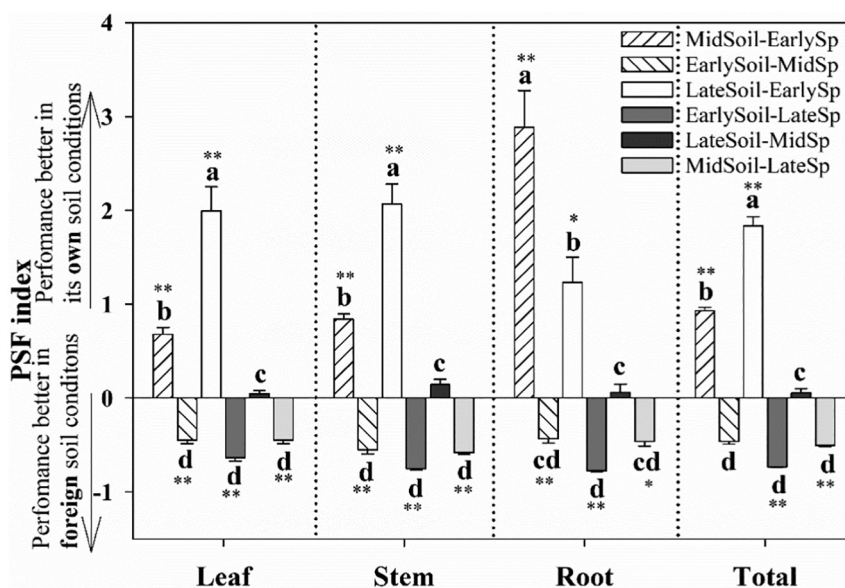
**Fig. 1.** Plant–soil feedback (PSF) index values calculated using the biomass of early-, mid- and late-successional species grown under monocultures using a soil conditioned by these plant species communities. Plant–soil feedback values indicate how soil conditioning affects plant growth, with positive values indicating better performance in its own soil conditions, and negative values indicating better performance in foreign soil conditions. Error bars indicate $\pm$ SE ($n = 4$). Different lowercase letters indicate significant differences ($P < 0.05$) in the three pairwise feedback trials. Asterisk (*) indicates that the response differed significantly from zero: *$P < 0.05$, **$P < 0.01$. MidSoil-EarlySp, the effects of a soil conditioned by mid-successional species on early-successional species; EarlySoil-MidSp, the effects of a soil conditioned by early-successional species on mid-successional species; LateSoil-EarlySp, the effects of a soil conditioned by late-successional species on early-successional species; EarlySoil-LateSp, the effects of a soil conditioned by early-successional species on late-successional species; LateSoil-MidSp, the effects of a soil conditioned by late-successional species on mid-successional species; MidSoil-LateSp, the effects of a soil conditioned by mid-successional species on late-successional species.

biomass to soil feedback were consistent with their total biomass in early-, mid- and late-successional species.

### 3.2. PSF index of fungal and bacterial communities

The PSF index values of fungal Chao1 and ACE in soils conditioned by mid- and late-successional species were positive and changed significantly following the growth of early- and mid-successional species, respectively (Fig. 2). Similarly, the PSF index values for the Shannon and Simpson indices were positive and also changed significantly in soils conditioned by early-successional species, following the growth of late-successional species. When considering significant changes, the pairwise trials were mainly negative for PSF index values calculated using the relative abundance of fungal phylum, class, order, family, and genus in soils (Fig. 2, Table S1). Positive PSF index values were mainly found at the class level, with the corresponding values for fungal classes in soils conditioned by early-successional species following the growth of late-successional species. Positive and negative PSF index values were observed in 50% of the fungal class, order and genus for partial treatments. They were fungal class and order in soils conditioned by late- and early-successional species, following the growth of early- and late-successional species, respectively; and fungal classes and genera in soils conditioned by late-successional species following the growth of mid-successional species. The PSF index values of fungal phyla in soils conditioned by early-, mid-, and late-successional species did not change significantly following the growth of mid-, late-, and early-successional species, respectively. Neither did the corresponding values for fungal genera in soils conditioned by early-successional species change significantly after the growth of late-successional species.

With respect to soil bacterial community, only the PSF index value for Shannon, Chao1, and ACE indices in soils conditioned by mid-successional species changed significantly under the feedback of early- and late-successional specie (Fig. 3). The PSF index value of Shannon in soil conditioned by late-successional species under the feedback of mid-successional species was also negative. The PSF index calculated using the relative abundance of bacterial phylum, class, order, and family in soils for the pairwise trials were mainly negative (Fig. 3, Table S2–6). Interestingly, a very different result was obtained at the genus level, with positive and negative PSF index values of 50% obtained in the pairwise trials. These were bacterial genera in soils conditioned by early-, mid-, and late-successional species following the growth of late-, late-, and early-successional species, respectively.

### 3.3. Relationship between plant biomasses and the soil microbial community

Constrained RDA revealed the relationships between plant biomass and relative abundance (phylum level) of the soil fungal and bacterial communities (Fig. 4). *Acidobacteria*, *Actinobacteria*, and *Thermomicrobia* were the most significant group that affected the biomasses of the early-, mid-and late-successional species and explained 53.9% ($P = 0.006$), 66.4% ($P = 0.002$) and 53.9% ($P = 0.008$) of the total variance, respectively. The *Chloroflexi*, *Proteobacteria*, and *Basidiomycota* were the next most significant microbial groups affecting the biomasses of the early-, mid- and late-successional species, respectively.

## 4. Discussion

### 4.1. Plant performance was differentially altered by soil feedback

This study demonstrated that the PSF index values calculated using plant biomass of subsequent successional species revealed a positive feedback when the plants grew in a soil conditioned by the pre-successional species. In contrast, the reverse was negative feedback, supporting our first hypothesis (Fig. 1). However, countering our hypothesis, the feedback from a soil conditioned by late-successional species to mid-successional species was not significant. Consistently with previous reports, early-, mid-, and late-successional species exhibited a variety of responses to soil feedback (Jing et al., 2015); however, in contrast to previous studies (Kardol et al., 2006; Jing et al., 2015), the data reported herein showed that soil feedback on plant species, either positive or negative, was closely related to succession stage.

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Furthermore, our results indicated that soil feedback significantly affected plant growth at different successional stages. In comparison, previous studies found that species at the late-successional stage were affected by soil feedback but not those at early- or mid-successional stages (Kardol et al., 2006). The difference between the two studies might be attributed to the differences in the experimental setup (Kardol et al., 2006). Specifically, in our greenhouse experiment, the soils used were directly collected from the field, whereas the soils used in previous studies were developed under laboratory conditions.



**Fig. 2.** Plant–soil feedback (PSF) index calculated using the diversity index (Shannon, Simpson, Chao1, and ACE) and relative abundance (phylum level) of soil fungal communities of early-, mid-, and late-successional species, upon the growth of plant species at different successional stages. Error bars indicate ± SE (n = 4). Asterisk (*) indicates that the response differed significantly from zero: *P < 0.05, **P < 0.01. MidSp-EarlySoil, the effects of mid-successional species on a soil conditioned by early-successional species; EarlySp-MidSoil, the effects of early-successional species on a soil conditioned by mid-successional species; LateSp-EarlySoil, the effects of late-successional species on a soil conditioned by early-successional species; EarlySp-LateSoil, the effects of early-successional species on a soil conditioned by late-successional species; LateSp-MidSoil, the effects of late-successional species on a soil conditioned by mid-successional species; MidSp-LateSoil, the effects of mid-successional species on a soil conditioned by late-successional species. A positive PSF index value indicated that plant species reduced the abundance or diversity of the soil microbial communities, whereas a negative PSF index value indicated that plant species increased the abundance or diversity of the soil microbial communities. The same below.

When plants grow better in soil conditioned by conspecific species compared to that conditioned by interspecific species, PSF is positive; conversely, PSF is negative (Kulmatiski et al., 2010; Baxendale et al., 2015). Thus, the paired PSF could be used to predict competitive exclusion or coexistence between specific plant species (Bever, 2003; Kulmatiski et al., 2010). The feedback effect of the soil microbial community associated with late-successional species on mid-successional species was not significant (Fig. 1). This phenomenon explains the relationship whereby mid- and late-successional species coexist successfully (consistent with field survey results). Positive feedback from the soil conditioned by mid-successional species might enhance the dominance of late-successional species (Klironomos, 2002), thus accelerating the replacement of the mid-successional plants with the late-successional species (Kardol et al., 2006; Kardol et al., 2007; Kulmatiski et al., 2010; Pendergast et al., 2013). In the species succession sequence, late-successional species have a competitive advantage over pre-successional species. Concomitantly, species with competitive advantages experience significant positive feedback when growing in the soil conditioned by plants of competitor species (Pendergast et al., 2013), and the opposite was true (Kardol et al., 2006; van de Voorde et al., 2011), supporting our first hypothesis. The PSF index values calculated using the total plant biomass of early-successional species in a soil conditioned by late-successional species were more strongly positive than those in a soil conditioned by mid-successional species (Fig. 1). Therefore, the growth of early-successional species in soils conditioned by late-successional species was more severely hindered than their growth in soils conditioned by mid-successional species (Pendergast et al., 2013; Baxendale et al., 2015). Thus, our study demonstrated the direction of species succession, with positive feedback from soils conditioned by the pre-successional species on the subsequent successional species. In comparison, negative feedback was obtained from soils conditioned by the subsequent successional species on the pre-succession species, indicating that the reverse direction of species succession was effectively inhibited. Thus, phased plant-soil feedback might be important for predicting the rate of succession, which in turn might be utilized in designing ecological restoration practices (Young et al., 2005).

In contrast with a previous study, the PSF index varied consistently for leaf, stem, root, and total biomass (Baxendale et al., 2015). This contrasting result further confirmed that calculating plant feedback with total biomass concealed the influence of plant organs under soil feedback, which was not conducive to elucidating the plant response mechanism. The strong influence of soil feedback on plant performance reaffirmed the soil community feedback theory established based on monoculture (Pendergast et al., 2013).

### 4.2. Changes in microbial community during plant growth

Positive feedback from the subsequent successional species to the soil bacterial community conditioned by the pre-successional species supported our second hypothesis (Fig. 3). However, feedback from
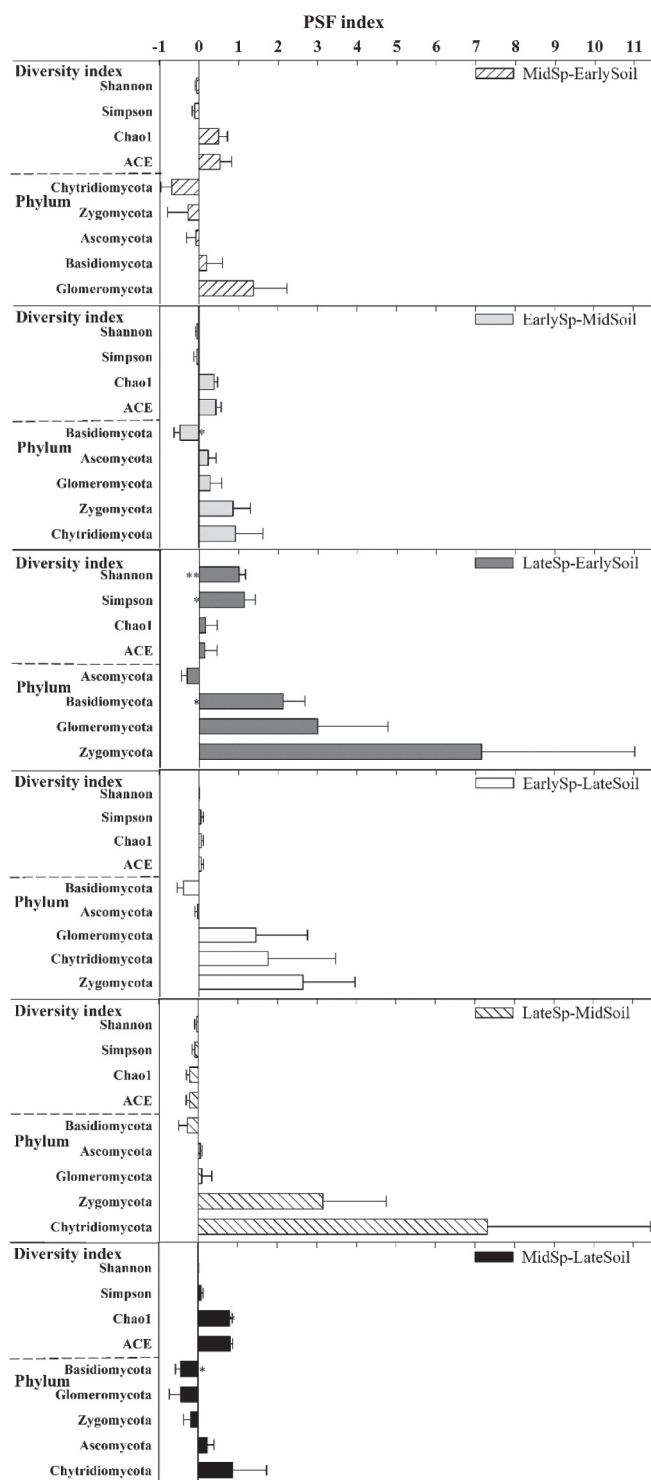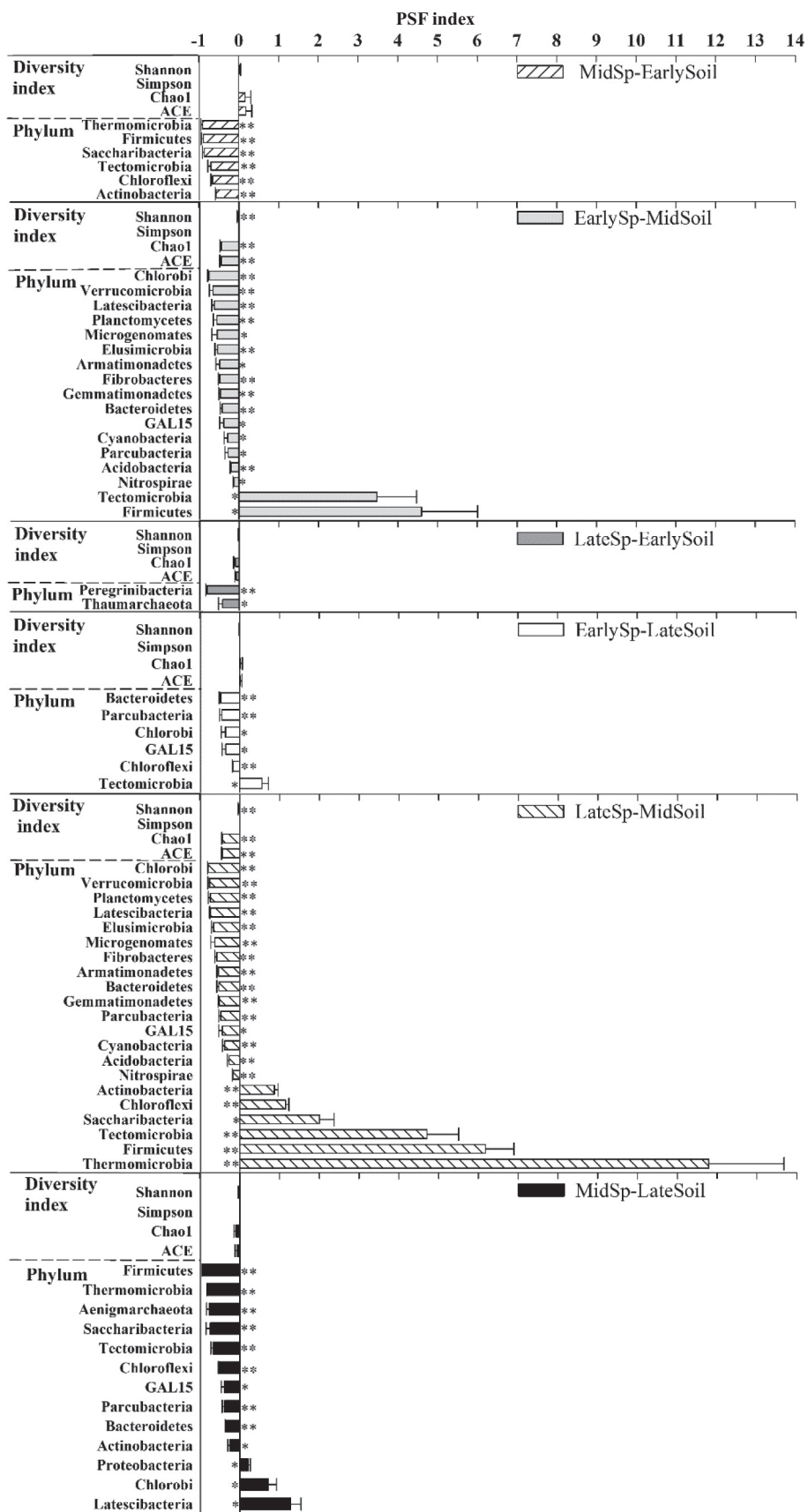
**Fig. 3.** Plant–soil feedback (PSF) index calculated using the diversity index (Shannon, Simpson, Chao1, and ACE) and relative abundance (phylum level) of soil bacterial communities of early-, mid- and late-successional species, upon the growth of plant species at different successional stages. Error bars indicate ± SE (n = 4).
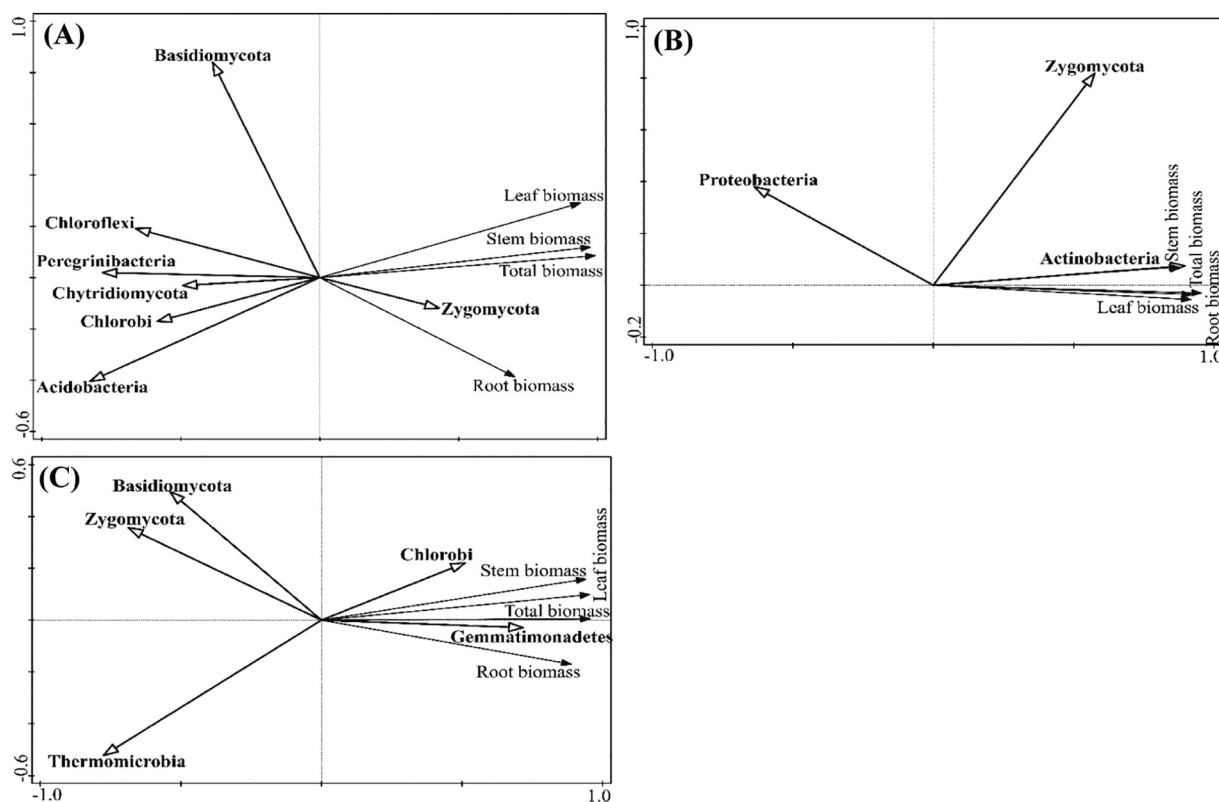
**Fig. 4.** Biplots from redundancy analysis representing the relationship between the biomasses of early-(A), mid-(B), and late-successional species(C) and relative abundance (phylum level) of soil fungal and bacterial communities.

late-successional species to the bacterial genus in soil conditioned by early- and mid-successional species was not consistent with this hypothesis (Table S4–5). Similarly, positive feedback from presuccessional species to the soil bacterial community of the subsequent successional species was not consistent with our second hypothesis, either. However, interestingly, positive feedback from early-successional species to the bacterial genera in soil conditioned by late-successional species supported our second hypothesis (Table S4). Compared with bacterial communities, the effects of plant species on soil fungal communities at different successional stages showed greater variability. Therefore, overall, our results supported the second hypothesis only partially. Similarly, previous studies partially support our findings for *Acidobacteria* and *Proteobacteria* phyla, *Bacteroidetes*, *Firmicutes*, and *Betaproteobacteria* classes (Fig. 4, Table S7) (Lozano et al., 2014; Zhang et al., 2016a; Zhao et al., 2019; Zhong et al., 2020), which may play an important role in plant-microbial feedback, especially in secondary succession. In general, the feedback of early-, mid-, and late-successional species to the soil microbial community during secondary succession was mainly positive, especially the bacterial community. However, feedback did not consistently affect the soil microbial community across all taxonomic levels.

### 4.3. Microbial community influenced species replacement during succession

It is generally assumed that early succession species experience more negative PSF (Cortois et al., 2016; Koziol and Bever, 2019). Such negative plant-microbial feedback might promote the replacement of plant species during early succession (Kulmatiski et al., 2010). In the present study, we found that the bacterial community was negatively correlated with the biomass of early-successional species, which was one of the main factors affecting biomass variation in early-successional species. The extent to which the fungal community explained the total plant biomass variables during

early succession was much less than the extent to which the bacterial communities explained those variables (Fig. 4, Table S7). This result indicated that early-successional species are less dependent on fungi, with the accumulation of fungi producing a larger positive PSF (Zhang et al., 2016b). Furthermore, positive plant-microbial feedback might strongly contribute to plant communities during early succession (Bever, 2003; Reynolds et al., 2003). We found that the bacterial community also showed positive PSF to early-successional species, despite negative plant-microbial feedback remaining the most influential feedback (Fig. 4, Table S7). Therefore, negative PSF is common in grassland ecosystems, representing an important mechanism for maintaining species diversity and promoting the replacement of species during succession (Kardol et al., 2006; Kulmatiski et al., 2010).

The plant-microbial feedback changes over the sequence of succession. It is generally assumed that late-successional species experience positive plant-microbial feedback (Van der Putten, 2003; Kardol et al., 2006), with fungal positive PSF promoting the successful replacement of late-successional species (Koziol and Bever, 2016; Zhang et al., 2016b; Koziol and Bever, 2019). Further, negative PSF is also important during late succession (Bever, 2003). In our study, the feedback of soil microbial communities to late-successional species was mostly positive (Fig. 4, Table S7). Nonetheless, it varied significantly across taxonomic levels of microbial communities. Thus, for example, bacterial communities had a positive feedback effect and were a central component of late-successional species at the class and order levels, although they had a negative feedback effect at the phylum and genus levels. On the other hand, fungal communities were the main factor affecting the changes in biomass of late-successional species at the family level.

Few studies have evaluated the feedback relationship between intermediate succession species and microbial communities. Previous studies have shown that PSF is mainly neutral for the mid-successional stage (Kardol et al., 2006), and the legacy of plant-soil

interaction is largely driven by changes in soil microbial communities (Kardol et al., 2013; Bailey and Schweitzer, 2016). Therefore, the main plant-microbial feedback of mid-successional species might be neutral. However, the RDA showed that the bacterial community remained the main variable affecting changes in mid-successional species biomass and mainly showed a positive effect except at class level (Fig. 4, Table S7). The microbial community is an important limiting factor for restoring and reconstructing local vegetation; furthermore, it is an important component affecting PSF (Kardol et al., 2007; Pendergast et al., 2013). *Acidobacteria* might represent an important microbial factor affecting the shift of early-successional species to mid-successional species. The genus *Variibacter* might strongly contribute to the shifts from early-successional to late-successional species (Fig. 4, Table S7). Furthermore, our results demonstrated that the key microbial communities affecting the succession sequence of plant populations were not always consistent across the taxonomic levels but varied between them.

## 5. Conclusions

Plant–soil feedback represents an important mechanism driving the changes in the plant community along secondary successional gradients. This study identified key fungal and bacterial populations that contribute to this process in grasslands. We showed that the main populations of soil fungal and bacterial communities affecting the replacement of plant species during succession varied across taxonomic levels. Additionally, we showed that the feedback of early-, mid-, and late-successional species to soil fungi and bacterial communities was mainly positive. Our results provide new insights into the mechanisms whereby the soil microbial community influences PSF responses in grasslands. We showed that soil conditioned by pre-succession species influenced subsequent plant growth with positive PSF index values and vice versa. Our findings lend support to the idea that the paired PSF among plant species at different stages of succession might be used to predict the direction of species replacement. Future studies should investigate the manner in which plant–soil feedback influences the mechanisms of species succession through changes in the soil microbial community under natural field conditions across ecosystems.

## CRediT authorship contribution statement

**Jiaoyang Zhang:** Investigation, Resources, Data curation, Writing - original draft. **Zemin Ai:** Conceptualization, Methodology, Writing - review & editing, Investigation, Resources. **Hongwei Xu:** Investigation, Resources. **Hongfei Liu:** Investigation, Resources. **Guoliang Wang:** Conceptualization, Methodology, Writing - review & editing. **Guobin Liu:** Conceptualization, Methodology, Writing - review & editing. **Sha Xue:** Conceptualization, Methodology, Writing - review & editing, Investigation, Resources.

## 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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## Data accessibility

Data are deposited in the Mendeley Data https://data.mendeley.com/datasets/9r6pd4mxsm/1

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