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Effects of rhizosphere interactions of grass interspecies on the soil microbial properties during the natural succession in the Loess Plateau

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ABSTRACT

Natural vegetation succession is a process of interactions between plants and various environmental factors. The dynamics of soil microbes during successional stages have been intensively explored in the past two decades. The effect of rhizosphere interactions of interspecies on microbial properties, however, has received less attention. During the early stage of succession on a Loess Plateau soil from China, we investigated microbial properties in the rhizospheres of three dominant grass species: *Artemisia capillaris*, *Artemisia sacrorum* and *Stipa bungeana*. Experiments with monocultured and polycultured potted plants were designed to determine the effects of rhizosphere interactions on plant growth and soil microbial properties. The results showed that root biomass, shoot biomass, plant height, microbial biomass carbon (C), microbial biomass nitrogen (N), invertase and urease activities, Gram-negative and Gram-positive bacteria were significantly higher for *A. capillaris* than for *A. sacrorum* and *S. bungeana* in both monocultures and polycultures. Lower root biomass, shoot biomass, plant height, microbial biomass C, microbial biomass N, invertase and alkaline phosphatase activities, and levels of microbial phospholipid-derived fatty acids in polycultures relative to the monocultures indicated that rhizosphere interactions led to lower plant growth and soil microbial activities. Our study suggests that rhizosphere interactions had a significant effect on the microbial properties of plant rhizospheres. The advantage of *A. capillaris* over *A. sacrorum* and *S. bungeana* in growth characteristics and rhizosphere microbial conditions is likely responsible for the dominance of *A. capillaris* in the early stage of succession in abandoned cropland on the Loess Plateau.

1. Introduction

The deterioration of natural ecosystems has accelerated during the last century due to increasing human activity and the extensive use of natural resources [1]. Natural recovery without further anthropogenic disturbance has been assumed to be the most effective way to restore disturbed soil ecosystems, because native plant species are better able to adapt to poor and changeable environmental conditions [2]. Natural succession is generally characterized by an initial dominance of some species, subsequent co-occurrence with other species and substitution by another dominant species with time. Finally, the ecosystem develops to a stable community of species adapted to the biotic and abiotic conditions. Vegetation succession is a slow ecological process and is completely dependent on the interactions between vegetation and the various environmental factors [3].

The rhizosphere is the soil region influenced by plant roots and characterized by high microbial activity [4]. Rhizosphere microbial activities are critical for the establishment of vegetation and soil nutrient cycling [5,6]. For instance, microbial biomass, as one of the living component of soil organic matter, responds rapidly to changes in the soil environment because of this high turnover rate [7]. Basal respiration is widely used as an indicator of microbial activity. Invertase is a type of hydrolase enzyme involved in the decomposition of organic matter and releases reducing sugars as end products of the carbon (C) cycle [8]. Alkaline phosphatase participates in soil phosphorus (P) cycling and transforms organic P into inorganic P, which is then available to plants. Urease has a vital role in soil nitrogen (N) cycling [9]. Given these important functions, there is considerable interest in understanding the linkage between microbial performance, soil variables and plant growth. As an available approach for determining microbial community composition, analysis of phospholipid fatty acids (PLFAs) uses the lipids of microbial membranes as biomarkers for specific groups of microorganisms [10]. Rapid changes in soil microbial community structure can be effectively detected by changes in PLFA patterns. In general, microorganisms play an important role in the re-establishment of vegetation and long-term ecosystem stability; however, recent evaluation of the success of revegetation by natural succession has been limited to visual inspection of aboveground indicators and to

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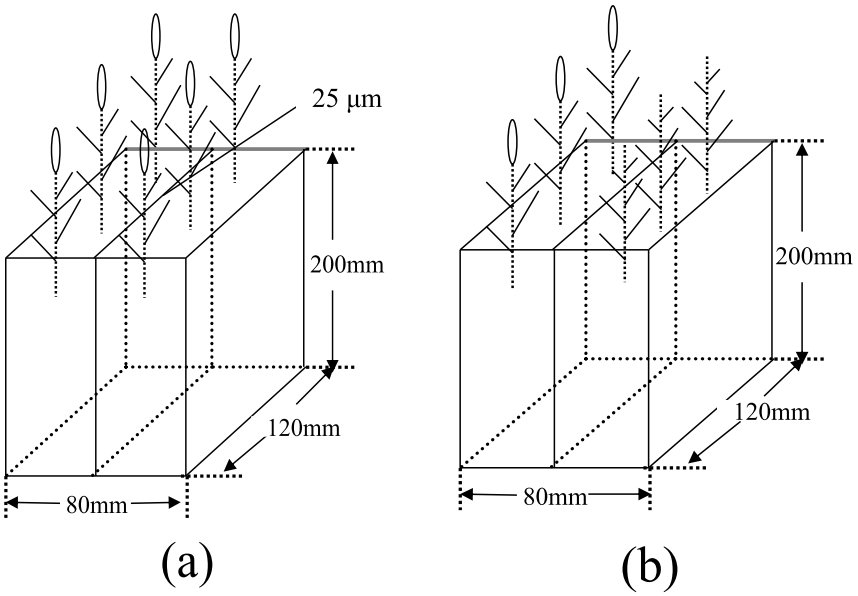

Fig. 1. Diagram of the rhizobox. (a) monoculture; (b) polyculture.

monitoring of soil parameters. Little attention has been given to microbial rhizosphere interactions between plants, and lack of such knowledge may lead to an incomplete understanding of ecosystem recovery.

The Loess Plateau of China has experienced a significant change in land use during the past 50 years. Historically, the native vegetation was destroyed to meet the food supply needs of an expanding population, resulting in severe soil erosion and land degradation. An ambitious conservation project, Grain for Green, was implemented by the Chinese government in 1999 to control erosion and restore soil quality, and large areas of sloping cropland have been restored to grassland or forest. The sloping cropland was abandoned for natural recovery without further human disturbance as an important measure of restoration. This natural restoration has led to an increase in vegetation cover as the abandoned fields were naturally recolonized by surrounding vegetation [11]. Intensive studies have indicated that this type of conversion can greatly improve soil aggregate characteristics [12,13]; C, N and P supply [14,15]; enzyme activities [16]; and microbial compositions [11] of the abandoned sloping cropland. These studies, however, mainly focused on the outcome of natural succession, such as the effect of recovery on vegetation communities and soil conditions, but the causes of the successional order and the interactions of microbial communities of plants remain unknown. Such information is important for a better understanding of natural succession and of the interactions among plant communities and for appropriately managing the ecological environment.

Our recent studies have shown that *Artemisia capillaris*, *Artemisia sacrorum* and *Stipa bungeana* are the three dominant species in communities during the early stage of succession on abandoned cropland of the Loess Plateau [17]. Notably, in the initial successional stage (1–10 years), *A. capillaris* was dominant, and *A. sacrorum* and *S. bungeana* were subdominant. The present study investigated the microbial properties of *A. capillaris, A. sacrorum* and *S. bungeana* rhizospheres in experiments with potted plants. We hypothesized that (i) rhizosphere interactions of interspecies could lead to a great variation in rhizosphere microbial properties and (ii) the *A. capillaris* rhizosphere would have higher levels of microbial biomass, enzyme activities and PLFAs compared with *A. sacrorum* and *S. bungeana*.

2. Materials and methods

2.1. Soils

Pot experiments were conducted in a greenhouse at the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, China. The soil for the experiments was collected from the Ansai Research Station of Soil and Water Conservation in the Dunshan watershed, Shaanxi Province, on the northern Loess Plateau (109°19′23″E, 36°51′30″N). All soils were taken from the surface layer (0–20 cm) of abandoned cropland that had been allowed to experience natural succession for five years. The soils were air-dried, ground and sieved through a 2 mm plastic mesh. The chemical properties of soil were as follows (means of three determinations ± standard deviations): organic C, 3.24 ± 0.24 g kg^{-1}; total N, 0.29 ± 0.02 g kg^{-1}; total P, 0.51 ± 0.02 g kg^{-1}; available P, 1.17 ± 0.09 mg kg^{-1}; available N, 43.79 ± 3.61 mg kg^{-1}; and pH, 8.55 ± 0.14.

2.2. Experimental design

Rhizoboxes are increasingly used to investigate the properties of rhizospheres, because they avoid the overestimation of microbial biomass common to the root-shaking method and overcome the disadvantage of removing soluble material by washing roots [18,19]. We constructed rhizoboxes to investigate the properties of the rhizospheres of monocultured and polycultured plants. Three plant species common in the early stage of natural succession of abandoned cropland on the Loess Plateau (*A. capillaris*, *A. sacrorum* and *S. bungeana*) were planted in rhizoboxes as either monocultures or polycultures.

2.2.1. Monoculture experiment

The dimensions of monoculture rhizoboxes were 120 × 80 × 200 (length × width × height, mm) (Fig. 1a). The boxes were divided into left and right rhizosphere zones (40 mm in width) separated by 25-μm mesh nylon cloth. This design separated the soil zones and successfully prevented root hairs from entering the adjacent soil zones but permitted the transfer of soil microfauna and root exudates between the zones. Three species were respectively monocultured: *A. capillaris*, *A. sacrorum* and *S. bungeana*. A pot experiment without planting was conducted as a reference. Each rhizobox was filled with 3 kg of treated soil. Seeds of each species were thoroughly rinsed with water, and germinated on filter paper. After seeds were germinated for 24 h at 28 °C in darkness,

30 germinated seeds with the radicle emerged were sown in rhizosphere zone soil. The seedlings were subsequently thinned to 15 plants after emergence in each zone. Each treatment had five replicates and there were 15 pots in total.

2.2.2. Polyculture experiment

To investigate the rhizosphere interaction of interspecies on soil microbial properties, the three plant species were polycultured with each other as follows: *A. capillaris* with *A. sacrorum*; *A. capillaris* with *S. bungeana*; and *A. sacrorum* with *S. bungeana*. The polyculture rhizoboxes were of the same design as the monoculture rhizoboxes, with the only difference that each of the two zones was sown with a different species (Fig. 1b), so that soils of both zones both contained rhizosphere soils. Each zone was sown with 30 germinated seeds and subsequently thinned to 15 plants per species. Each rhizobox was filled with 3 kg of treated soil. Each treatment had five replicates and there were 15 pots in total.

2.2.3. Experimental conditions

All pot experiments were conducted during April–October 2012 under greenhouse conditions of a temperature range of 20–25.8 °C for the day and 8–15.8 °C for night. Evaporative cooling and shade cloth were used to prevent excessively high temperatures on sunny days. Rhizoboxes were arranged in a randomized design within the greenhouse and their position was rotated regularly to ensure uniform conditions. No nutrients were added to the soils in order to simulate the nutrient levels of the early stage of natural succession. Soil moisture was maintained at 11–13% by daily additions of deionized water, consistent with the moisture level in natural fields.

At the end of the experiments, plant height was recorded, and the aboveground parts were clipped as shoots and dried at 65 °C in an oven for 72 h to obtain the dry weight. The boxes were then dismantled, and the roots were manually separated from soils, rinsed with deionized water and dried at 60 °C for 48 h to obtain the dry weight. The rhizosphere soils were sieved gently to remove any roots, keeping the root mass as intact as possible. Rootlets that passed through the sieve were subsequently removed with forceps. The soil samples from the different soil zones of each box were homogenized separately before analysis. Each soil sample was divided into two parts: one part was stored at 4 °C for measurement of microbial biomass, respiration and enzymatic activities; and the other part was frozen at −20 °C, and then freeze-dried for PLFA determination. Microbial biomass, respiration, enzymatic activities and PLFAs were determined within a week.

2.3. Laboratory analysis

Soil organic C was measured using the Walkley–Black method and total N by the Kjeldahl method. Total P was measured colorimetrically after wet digestion with H_2SO_4 + $HClO_4$, and available P was measured by the Olsen method. Available N was measured with a micro-diffusion technique after alkaline hydrolysis. An automatic acid-based titrator (Metrohm 702, Swiss) was used to measure soil pH in 1:2.5 soil: water suspensions.

2.3.1. Microbial biomass and basal respiration

Microbial biomass C and N were measured by fumigation/extraction method [20]. Twenty-five grams of fresh soil were fumigated with $CHCl_3$ for 24 h at 25.8 °C. Following fumigant removal, the soil was treated with 100 ml of 0.5 M K_2SO_4 by horizontal shaking for 1 h at 200 rpm and then filtered. An additional 25 g of non-fumigated soil was extracted at the same time that fumigation commenced. Organic C in the extracts was measured using a Liqui TOCII analyzer (Elementar, Germany). Total N in the extracts was measured using the Kjeldahl method. Soil basal respiration was estimated by CO_2 release at 25.8 °C in samples incubated for 14 days, adjusted to 50% of field water-holding capacity [21]. The CO_2 released was trapped in NaOH, and the residual NaOH was titrated with HCl.

2.3.2. Enzymatic activities

The activities of invertase, urease, alkaline phosphatase and catalase were determined as previously described [22]. The moisture content was determined before all enzyme assays, and the controls were included without substrate and without soil samples. Invertase activity was determined using sucrose as the substrate; 3-amino-5-nitro-salicylic-acid released was assayed colorimetrically at 508 nm and results expressed as mg glucose g^{-1} soil h^{-1}. Urease activity was measured using urea as the substrate, and the released ammonium was assayed colorimetrically at 578 nm, with results expressed as mg ammonium-N g^{-1} soil h^{-1}. Alkaline phosphatase activity was determined with disodium phenyl phosphate colorimetry and the released phenol was assayed colorimetrically at 660 nm, with results expressed as mg phenol g^{-1} soil h^{-1}. Catalase activity was measured by the H_2O_2 consumed by soil, and results expressed as mol $KMnO_4$ g^{-1} soil h^{-1}.

2.3.3. Microbial community structure – PLFAs

Microbial community structure was investigated by determining the relative abundances of PLFAs of the different microbial groups in the soils. The three-step procedure involved extraction, fractionation and quantification of soil phospholipids, and was based on the method of Bligh and Dyer [23] and modified by Bardgett et al. [24]. Briefly, 3-g soil samples were shaken for 2 h in a buffer solution of $CHCl_3$: methanol: citrate (1:2:0.8). Then, equal volumes of $CHCl_3$ and citrate were added, and the two phases separated overnight. The $CHCl_3$ phases were reduced by evaporation. The lipids were then split into neutral, glycolipids and phospholipids with silicic acid columns by eluting $CHCl_3$, acetone and methanol, respectively. The separated PLFAs were subjected to mild alkaline methanolysis at 50 °C, and the resulting fatty-acid methyl esters (FAMEs) were detected with an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector. A mixture of commercial FAMEs (Supelco UK, Poole, Dorset, UK) ranging from C11 to C20 was used as a qualitative standard to identify the separated FAMEs. The concentrations of individual FAMEs were calculated using the internal standard (19:0) peak as a reference. The fatty-acid nomenclature used was that of Frostegård et al. [25]. The polyenoic, unsaturated PLFA 18:2w6 was used as the indicator of fungal biomass [26]. Gram-positive (Gram+) bacteria were represented by iso- and anteiso-branched fatty acids including 13:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 anteiso, 17:0 iso, 19: 0 anteiso and 22:0 iso. Gram-negative (Gram−) bacteria were represented by monounsaturated fatty acids including i12:1 w4c, 14:1 w5c, 15:1 iso w9c, 16:1 w7c, 17:1 iso w9c, 17:1 w8c, 17:0 cyclo w7c, 17:1 w5c, 18:1 w9c, 18:1 w7c, 20:1 w9c, 22:1 w9c, 22:1w6c and 22:1w3c. The bacterial:fungal PLFA ratio was used as an indicator of changes in the relative abundance of these two microbial groups [24].

2.4. Statistical analysis

The data were expressed as means ± standard deviations. Rhizosphere microbial properties between monocultures and between polycultures was compared using one-way analyses of variance, followed by Duncan's multiple comparison test at $P < 0.05$. Pearson's correlation coefficient was used to evaluate correlations between plant biomass and rhizosphere microbial properties. All statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Rhizosphere microbial properties of plants in monocultures

The three grass species differed significantly in their growth ($P < 0.05$, Table 1). The *A. capillaris* had the highest root and shoot biomass, followed by *A. sacrorum* and *S. bungeana*; *A. capillaris* was also

Table 1
Means ± standard deviations (n = 5) of the rhizospheric microbial properties of the species in the monocultures.

Properties	*A. capillaris*	*A. sacrorum*	*S. bungeana*	*Soil without plant*
Root biomass (g)	61.5 ± 2.1 a	43.7 ± 2.3 b	38.2 ± 1.8 c	–
Shoot biomass (g)	44.4 ± 2.0 a	39.2 ± 1.8 b	32.1 ± 2.1 c	–
Height (cm)	33.8 ± 2.3 a	19.8 ± 1.3 c	26.8 ± 1.6 b	–
Microbial biomass C (mg kg^{-1})	235.9 ± 13.5 a	206.2 ± 16.1 b	133.9 ± 18.3 c	62.4 ± 6.84 d
Microbial biomass N (mg kg^{-1})	69.0 ± 3.4 a	61.2 ± 4.5 b	49.8 ± 5.1 c	27.5 ± 4.9 d
Basal respiration (mg CO_2-C kg^{-1} day^{-1})	32.2 ± 5.5 b	50.9 ± 6.8 a	30.6 ± 6.1 b	11.7 ± 4.4 d
Invertase (mg glucose g^{-1} h^{-1})	5.43 ± 0.36 a	4.62 ± 0.41 b	2.99 ± 0.35 c	0.84 ± 0.17 d
Urease (mg NH_4-N g^{-1} h^{-1})	0.49 ± 0.04 a	0.30 ± 0.03 b	0.25 ± 0.04 c	0.10 ± 0.03 d
Alkaline phosphatase (mg phenol g^{-1} h^{-1})	0.31 ± 0.03 c	0.49 ± 0.04 a	0.41 ± 0.03 b	0.18 ± 0.05 d
Catalase (ml 0.1N $KMnO_4$ g^{-1})	0.50 ± 0.02 a	0.50 ± 0.03 a	0.52 ± 0.03 a	0.26 ± 0.08 d
Gram- PLFA (nmol g^{-1})	3.56 ± 0.26a	3.01 ± 0.22 b	3.12 ± 0.19 b	1.69 ± 0.34 d
Gram + PLFA (nmol g^{-1})	0.24 ± 0.03 c	0.46 ± 0.01 a	0.32 ± 0.03 b	0.11 ± 0.02 d
Bacterial PLFA (nmol g^{-1})	8.14 ± 0.51 a	6.15 ± 0.32 b	5.34 ± 0.38 c	3.05 ± 1.04 d
Fungal PLFA (nmol g^{-1})	0.41 ± 0.05 a	0.38 ± 0.02 a	0.37 ± 0.06 a	0.17 ± 0.05 d
Bacterial: Fungal PLFA ratio	20.35 ± 1.51 a	16.18 ± 0.89 b	14.43 ± 0.66 c	17.9 ± 2.1 ab
Gram-: Gram + PLFA ratio	14.83 ± 1.22 a	6.54 ± 0.79 c	9.75 ± 1.31 b	15.3 ± 1.98 a

Different letters in the row indicated the significant difference between the monocultures at the 0.05 level.

significantly taller ($P < 0.05$).

Microbial properties in rhizosphere soil of the three species, including microbial biomass, basal respiration, enzyme activities and microbial groups indicated by PLFAs, were significantly higher ($P < 0.05$) compared to soil without plants (Table 1). Among the plants, *A. capillaris* had the highest contents of microbial biomass C and N, followed by *A. sacrorum* and *S. bungeana*. Respiration was significantly higher ($P < 0.05$) for the *A. sacrorum* rhizosphere than for the other species. The activities of microbial enzymes in the rhizospheres differed significantly among species, with the exception of catalase ($P = 0.64$). The relative activities of invertase and urease were similar, with the highest activities in the *A. capillaris* rhizosphere; however, *A. sacrorum* had the highest alkaline phosphatase activity.

The *A. capillaris* had significantly higher Gram− and bacterial PLFAs, bacterial: fungal PLFA ratio and Gram−: Gram + PLFA ratio than *A. sacrorum* and *S. bungeana* (Table 1). The *A. sacrorum* rhizosphere had the highest level of Gram + PLFAs, followed by *S. bungeana* and *A. capillaris*.

3.2. Rhizosphere microbial properties of plants in polycultures

Three plant species were polycultured with each other to investigate the dynamic rhizosphere microbial properties (Figs. 2–5). In the *A. capillaris/A. sacrorum* polyculture, *A. capillaris* had significantly greater root biomass, shoot biomass and height than *A. sacrorum* (Fig. 2). The microbial properties showed a similar trend that microbial biomass C and N and the activities of invertase and urease were 44.1, 41.3, 51.8 and 26.7% higher, respectively, in the rhizosphere of *A. capillaris* compared with *A. sacrorum*. Basal respiration, however, was 38.2% lower in the *A. capillaris* rhizosphere (Fig. 3). Alkaline phosphatase and catalase activities did not differ significantly between the two rhizospheres (Fig. 4). Total PLFA was significantly higher in the *A. capillaris* rhizosphere, due to higher Gram−, Gram+, bacterial and fungal PLFAs, and the bacterial: fungal PLFA ratio (Fig. 5).

Compared to *S. bungeana*, root biomass, shoot biomass, height, microbial biomass C, microbial N, respiration, invertase activity, urease activity, Gram− PLFAs, Gram + PLFAs, bacterial PLFAs and the bacterial: fungal PLFA ratio were significantly higher for *A. capillaris* in the *A. capillaris/S. bungeana* polyculture. Fungal PLFAs and the bacterial: fungal PLFA ratio did not differ significantly ($P = 0.48$ and 0.37, respectively) between *A. capillaris* and *S. bungeana*. In the *A. sacrorum/S. bungeana* polyculture, no significant differences were found between the two species in root biomass, shoot biomass, height and soil microbial variables including microbial biomass C, microbial biomass N, Gram− and fungal PLFAs, and activities of urease, alkaline phosphatase and catalase.

3.3. Rhizosphere interactions and soil microbial properties

The growth characteristics of plants and the microbial properties of rhizospheres in monocultures and polycultures are shown in Table 2. Root biomass, shoot biomass, height, microbial biomass C and N, and activities of invertase, urease and alkaline phosphatase were significantly lower ($P < 0.05$) in polycultures for all species relative to monocultures. The activities of catalase did not substantially differ between the cultures ($P > 0.05$). The compositions of the microbial communities were also affected by competition. For *A. sacrorum*, all rhizosphere microbial PLFAs were significantly lower in polyculture than monoculture. For *S. bungeana*, only the Gram− and Gram + bacterial PLFAs were higher in polyculture than monoculture; the other microbial PLFAs showed no significant differences.

The rhizosphere of *A. capillaris* had different microbial community compositions in the two polycultures. In the *A. capillaris/A. sacrorum*, the Gram−: Gram + PLFA ratio was much lower in polyculture than monoculture, but the other microbial PLFAs, such as Gram− and bacterial PLFAs, did not significantly differ. In the *A. capillaris/S. bungeana* polyculture, however, the Gram− and bacterial PLFAs in the *A. capillaris* rhizosphere were significantly lower in polyculture than monoculture.

3.4. Correlations between plant biomass and rhizosphere microbial properties

Correlations between root biomass, shoot biomass and rhizosphere microbial properties are shown in Table 3. Root and shoot biomass were positively correlated with microbial biomass, invertase activity, urease activity and Gram−, Gram+, bacterial and fungal PLFAs ($P < 0.05$), but were negatively correlated with basal respiration ($P < 0.05$). Among the microbial properties, microbial biomass C and N were positively correlated with invertase activity, urease activity and PLFAs of Gram−, Gram+, bacteria and fungi ($P < 0.05$); however, no significant relationship was found between microbial biomass and basal respiration. There was a positive relationship among invertase, urease activities and the different microbial groups. Alkaline phosphatase was only significantly correlated with fungal PLFAs ($P < 0.05$).

4. Discussion

4.1. Basic growth characteristics

Interspecific interaction is a phenomenon in which one species affects the survival, growth or fitness of another, and for plants is reflected in their aboveground and belowground characteristics. We

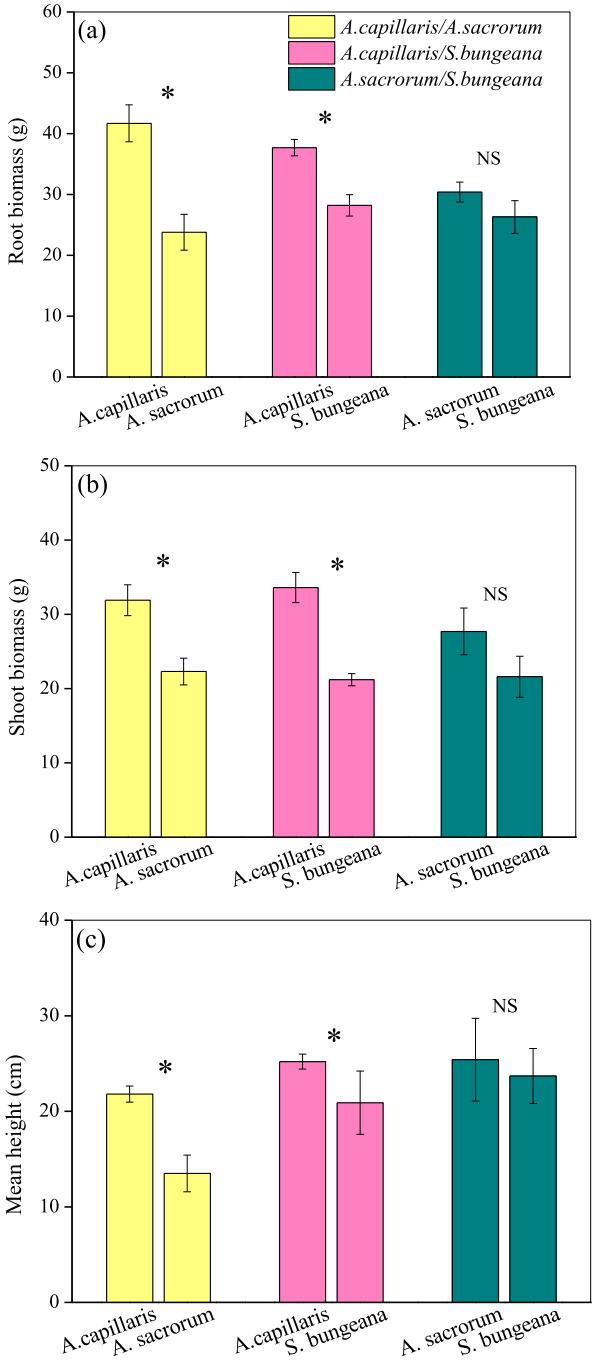

Fig. 2. Means ± standard deviations for (a) root biomass, (b) aboveground biomass and (c) height of the species in the polycultures. * indicate significant differences at $P < 0.05$. NS: Not significant.

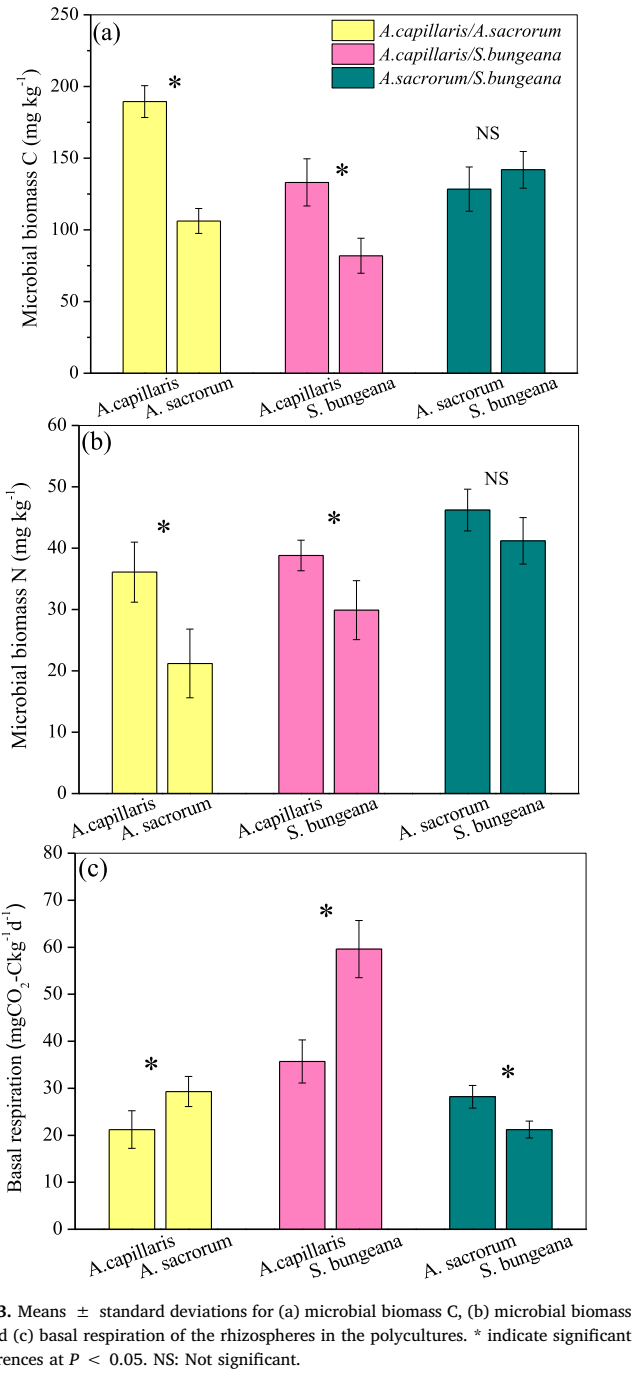

Fig. 3. Means ± standard deviations for (a) microbial biomass C, (b) microbial biomass N and (c) basal respiration of the rhizospheres in the polycultures. * indicate significant differences at $P < 0.05$. NS: Not significant.

found higher root and shoot biomasses and height of *A. capillaris* than *A. sacrorum* and *S. bungeana* in both monocultures and polycultures (Table 1), demonstrating growth advantages of *A. capillaris* over the other two species when they interacted. Because the experimental soil was collected from an early successional stage of a field that had been abandoned for five years, the result could indicate that *A. capillaris* is more adaptive to early soil conditions during natural succession on the Loess Plateau. The roots of sympatric plant species may avoid each other and hence compete for nutrients through root segregation by occupying different spatial locations or niches in the soil profile [27]. This segregation can lead to more biomass relative to monocultured plants. Mommer et al. [28] reported that a higher root biomass (measured using real-time PCR) was due to the enhanced growth of one species in a polyculture. In contrast, we found that root and shoot biomass and height were lower in polycultures than monocultures for the three species, suggesting that rhizosphere interactions strongly negatively affected the plant growth, perhaps due to the competition for nutrients by roots. Plants can alter their ability to acquire nutrients depending on the other plant species present [29]. Jumpponen et al. [30] found that *Achillea millefolium* grown in monoculture acquired more N than when grown with *Festuca ovina*, and a similar phenomenon was reported by Veresoglou and Fitter [31]. The uptake of nutrients by roots can clearly be affected by interactions between plants, so polyculture might also reduce nutrient uptake in a species. Competitively dominant plants may use the most abundant nutrients in the soil, especially when growing with competitively inferior species [32].

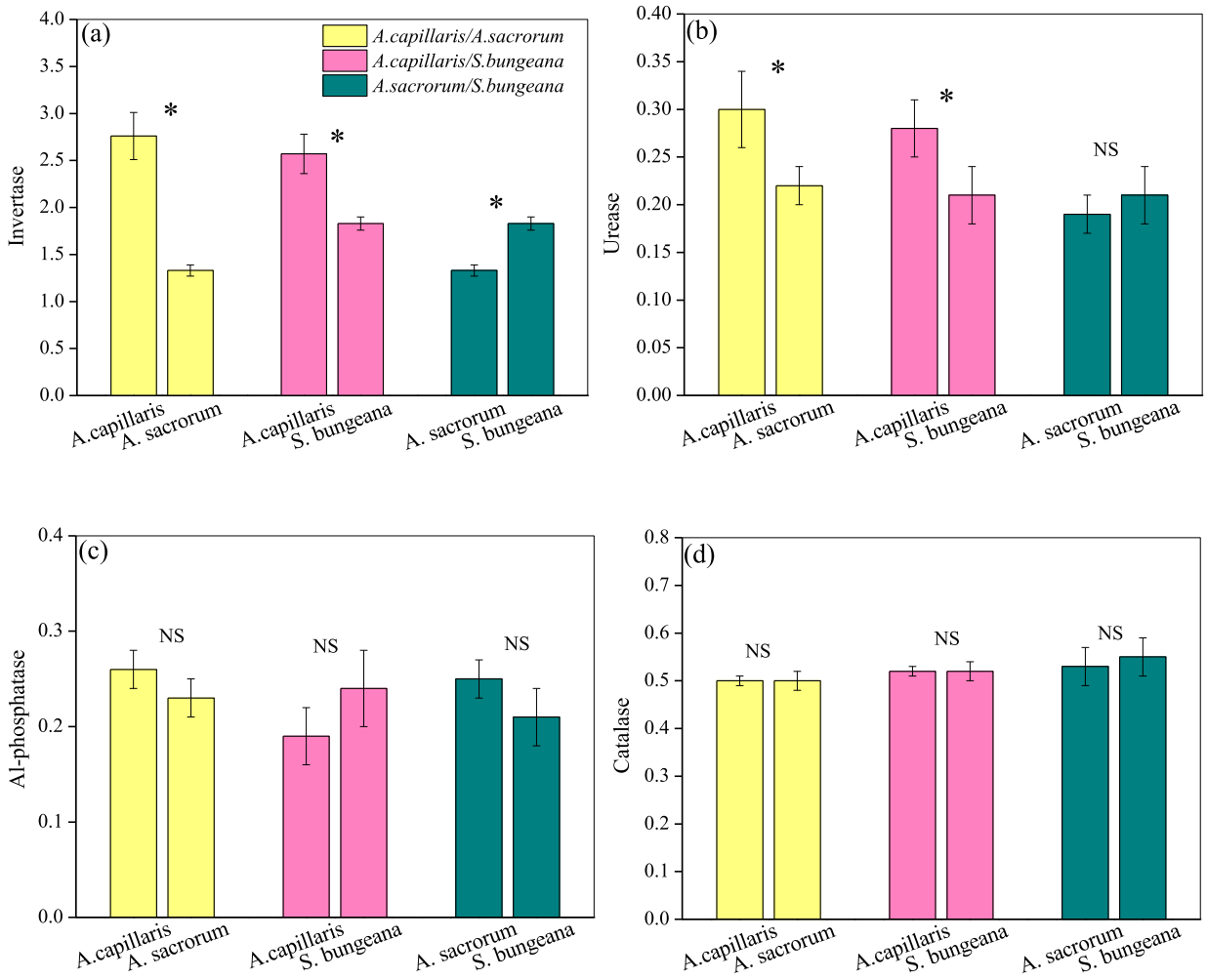

Fig. 4. Means ± standard deviations for the enzymatic activities of (a) invertase (mg glucose g^{-1} h^{-1}), (b) urease (mg NH_4-N g^{-1} h^{-1}), (c) alkaline phosphatase (ml 0.1N $KMnO_4$ g^{-1}) and (d) catalase (mg phenol g^{-1} h^{-1}) of the rhizospheres in the polycultures. * indicate significant differences at $P < 0.05$. NS: Not significant.

4.2. Microbial biomass

Plants cause changes in soil properties that lead to complex local interactions between vegetation and soil [32,33]. A comparison of microbial quantity and diversity could be useful in understanding how rhizosphere communities mediate impacts of plants on the soil environment [7]. Our study showed that the rhizosphere soils had higher microbial biomass C and N contents and increased respiration compared to soil without plants. These results were consistent with those of Garcia et al. [34], suggesting that microorganisms in the rhizosphere were more active than those in bulk soil. The high microbial properties are attributed to root exudation of substrates such as sugars, acids, hormones, mucilage, sloughed root cells and C allocated to root-associated symbionts. These substrates provide favorable resources for the microbial population. We also found a significant difference in microbial biomass C and N among rhizospheres of the three species in monocultures (Table 1), indicating that the microbial biomass was strongly species-specific, as previously suggested [22,35]. The colonization and establishment of microorganisms in rhizospheres is affected by many factors, such as the quantity and quality of root exudates secreted by particular species, soil physicochemical properties and climatic conditions. Rhizospheres are thus likely to develop microenvironments under the continuous effects of root exudates, soil characteristics and climatic factors, providing an opportunity for the development of specialized rhizoflora. The rhizosphere microbial biomasses also differed among the species in the polycultures (Fig. 3), in accordance with a previous report in which plant species was the most important factor affecting microbial biomass [36]. Likely because the higher root biomass could provide more root exudates and so support more microorganisms, *A. capillaris* had higher microbial biomass C and N than the other two plants in both monocultures and polycultures.

Interactions between plants affected both plant growth and microorganisms in the rhizospheres. Similar to root and shoot biomass, the rhizosphere microbial biomass of the three species was significantly lower in the polycultures than monocultures (Table 2), suggesting that rhizosphere interactions negatively affected microbial activities in the rhizospheres. The interspecific rhizosphere interactions may have decreased soil microbial quantities because plants in interacting systems exert species-specific effects on the rhizosphere microbial community and quantity as a result of differences in amount and composition of root exudates [37]. The quantities of root exudates released by plants may have been generally lower than in monocultures due to competition for nutrients. The lower microbial biomasses in the rhizospheres of plants in polycultures were thus likely due to lower levels of root exudates – a hypothesis that warrants further investigation. Garcia et al. [34] demonstrated that microbial biomass was highly correlated with basal respiration; however, in our study, basal respiration and microbial biomass varied differently. Part of the microbial biomass may thus have no direct relationship with microbial activity on this Loess Plateau soil. Rhizosphere interactions differentially affected basal respiration in various rhizospheres. For example, basal respiration was lower in the *A. sacrorum* rhizosphere in polycultures than monocultures, but was

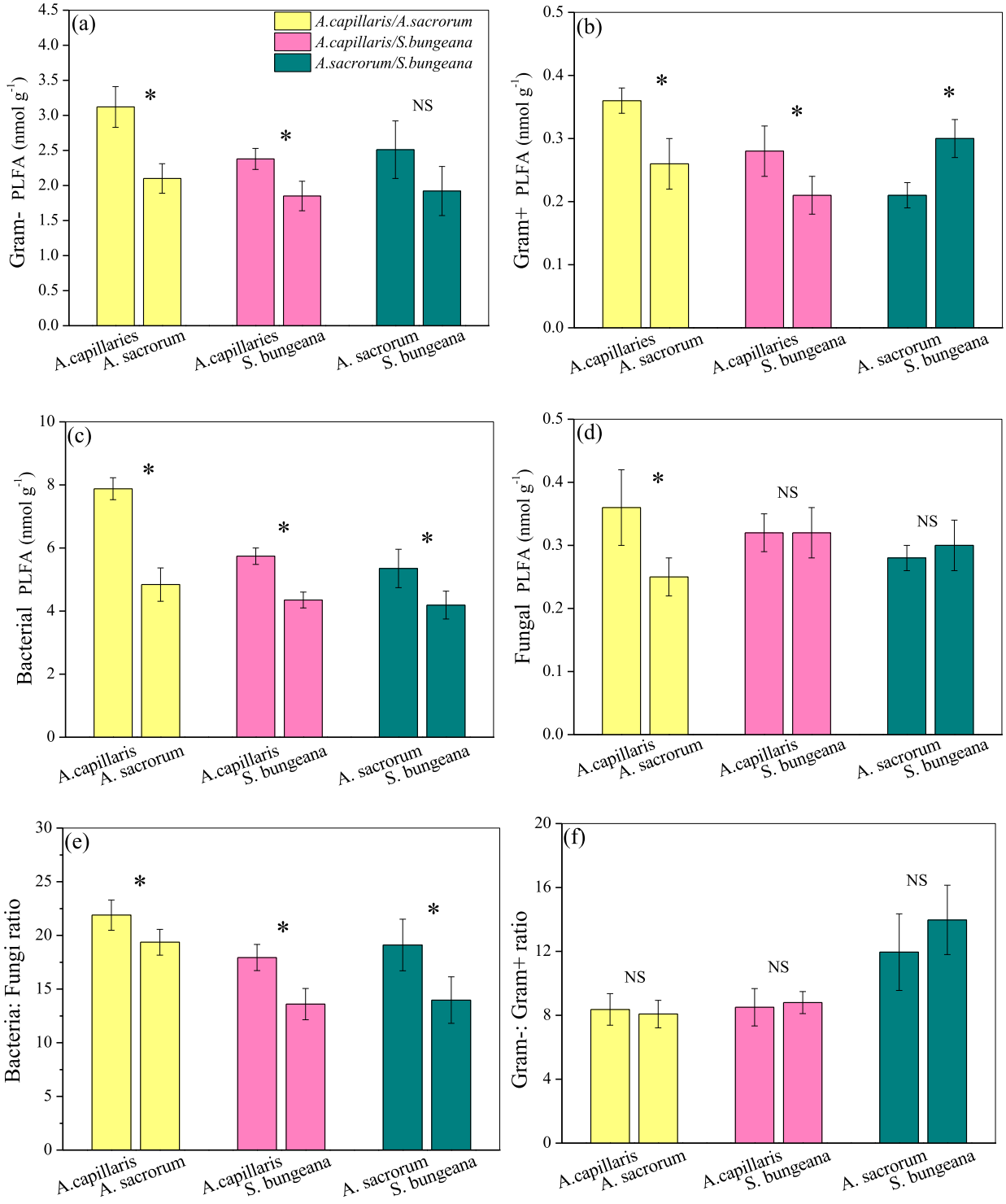

Fig. 5. Means ± standard deviations for the abundance of identified PLFAs for (a) Gram-bacteria, (b) Gram + bacteria, (c) all bacteria and (d) all fungi and the PLFA ratios for (e) bacteria and fungi and (f) Gram- and Gram + bacteria of the rhizospheres in the polycultures. * indicate significant differences at $P < 0.05$. NS: Not significant.

higher in the *S. bungeana* rhizosphere in polyculture. These results support the finding of instability of basal respiration in arid areas [38].

4.3. Enzymatic activities

Vegetation had a significant effect on enzyme activities in the soil studies. Our results were accordance with the observation by Garcia et al. [34] of 25–55% higher enzyme activities in rhizosphere soil of plants compared with bare soil, indicating a positive effect of plants on soil enzyme activities. This result also suggested the amount of energy flow and material exchange present in the rhizosphere relative to the bulk soil because enzyme activity indicates the soil potential to support biochemical processes. In addition, our study showed higher invertase and urease activities in the *A. capillaris* rhizosphere and higher phosphatase activity in the *A. sacrorum* rhizosphere in monocultures (Table 1). These discrepancies may be related to the different roles of

Table 2
Comparison of plant characteristics and rhizospheric soil properties between polycultures and monocultures.

Parameters	*A. capillaris*			*A. sacrorum*			*S. bungeana*		
	AC/AS	*AC/SB*	*AC*	*AS/AC*	*AS/SB*	*AS*	*SB/AC*	*SB/AS*	*SB*
Root biomass (g)	41.7 b	37.7 b	61.5 a	23.8 c	30.4 b	43.7 a	28.2 b	26.3 b	38.2 a
Shoot biomass (g)	31.9 b	33.6 b	44.4 a	22.3 b	22.7 b	39.2a	21.2 b	21.6 b	32.1 a
Height (cm)	21.8 c	25.2 b	33.8 a	13.5 c	25.4 a	19.8 b	20.9 b	23.7 ab	26.8 a
Microbial biomass C (mg kg^{-1})	189.5 b	133.1 c	235.9 a	106.2 b	128.4 b	206.2 a	81.9 b	141.9 a	133.9 a
Microbial biomass N (mg kg^{-1})	36.1 b	38.8 b	69.0 a	21.2 c	46.2 b	61.2 a	29.9 c	41.2 b	49.8 a
Basal respiration (mg CO_2-C kg^{-1} day^{-1})	21.2 b	35.7 a	32.2 a	29.3 b	28.2 b	50.9 a	59.6 a	21.2 c	30.6 b
Invertase (mg glucose g^{-1} h^{-1})	2.76 b	2.57 b	5.43 a	1.33 b	1.33 b	4.62 a	1.83 b	1.84 b	2.99 a
Urease (mg NH_4-N g^{-1} h^{-1})	0.30 b	0.28 b	0.49 a	0.22 b	0.19 b	0.30 a	0.21 a	0.21 a	0.25 a
Al-phosphatase (mg phenol g^{-1} h^{-1})	0.26 b	0.19 c	0.31 a	0.23 b	0.25 b	0.49 a	0.24 b	0.21 b	0.41 a
Catalase (ml 0.1N $KMnO_4$ g^{-1})	0.50 a	0.52 a	0.50 a	0.50 a	0.53 a	0.50 a	0.52 a	0.55 a	0.52 a
Gram- PLFA (nmol g^{-1})	3.12 b	2.38 c	3.56 a	2.10 c	2.51 b	3.01 a	1.85 b	1.92 b	3.12 a
Gram + PLFA (nmol g^{-1})	0.36 a	0.28 b	0.24 b	0.26 b	0.21 b	0.46 a	0.21 b	0.30 a	0.32 a
Bacterial PLFA (nmol g^{-1})	7.88 b	5.74 c	8.14 a	4.84 b	5.35 ab	6.15 a	4.35 b	4.19 b	5.34 a
Fungal PLFA (nmol g^{-1})	0.36 ab	0.32 b	0.41 a	0.25 b	0.28 b	0.38 a	0.32 ab	0.30 b	0.37 a
Bacterial: Fungal PLFA ratio	21.9 b	17.9 b	20.35 a	19.4 a	19.1 a	16.18 b	13.6 a	14.0 a	14.43 a
Gram-: Gram + PLFA ratio	8.4 b	8.5 b	14.83 a	8.1 b	11.9 a	6.54 c	8.8 b	13.9 a	9.75 b

Different letters indicated the significant difference between polycultures and monocultures at the 0.05 level. AC: *A. capillaris*; AS: *A. sacrorum*; SB: *S. bungeana*.

the enzymes in the cycling and conversion of materials. Invertase is a type of hydrolase involved in the decomposition of organic matter, and urease contributes to soil N cycling [9]. The differences in the enzymatic activities in the rhizospheres of the three species may thus indicate status of the various nutrient cycles in rhizospheres. The higher invertase and urease activities of the *A. capillaris* rhizosphere were probably due to the higher amounts of carbohydrates available for decomposition and to the higher amounts of adsorption of organic N or depletion of available N by the roots. In contrast to monocultures, the polycultures presented differences in enzymatic activities. The rhizospheres of polyculture *A. capillaris* had significantly higher invertase activities than polyculture *A. sacrorum* and *S. bungeana*, indicating a competitive advantage of *A. capillaris* in invertase when interacting with *A. sacrorum* or *S. bungeana*. The lower invertase, urease and alkaline phosphatase activities for all three species' rhizospheres in polycultures compared with monocultures indicated a negative effect of rhizosphere interactions on the enzyme activities. In contrast to the other enzymes, the activity of catalase, an important enzyme in soil biochemical reactions, did not significantly differ between the monocultures and polycultures, suggesting that catalase may not have a close relationship with plant species and competition in arid areas. The soil is one of many important factors determining enzymatic activities [39]. In our study, all cultures used the same soil, which may have contributed to the lack of differences in catalase activity.

4.4. Microbial community structure

In agreement with our previous work [11] in which higher contents of PLFA markers characteristic of Gram− bacteria were found in *A. capillaris* rhizospheres in natural fields, we observed the highest abundance of Gram− bacteria in the *A. capillaris* rhizospheres in both monocultures and polycultures. The clear distinction in microbial community structure was likely due mainly to the variation in the amounts and chemical compositions of the rhizodeposits. Our findings were consistent with previous reports that plant species was the most important factor determining microbial community composition [40,41]. Gram− bacteria are more frequent in rhizospheres, preferably growing on plant labile C, whereas Gram + bacteria may be dominant in soils with relatively less available C [42]. The higher levels of Gram− bacterial PLFAs in the rhizosphere of *A. capillaris* thus suggested abundant rhizodeposition by roots.

The rhizosphere fungal communities of the three species were generally similar in the monocultures, suggesting little influence of plant species. Innes et al. [43] also found that the plant species had no significant effect on contents of 18w:6 PLFA, a marker of fungal biomass. Root interactions usually significantly influence the microbial

Table 3
Correlation matrix between plant biomass and microbial properties.

Parameters	Root biomass	Shoot biomass	MBC	MBN	Basal respiration	Invertase	Urease	Alkaline phosphatase	Catalase	Gram- PLFA	Gram + PLFA	Bacterial PLFA	Fungal PLFA
Root biomass	1.00	0.92**	0.79**	0.62*	−0.73*	0.80**	0.75*	0.51	0.19	0.82**	0.81**	0.85**	0.69*
Shoot biomass		1.00	0.86**	0.68*	−0.77**	0.74*	0.80**	0.56	0.09	0.87**	0.79**	0.79**	0.71*
MBC			1.00	0.90**	−0.57	0.75*	0.79**	0.49	0.32	0.92**	0.81**	0.86**	0.76*
MBN				1.00	−0.64*	0.66*	0.67*	0.27	0.35	0.79**	0.72*	0.82**	0.80**
Basal respiration					1.00	0.38	0.33	−0.05	−0.24	−0.78**	−0.74*	−0.69*	−0.77**
Invertase						1.00	0.75*	−0.53	0.51	0.79**	0.69*	0.82**	0.71*
Urease							1.00	−0.17	−0.04	0.81***	0.72*	0.70*	0.76*
Alkaline phosphatase								1.00	0.58	−0.09	0.35	0.14	0.67*
Catalase									1.00	0.32	0.14	0.19	0.58
Gram- PLFA										1.00	0.81**	0.88**	0.72*
Gram + PLFA											1.00	0.77**	0.68*
Bacterial PLFA												1.00	0.64*
Fungal PLFA													1.00

MBC: microbial biomass C; MBN: microbial biomass N. *Correlation is significant at the $p < 0.05$ level (2-tailed); **Correlation is significant at the $p < 0.01$ level (2-tailed).

community composition due to intrinsic differences in root morphology and their inability to fix nutrient elements [44]. Consistently, the *A. capillaris* rhizosphere had greater abundances of Gram− and Gram + bacteria compared with *A. sacrorum* and *S. bungeana*, perhaps due to the release of different qualities and quantities of root exudates favorable to different bacterial species. This demonstrates that plant identity and interspecific interactions are important in controlling soil microflora. The high bacterial: fungal PLFA ratios for *A. capillaris* in both cultures were consistent with the result of bacterial PLFAs, and further supported the high abundance of bacteria in the *A. capillaris* rhizosphere. A higher Gram−:Gram + ratio in the rhizosphere during the development of an ecosystem suggests a shift in composition from a Gram + -dominated bacterial community to one dominated by Gram−. Correlation analysis revealed that the levels of bacteria, fungi, urease activity, invertase activity and microbial biomass in the rhizosphere, were all positively or significantly positively related to biomasses of root and shoot. This result indicated the dependence of microbial rhizosphere interactions on plant roots, likely because rhizospheric soil microbial quantity and enzyme activities are strongly linked to root exudates. Commonly, plant roots release 17% of the photosynthate captured into rhizosphere zones, most of which is available to the microbial community [45].

5. Conclusions

We tested the hypothesis that rhizosphere interactions led to a change in soil microbial activities, including microbial biomass, enzyme activities and level of PLFAs. There were higher microbial biomass, invertase and urease activities, and PLFAs for *A. capillaris* compared with *A. sacrorum* and *S. bungeana* in both monocultures and polycultures, which probably accounts for the dominance of *A. capillaris* in the early succession of abandoned cropland on the Loess Plateau. Our study provided some insight into the effects of plant interaction on microbial communities in the rhizosphere, but we still do not know the exact mechanisms through which compositions of microbial populations are affected. Moreover, much research is still required to examine the dynamics of soil microbial diversity during the period of plant growth. Subsequent research should focus on the influence on microorganism diversity of the quality and quantity of root exudates that monoculture and polyculture plants release into soil.

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