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RESEARCH ARTICLE



## Positive effects of plant diversity on soil microbial biomass and activity are associated with more root biomass production

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### ABSTRACT

This study aims to explore relationships between plant diversity and soil microbial function and the factors that mediate the relationships. Artificial plant communities (1, 2, 4 and 8 species) were established filled with natural and mine tailing soils, respectively. After 12 months, the plant species richness positively affected the soil microbial functional diversity in both soil environments but negatively affected microbial biomass and soil basal respiration in the natural soil. The root biomass positively correlated with the microbial biomass, cultural bacterial activity and soil basal respiration in both soil environments. Moreover, the  $D_i$  (deviations between observed performances and expected performances from the monoculture performance of each species of mixture) of microbial biomass, cultural bacterial activity and soil basal respiration positively correlated with the  $D_i$  of root biomass in both soil environments. Consistent with stress-gradient hypothesis, the  $D_{mix}$  (over-function index) of aboveground biomass positively correlated plant species richness in the mine tailing soil. Results suggest that the root biomass production is an important mechanism that affects the effects of plant diversity on soil microbial functions. Different responses of soil microbial function to increasing plant diversity may be due to root biomass production mediated by other factors.

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### KEYWORDS

Species richness; soil microbial function; root biomass; facilitation; mine tailing

### Introduction

Reductions in plant diversity have been shown to negatively affect terrestrial ecosystem function (Loreau et al. 2001; Cardinale et al. 2007). Most of these studies focused on the effect of change in plant diversity on primary productivity (Fukami and Morin 2003; Grace et al. 2007), nutrient cycling (Niklaus et al. 2007) and invasibility (Fargione and Tilman 2005). Although soil micro-organisms play a pivotal role in the function of terrestrial ecosystems (Fierer et al. 2009; Eisenhauer et al. 2010), relatively few studies have focused on the effect of change in plant diversity on soil microbial communities (Lamb et al. 2011). Plant diversity mainly affects the soil microbial function by changing the rhizodeposits and litter (Fanin et al. 2011; Lange et al. 2014, 2015), which may be mediated by species identity, biomass production and soil environments. However, how these potential factors affect the effects of plant diversity on soil microbial communities is still unclear.

Theoretically, a decrease in plant diversity could reduce the biomass, activity and diversity of soil microbial communities due to the reduction in the amount and diversity of litter and rhizodeposits (Hooper et al. 2000; Knops et al. 2001). However, actual observations showed positive (Stephan et al. 2000; Zak et al. 2003; Eisenhauer et al. 2011; Lange et al. 2015), negative or no response (Johnson et al. 2008) of soil microbial biomass, activity and diversity to increasing plant diversity (Zhang et al. 2010; Rottstock et al. 2014). Soil micro-organisms are mostly heterotrophic; thus, they decompose plant material for food or use plant exudates (Loranger-

Merciris et al. 2006). Moreover, plants mainly provide carbon resources and other nutrients for soil microbial community in the form of plant litter and root exudates (Rodríguez-Loinaz et al. 2008). Especially, root exudates are readily available sources of carbon and energy for microbes (Paterson et al. 2007; Haichar et al. 2008). Moreover, microbial growth (Oger et al. 2004; Blagodatskaya et al. 2009) and activity (Nannipieri et al. 2012) can be stimulated by labile compounds (including enzymes) released by living roots. Consequently, how plant diversity affects roots may determine its effect on soil microbial communities

The stress-gradient hypothesis postulates that facilitation interactions should be common in communities in environments of high physical stress, while competitive interactions should be common in communities in benign environments (Bertness and Callaway 1994). Moreover, facilitation interactions and positive relationships between plant species richness and productivity have been found in many plant communities under stressful environments (Mulder et al. 2001; Callaway et al. 2002; Wang et al. 2011, 2013; Steudel et al. 2012). Although the stress-gradient hypothesis helps predict the facilitative effects of plant diversity on biomass production in stressful environments, how plant diversity affects soil microbial communities in stressful environments is unclear.

Mine tailings have no aggregate structure, low organic matter, low concentration of nutrients [nitrogen and phosphorus] and high concentrations of heavy metals (Wang et al. 2011). Compared to the natural soil, the mine tailing

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soil represents a high stressful condition. The present study utilized an artificial plant system to test the effects of plant diversity on soil microbial communities in the natural and mine tailing soils. Soil microbial function and structure diversity were, respectively, determined using BIOLOG Ecoplate and phospholipid fatty acid (PLFA) analyses. Measures of soil total nitrogen, total phosphorus, organic matter and water content reflected the change in soil resource. We test the hypothesis that the effects of plant diversity on soil microbial communities should depend on its effect on root biomass production, and we raise the question of whether facilitative effects of plant diversity in stressful environments also promote the soil microbial functions.

## Methods

### Experimental design

Based on a plant species survey conducted at the Huangyan Pb/Zn mine tailings (28°34'23"N, 120°53'44"E) in Zhejiang, China (Table S1), eight plant species were randomly collected from common species on the mine tailings. The characteristics of plant communities on the mine tailings were (average value  $\pm$  SD,  $n = 10$ ): 736.54  $\pm$  283.40 g (dry weight) per m<sup>2</sup> of aboveground biomass, 6.76  $\pm$  2.41 per m<sup>2</sup> of species richness, 118.50  $\pm$  25.57 cm of height, 53.75  $\pm$  8.40% of cover and 34.83  $\pm$  7.83 plants per m<sup>2</sup> of density. The eight species included six annual species (*Bidens pilosa* Linn., *Brassica campestris* Linn., *Chenopodium ambrosioides* Linn., *Commelina communis* Linn., *Solanum nigrum* Linn. and *Xanthium sibiricum* Patr. ex Widder) and two perennial species (*Mirabilis jalapa* Linn. and *Phytolacca americana* Linn.). Mine tailing soil was collected from the Huangyan Pb/Zn mine tailings, and natural soil was collected from a nearby mountain of Linhai city (Beigu Mountain, 28°51'16.86"N, 121°06'29.28"E). Compared to the natural soil, the mine tailing soil had much lower concentrations of OM, total P and total N (Table S2). Moreover, there were high levels of heavy metals in the mine tailing soil. Consequently, the mine tailing soil should represent a high stressful condition.

A controlled experiment was used to manipulate plant diversity in experimental pots filled with either natural or mine tailing soil (Wang et al. 2013). Briefly, plant communities were constructed with four species richness levels for a total of 23 pots per soil type (Table 1). We did not use the alternative technique of a random selection of species from a total pool because, with a limited series of communities, equal representation of species at each species richness level was not guaranteed. Instead, combinations were chosen

**Table 1.** Experimental plant community composition manipulated with different plant species richness levels.

Species richness			
1	2	4	8
BP	BP + XS	BP + CC + CA + SN	BP + PA + CC + MJ +
PA	PA + MJ	PA + MJ + CA + BC	CA + SN + BC + XS
CC	CC + CA	XS + PA + SN + MJ	
MJ	BP + PA	PA + BP + CC + BC	
CA	SN + BC	BP + CC + MJ + CA	
SN			
BC			
XS			

Note: BP: *Bidens pilosa*, PA: *Phytolacca americana*, CC: *Commelina communis*, MJ: *Mirabilis jalapa*, CA: *Chenopodium ambrosioides*, SN: *Solanum nigrum*, BC: *Brassica campestris*, XS: *Xanthium sibiricum*.

to guarantee every species could be selected at least once at each of four diversity levels. The natural and mine tailing soils were used to represent lower and higher stressful environment, respectively. Natural and mine tailing soils were fully mixed and added to plastic pots (80 cm  $\times$  80 cm  $\times$  60 cm) in June 2009. Seeds of the eight species were collected from plants growing on the Huangyan Pb/Zn mine tailings or its surrounding area. The seeds of these species were sown in trays in April 2009, and 32 seedlings were transplanted into each pot at 2 months after germination. The plant density was similar to the natural density of plant communities in the mountain area around Linhai city. Each species in the mixtures was planted at equal partial density. The pots were weeded weekly during the experimental period to remove any naturally colonizing species. In addition to natural rainfall, water was added by artificially spraying during dry periods.

### Plant and soil samples

Plants were harvested in May 2010, about 12 months after the plant community construction. The experimental pots were disassembled. Soil and plants of each pot were placed on gauze and then washed with water. After soils were washed, the roots of each plant were carefully separated. The dead plants and their roots were excluded. Some fine roots were washed out and then gathered. The dry weight of the fine roots represented only 3–5% of all dry roots for each pot (data were calculated from 10 pots randomly selected during biomass harvest). All the aboveground and root biomass (living plants) of each pot were sorted separately into species, oven dried (80°C) to constant weight and weighed. Before the plant harvest, five soil cores (0–20 cm) were randomly collected and combined into one sample. A part of each soil sample was air-dried and homogenized and sieved (<2 mm) to remove plant roots and small stones, and was used to analyze soil total nitrogen, total phosphorus and organic matter. Another part of each soil sample was homogenized and plant roots and small stones were removed. Some of this soil was placed immediately in plastic ziplock bags and stored in a freezer at 4°C until analysis of microbial functional diversity. Some others were placed immediately in plastic bags and stored in a freezer at –20°C until analysis of PLFA.

### Soil total nitrogen, total phosphorus, organic matter and water content

Soil organic matter was determined using the Walkley–Black method (Nelson and Sommers 1982). Briefly, 10 mL of 1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 20 mL of H<sub>2</sub>SO<sub>4</sub> were added to 1 g of soil in a 500-mL Erlenmeyer flask, and then diluted with 200 mL of deionized water. The indicator of 10 mL H<sub>3</sub>PO<sub>4</sub>, 0.2 g of NaF and 10 drops of C<sub>12</sub>H<sub>11</sub>N was added and then titrated with 0.5 N (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> solution. For the analyses of total nitrogen and total phosphorus, 1.0 g of K<sub>2</sub>SO<sub>4</sub> catalyst mixture and 5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> were added to 0.5 g of air-dried natural or mine tailing soil in 100-mL digestion tube. After heating soil to a milky white color, 20 mL of distilled water was added into the digestion tube. Total nitrogen was determined using the Berthelot reaction method and total phosphorus was determined using the molybdenum blue method (Page et al. 1982). The soil total nitrogen, total phosphorus, organic matter was measured in three repeats

per pot. Before the biomass harvesting, the water content of each pot was determined 5 d after they had all received the same amount of water through artificial spraying (no water was added during the final 5 d before harvesting). Five soil cores to a depth of 0–20 cm were randomly collected in each pot. Five replicated samples from each pot were combined into one sample. The water content was calculated by comparing the weight of undried and dried soil [(weight of undried soil – weight of dried soil)/weight of dried soil].

### BIOLOG analysis

The functional diversity of the soil microbial community was measured using BIOLOG Ecoplate (BIOLOG Inc., Hayward, CA, USA) following the method of Garland and Mills (1991). Fresh soil, equivalent to 10 g dry weight, was suspended in 100 mL of 0.85% NaCl on a rotary shaker for 30 min, and then serially diluted to  $10^{-3}$ . Each well of the plate was directly inoculated with 125  $\mu$ L of the diluted suspensions. Plates were incubated at 28°C. Absorbance ( $A_i$ ) of each well on the plate was measured after 144 h of incubation using a microplate reader (GENios ProTM, Tecan, Trading AG, Switzerland). The  $A_i$  was corrected against the control well on each plate before data analysis. Negative  $A_i$  values were set to zero. Average well color development (AWCD) was calculated as the sum of  $A_i$  per plate, divided by the 31 sources of carbon (C) (Garland 1996). AWCD was used to estimate cultural bacterial activity.

### PLFA analysis

Details of PLFA extraction and analysis were described in White et al. (1979). Briefly, a gas chromatograph (GC 7890, Agilent Technologies, Bracknell, UK) and a mass spectrometer (5973N MSD, Agilent Technologies) were used to analyze the final phospholipids, and an HP-5 MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) was used to separate the phospholipids fatty acids by automatic injection. Fatty acid contents were calculated from peaks generated by blanks containing an internal standard (methyl ester carbon 19:0, 10 ng  $\mu$ L<sup>-1</sup>, Sigma–Aldrich), as described previously (Abaye et al. 2005). The PLFAs identified in samples of the study were as follows: 12:1 3OH, 13:0 2OH, 14:0, 14:0a, 15:0 iso, 15:0a, 15:0i, 16:0, 16:0a, 16:0 N alcohol, 16:1w7c, 17:0, 17:0a, 17:1 iso, 18:0, 18:1w7c, 18:1w9c, 18:2w6, 9c, 18:3w6, 9, 12c, 19:1 iso and 19:0 10-methyl. Total PLFAs were used as an index of total living microbial biomass (Chapman and Newman 2010).

### Soil basal respiration

Soil basal respiration was determined following the method of Isermeyer (1952). Twenty grams of fresh soil was evenly distributed in a reagent bottle (500 mL). Approximately 20 mL of 0.05 M NaOH was dispensed into an open plastic bottle (50 mL), which was placed inside the reagent bottle. The apparatus was then closed and incubated for 24 h at 28°C. The CO<sub>2</sub> absorbed by the 0.05 M NaOH was precipitated as BaCO<sub>3</sub> by adding BaCl<sub>2</sub>. The remaining NaOH was titrated with 0.1 M HCl with phenolphthalein solution as the indicator.

### Analysis

The functional diversity (BIOLOG<sub>Shannon index</sub>) was calculated using the following equation:

$$H = - \sum p_i (\ln p_i),$$

where  $p_i$  is the proportional utilization of individual C source to all C sources.

Multiple regressions were used to predict soil microbial functions (BIOLOG<sub>Shannon index</sub>, total living microbial biomass, cultural bacterial activity and soil basal respiration) as a function of plant and soil factors (including the plant species richness, shoot biomass, root biomass, soil total nitrogen, total phosphorus, organic matter and water content). Backwards elimination was used to determine which factor was retained in the subset of variables that significantly ( $P < .05$ ) contributed to the regression. In the backwards elimination analysis, the least significant variable was eliminated from a multiple regression and a new multiple regression analysis was performed until all remaining variables were significant.

The deviation ( $D_i$ ) of function in a mixture could be expected based on monoculture function (Hector et al. 2002).

$$D_i = \frac{O_i - E_i}{E_i},$$

where  $O_i$  is the observed value in a mixture and  $E_i$  is the expected value, i.e. the monoculture function value multiplied by the initial proportion (relative abundance) of the species in the mixture. If  $D_i > 0$ , mixture expressed a better performance than the expected function; if  $D_i < 0$ , mixture expressed a worse performance than the expected function.

For each mixture, we calculated the over-function index  $D_{\text{mix}}$  as follows (Hector et al. 2002):

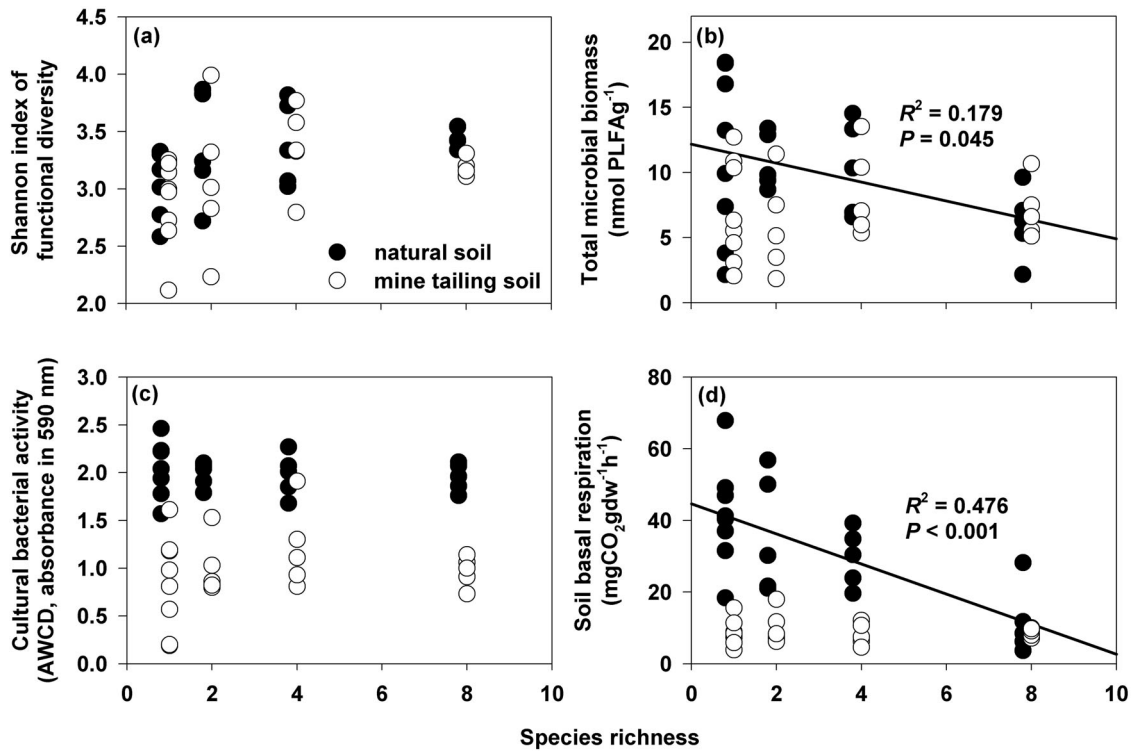
$$D_{\text{mix}} = \frac{O_T - M_{\text{max}}}{M_{\text{max}}}$$

where  $O_T$  was the observed value of functions (such as above-ground biomass, BIOLOG<sub>Shannon index</sub>, total living microbial biomass, cultural bacterial activity and soil basal respiration) in a mixture;  $M_{\text{max}}$  was the maximum function value grown in the monoculture. If  $D_{\text{mix}} > 0$ , facilitation should be the principal species interaction.

The dependence of soil microbial functions,  $D_i$  and  $D_{\text{mix}}$  on the plant species richness was analyzed using simple regression. The relationships between  $D_i$  of root biomass and  $D_i$  of soil microbial functions were analyzed using bivariate correlation. The analyses were conducted using SPSS 20.0.

### Results

The plant species richness marginally affected the BIOLOG<sub>Shannon index</sub> in both natural (Figure 1(a),  $r = 0.372$ ,  $n = 23$ ,  $P = .089$ ) and mine tailing soils ( $r = 0.381$ ,  $n = 23$ ,  $P = .072$ ). In the natural soil, the plant species richness negatively affected the total living microbial biomass (Figure 1(b), total living microbial biomass =  $-0.727$  plant species richness + 12.317,  $F = 4.564$ ,  $P = .045$ ) and soil basal respiration (Figure 1(d), soil basal respiration =  $-4.200$  plant species richness + 45.482,  $F = 19.057$ ,  $P < .001$ ), but had no significant effect on the cultural bacterial activity (Figure 1(c)). In the mine tailing soil, the plant species richness had no significant



**Figure 1.** The dependence of  $\text{BIOLOG}_{\text{Shannon index}}$  (a), total living microbial biomass (b), cultural bacterial activity (c) and soil basal respiration (d) on the plant species richness in the natural and mine tailing soils. The regression lines are shown and non-significant relationships are also shown. Solid lines natural soil, dotted lines mine tailing soil.  $R^2$  (regression coefficient) and  $P$ -values are shown next to significant fitted lines.

effects on the total living microbial biomass, cultural bacterial activity and soil basal respiration (Figure 1(b–d)).

Multiple regressions were used to analyze the relationships between soil microbial functions and plant species richness, biomass production and soil resources (Table 2). Shoot biomass, soil total nitrogen, total phosphorus, organic matter and water content were always deleted in the backwards elimination analysis and had no significant effects on the soil microbial functions. However, the root biomass was always retained as significant variables in both natural and mine tailing soils. Moreover, the root biomass positively affected the soil microbial functions. The plant species richness also

positively affected  $\text{BIOLOG}_{\text{Shannon index}}$  in both natural and mine tailing soils. However, the plant species richness negatively affected the total living microbial biomass and soil basal respiration in the natural soil.

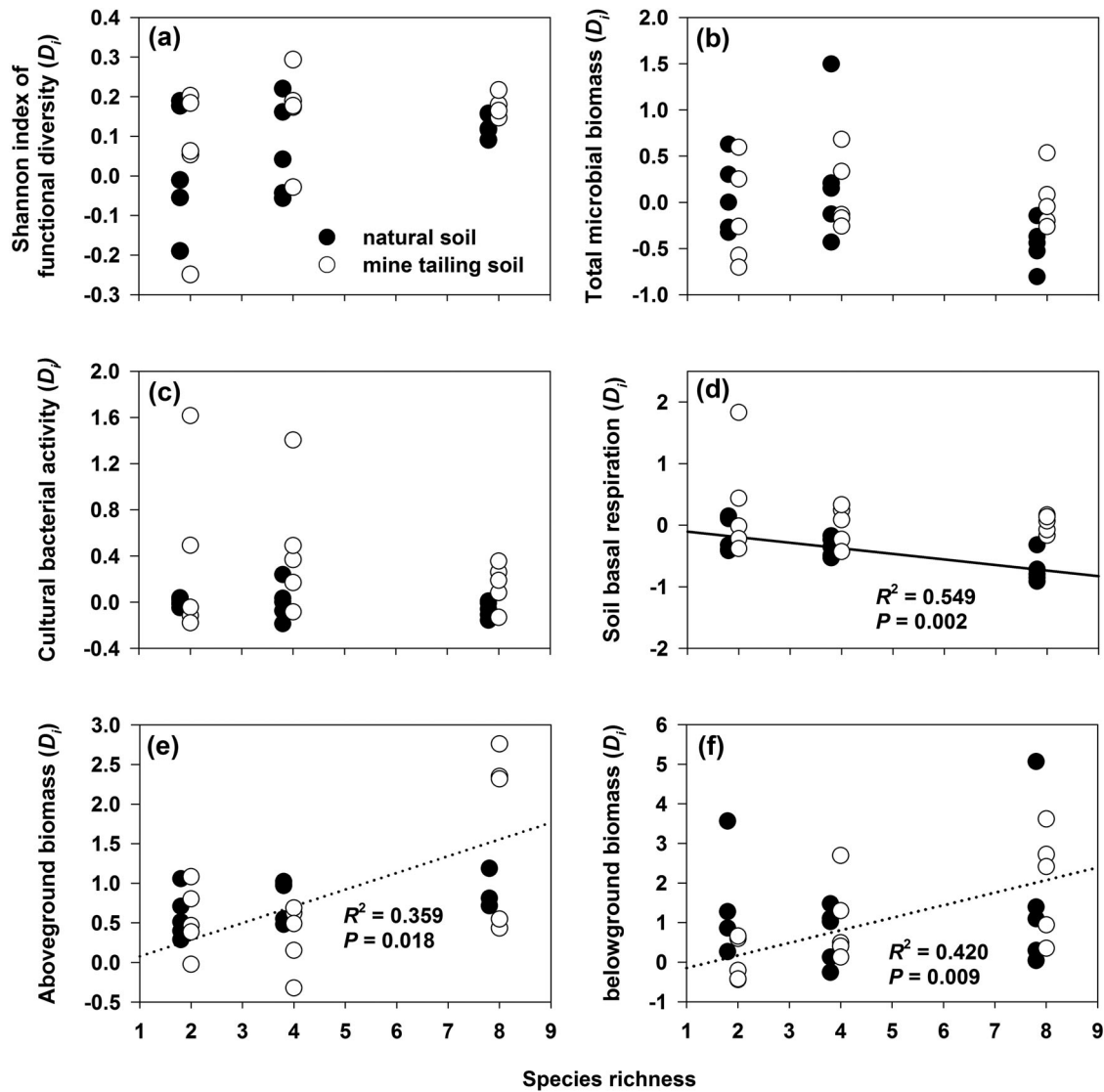
Linear regression analysis showed that the  $D_i$  of soil basal respiration negatively correlated with the plant species richness in the natural soil (Figure 2(d), soil basal respiration =  $-0.090$  plant species richness +  $0.004$ ,  $F = 15.798$ ,  $P = .002$ ). The  $D_i$  of aboveground biomass (Figure 2(e), aboveground biomass =  $-0.135$  plant species richness +  $0.211$ ,  $F = 7.294$ ,  $P = .018$ ) and belowground biomass positively correlated with the plant species richness in the mine tailing soil (Figure 2(f), belowground biomass =  $-0.466$  plant species richness +  $0.317$ ,  $F = 9.415$ ,  $P = .009$ ). The plant species richness also marginally affected the total microbial biomass (Figure 2(b),  $r = -0.101$ ,  $n = 15$ ,  $P = .083$ ) and aboveground biomass (Figure 2(e),  $r = 0.052$ ,  $n = 15$ ,  $P = .083$ ) in the natural soil.

The  $D_{\text{mix}}$  of aboveground biomass had no significant relationship with the plant species richness in the natural soil. Consistent with the stress-gradient hypothesis, the  $D_{\text{mix}}$  of aboveground biomass positively correlated with the plant species richness in the mine tailing soil (Figure 3(e), aboveground biomass =  $0.080$  plant species richness +  $0.842$ ,  $F = 7.081$ ,  $P = .020$ ). Moreover, in the mine tailing soil, the  $D_{\text{mix}}$  of aboveground biomass were higher than one in most of mixtures. Different from the aboveground biomass, the plant species richness had no positive relationships with the  $D_{\text{mix}}$  of soil microbial functions in the mine tailing soil (Figure 3(a–d)). Moreover, in the mine tailing soil, the  $D_{\text{mix}}$  of total living microbial biomass, cultural bacterial activity and soil basal respiration were lower than one in most of mixtures (Figure 3(b–d)).

The performances of soil microbial functions and root biomass in mixtures were estimated. The  $D_i$  of  $\text{BIOLOG}_{\text{Shannon index}}$  negatively correlated with the  $D_i$  of root biomass in the

**Table 2.** Multiple regression analyses between  $\text{BIOLOG}_{\text{Shannon index}}$ , total living microbial biomass, cultural bacterial activity and soil basal respiration and variables derived by backwards elimination from a model that initially contained plant species richness, shoot biomass, root biomass, soil total nitrogen, total phosphorus, organic matter and water content for natural soil and mine tailing soil treatments, respectively.

Soil type	Soil microbial function	Variable	Partial correlation coefficient	$P$ -value
Natural soil	$\text{BIOLOG}_{\text{Shannon index}}$	Species richness	0.417	.048
	Total living microbial biomass	Species richness	-0.393	.040
		Root biomass	0.430	.026
	Cultural bacterial activity	Root biomass	0.488	.018
	Soil basal respiration	Species richness	-0.872	<.001
		Root biomass	0.361	.008
Mine tailing soil	$\text{BIOLOG}_{\text{Shannon index}}$	Species richness	0.477	.013
	Total living microbial biomass	Root biomass	0.816	<.001
	Cultural bacterial activity	Root biomass	0.855	<.001
	Soil basal respiration	Root biomass	0.743	<.001



**Figure 2.** The dependence of  $D_i$  (BIOLOG<sub>Shannon index</sub> (a), total living microbial biomass (b), cultural bacterial activity (c), soil basal respiration (d), aboveground biomass (e) and belowground biomass (f) on the plant species richness in the natural and mine tailing soils. The regression lines are shown and non-significant relationships are also shown. *Solid lines* natural soil, *dotted lines* mine tailing soil.  $D_i$ ; deviations between observed performances and expected performances from the monoculture performance of each species of mixture.  $R^2$  (regression coefficient) and  $P$ -values are shown next to significant fitted lines.

natural soil (Figure 4(a),  $r = -0.669$ ,  $n = 15$ ,  $P = .006$ ). However, they had no significant relationship in the mine tailing soil. The  $D_i$  of total living microbial biomass, cultural bacterial activity and soil basal respiration positively correlated with the  $D_i$  of root biomass in both natural (Figure 4(b–d), total living microbial biomass,  $r = 0.526$ ,  $n = 15$ ,  $P = .044$ ; cultural bacterial activity,  $r = 0.520$ ,  $n = 15$ ,  $P = .047$ ; soil basal respiration,  $r = 0.621$ ,  $n = 15$ ,  $P = .014$ ) and mine tailing soils (total living microbial biomass,  $r = 0.665$ ,  $n = 15$ ,  $P = .007$ ; cultural bacterial activity,  $r = 0.538$ ,  $n = 15$ ,  $P = .039$ ; soil basal respiration,  $r = 0.519$ ,  $n = 15$ ,  $P = .047$ ).

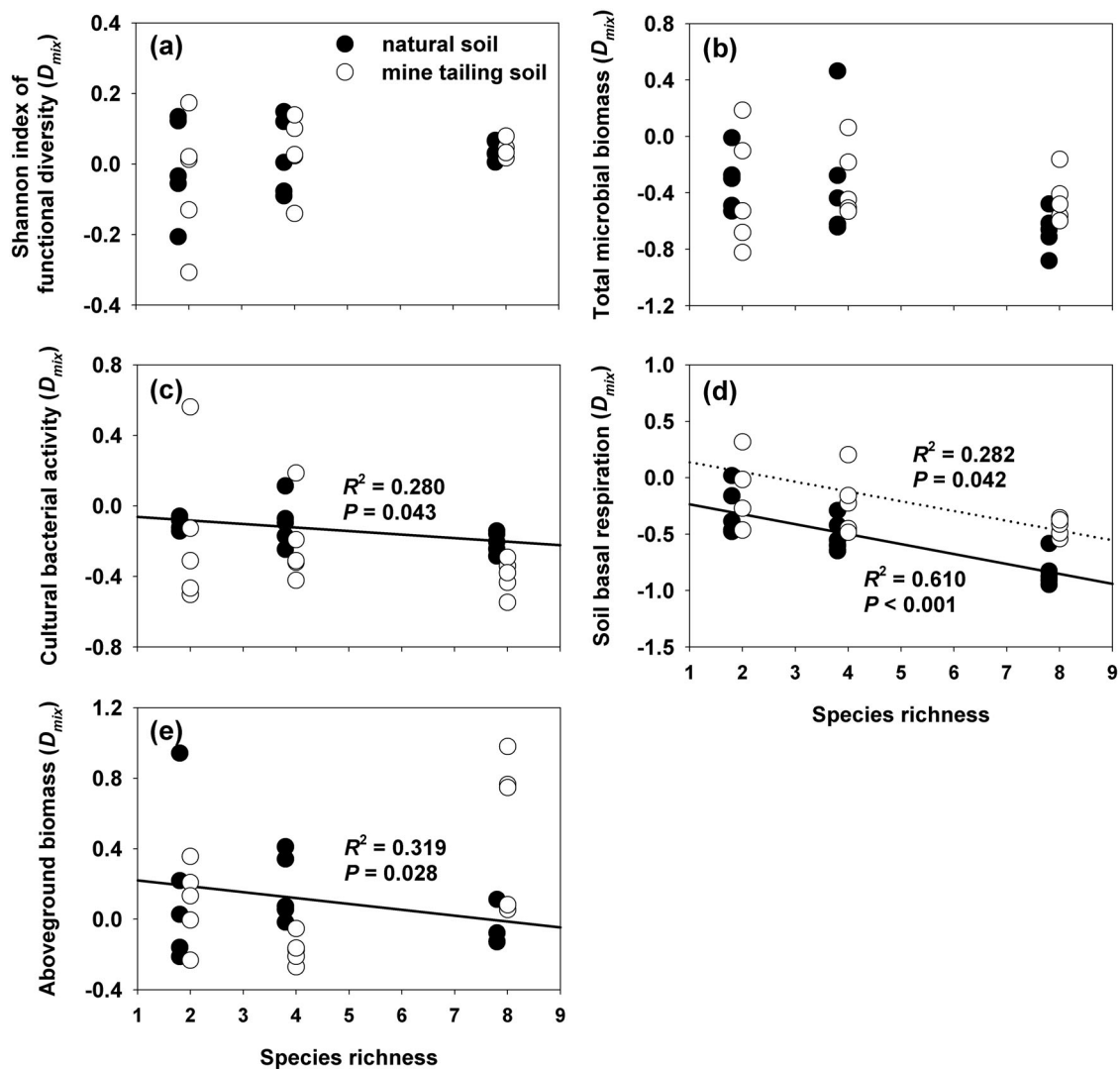
The plant species richness also had significant effects on the number of utilized carbon resources and the number of PLFA. Linear regression analysis showed that the number of utilized carbon resources (Figure 5(a), the number of utilized carbon resources in natural soil =  $0.624$  plant species richness +  $25.668$ ,  $F = 27.632$ ,  $P < .001$ ; the number of utilized carbon resources in mine tailing soil =  $0.415$  plant species richness +  $24.940$ ,  $F = 10.750$ ,  $P = .004$ ) and the number of PLFA (Figure 5(b), the number of PLFA in natural soil =  $0.815$  plant species richness +  $6.540$ ,  $F = 75.435$ ,  $P < .001$ ; the number of PLFA in mine tailing soil =  $0.675$  plant species richness +  $5.234$ ,  $F = 30.459$ ,  $P < .001$ ) positively correlated

with plant species richness in both natural and mine tailing soil.

## Discussion

In this study, the plant species richness had a marginally positive effect on the BIOLOG<sub>Shannon index</sub>. However, root biomass production rather than plant species richness positively determined the total living microbial biomass, cultural bacterial activity and soil basal respiration. Consequently, from the results of this study, we speculate that contrast results for the effects of plant diversity on soil microbial functions in prior studies may be due to a different underlying mechanism that determines specific soil microbial function.

Plant productivity covarying with plant diversity is an important factor that affects soil microbial communities (Zak et al. 2003). Many studies have found that plant diversity increased microbial biomass, activity and respiration mainly via increases in plant productivity (Zak et al. 2003; Johnson et al. 2008). In this study, consistent with our hypothesis, plant shoot biomass had no significant effect on the microbial biomass, activity and respiration, while root biomass



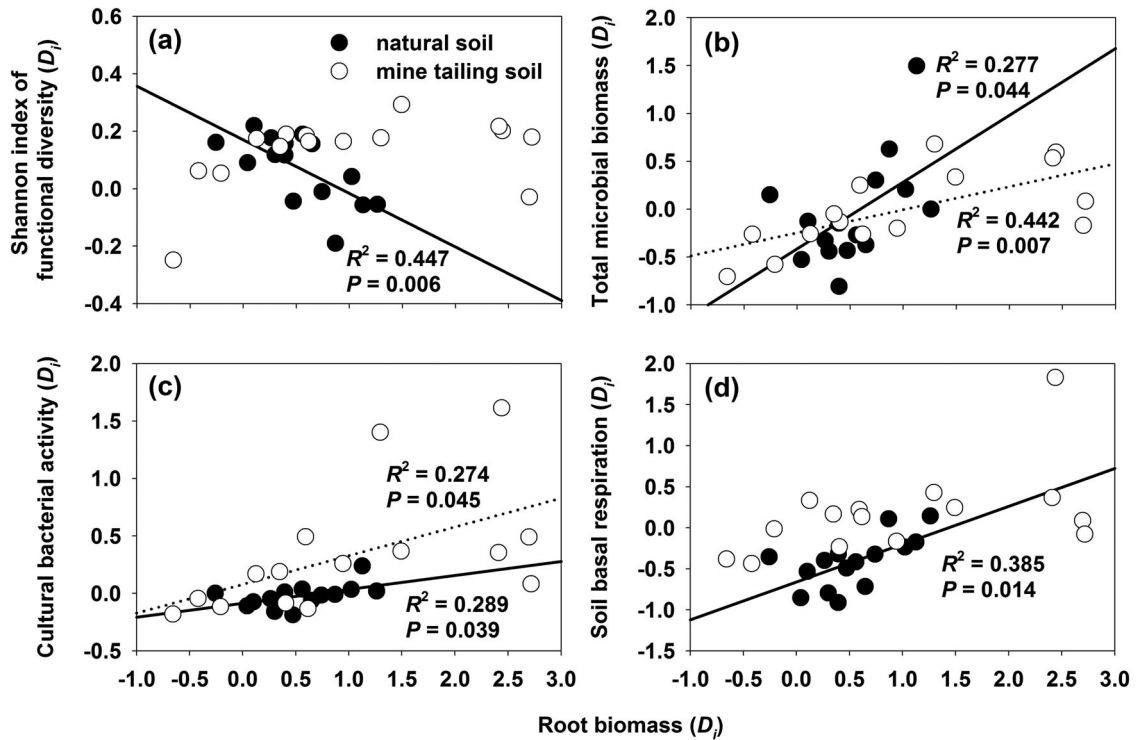
**Figure 3.** The dependence of  $D_{mix}$  (BIOLoG<sub>Shannon</sub> index (a), total living microbial biomass (b), cultural bacterial activity (c), soil basal respiration (d) and aboveground biomass (e) on the plant species richness in the natural and mine tailing soils. The regression lines are shown and non-significant relationships are also shown. *Solid lines* natural soil, *dotted lines* mine tailing soil.  $D_{mix}$ : over-function index, ratio of (the observed value of functions in a mixture – the maximum function value grown in the monoculture)/the maximum function value grown in the monoculture. If  $D_{mix} > 0$ , facilitation should be the principal species interaction.  $R^2$  (regression coefficient) and  $P$ -values are shown next to significant fitted lines.

positively affected them. Moreover, the performance of soil microbial biomass, activity and respiration in mixtures had a positive link with the performance of root biomass, which may be due to the stimulation of living root to microbial activity and growth (Oger et al. 2004; Blagodatskaya et al. 2009; Nannipieri et al. 2012). As the effect of each species on the soil microbial function in mixtures is difficult to separate, the effect mechanism (complementarity, facilitation and selection effects) of plant diversity on the soil microbial function is difficult to analyze. Consequently, we suggest that the effect mechanism of plant diversity on root biomass production will be helpful to explain how plant diversity affects the soil microbial function.

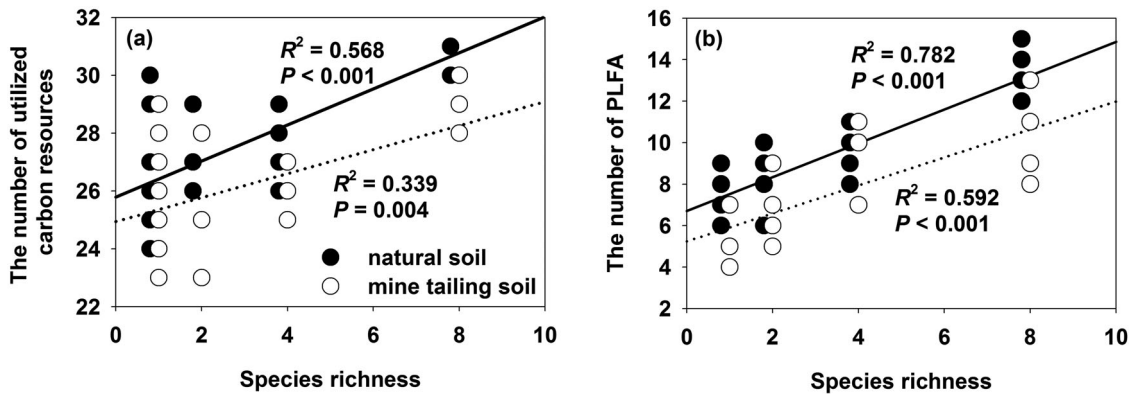
In the natural soil, the plant species richness negatively affected the total living microbial biomass and soil basal respiration. We speculate that the negative effects of plant species richness may be related to biomass partition between shoot and root. The theory of functional equilibrium postulates that biomass should be allocated to the organs with more resource competition (Brouwer 1983; Bloom et al. 1985; Wilsey and Polley 2004). In this study, *P. Americana* and *M. jalapa* are the tallest species and have broad leaves. We speculate that the mixtures with more species should

have a higher probability including these species (especially for eight-species mixtures). Light competition should be more intense in more diverse mixtures. Moreover, consistent with the result of Bessler et al. (2009), the root/shoot ratio negatively correlated with the plant species richness in the natural soil (Figure S1). Consequently, the negative effects of plant species richness on the total living microbial biomass and soil basal respiration may be related to less biomass allocation to root.

In the mine tailing soil, facilitative effects among species ( $D_{mix}$  of most of the mixture were higher than one) led to positive relationships between the plant species richness and the aboveground biomass. However, facilitative effects among species did not significantly influence the soil microbial functions. Moreover, the root/shoot ratio had no significant relationship with the plant species richness (Figure S1). The reason may be balance of competition between light and belowground resources. Mine tailings have no aggregate structure, low organic matter (OM), low concentration of nutrients [nitrogen (N) and phosphorus (P)] (Wang et al. 2011). More diverse mixture (have a higher probability including species with great absorption of belowground resources) may simultaneously have more intense



**Figure 4.** The relationships between  $D_i$  of root biomass and  $D_j$  of soil microbial functions (BIOLoG<sub>Shannon index</sub>) (a), total living microbial biomass (b), cultural bacterial activity (c) and soil basal respiration (d) in the natural and mine tailing soils. The regression lines are shown and non-significant relationships are also shown. Solid lines natural soil, dotted lines mine tailing soil.  $D_i$ : deviations between observed performances and expected performances from the monoculture performance of each species of mixture.  $R^2$  (regression coefficient) and  $P$ -values are shown next to significant fitted lines.



**Figure 5.** The dependence of the number of (a) utilized carbon resources and (b) PLFAs on the plant species richness in the natural and mine tailing soils. The regression lines are shown and non-significant relationships are also shown. Solid lines natural soil, dotted lines mine tailing soil.  $R^2$  (regression coefficient) and  $P$ -values are shown next to significant fitted lines.

competition in belowground resources and increased biomass allocation to root.

Different from the soil microbial biomass, activity and respiration, the plant species richness had a marginally positive effect on the BIOLoG<sub>Shannon index</sub>. Although the plant species richness did not significantly affect the BIOLoG<sub>Shannon index</sub>, the number of utilized carbon resources and the number of PLFAs significantly increased with increasing plant species richness (Figure 2). Grayston et al. (1997) found that, as different plant species add different carbon substrates to soil, the number (i.e. the diversity) of soil carbon substrates may also increase with increasing plant species richness. Moreover, increases of carbon substrate diversity most likely support more diversity microbial communities (Grayston et al. 1998). Soil microbial diversity is positively correlated with the multifunctionality of terrestrial ecosystem. The loss of soil microbial diversity will reduce the service functions

of terrestrial ecosystems, such as climate regulation, soil fertility and food production (Delgado-Baquerizo et al. 2016). Our results support the singular hypothesis for plant diversity (Eisenhauer et al. 2010), i.e. each plant species should have a unique effect on soil microbial communities, and may form the scientific basis for conservation of plant diversity in order to maintain soil microbial functional and structural diversity.

In this study, consistent with our hypothesis, soil microbial biomass, activity and respiration had positive link with root biomass production. Biomass partition between shoot and root can be affected by resource competition, such as light, soil moisture, mineral and nutrient competition (Wilson 1988; McConnaughay and Coleman 1999; Kahmen et al. 2005). Consequently, different responses of soil microbial biomass, activity and respiration to increasing plant diversity (Stephan et al. 2000; Zak et al. 2003; Johnson et al. 2008;



Zhang et al. 2010; Eisenhauer et al. 2011; Rottstock et al. 2014) may be due to root biomass production being mediated by other factors. Contrast to our question, facilitation among species did not lead to positive effects of plant diversity on the soil microbial functions. We also found that the plant species richness had a promotional effect on soil microbial functional diversity. Altogether, our findings provide strong empirical evidence that plant species diversity is critical for maintaining the soil microbial diversity of carbon resources utilization and soil microbial functional diversity. Especially in mine tailing soil, the protection of microbial diversity may be of great significance to the recovery of soil function.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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