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REGULAR ARTICLE

Copper tolerant *Elsholtzia splendens* facilitates *Commelina communis* on a copper mine spoil

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Abstract
Background and aims There is evidence that plant facilitation occurs in heavy metal wastelands, but the extent and mechanisms of facilitation are not known. The copper (Cu) tolerant *Elsholtzia splendens* is a dominant pioneer species during the secondary succession on copper mine spoils in eastern China. Species appearing later are often associated with patches of *E. splendens*. We hypothesize that *E. splendens* facilitates neighbors by modifying local soil properties.
Methods We conducted a field study on a heavy metal wasteland with local variation in soil Cu level to investigate the performance of a target species, *Commelina communis*, growing in open gaps vs. growing with *E. splendens*. Soil physicochemical and biological properties, biomass, plant interaction intensity as well as heavy metal concentration in *C. communis* were measured to study the effects of the presence of *E. splendens*.
Results Effects of the presence of *E. splendens* on *C. communis* were generally positive, but negative effects were sometimes observed. Positive effects of *E. splendens* increased with increasing soil Cu level. Soil microbial activity was higher in the presence of *E. splendens*. Our results are consistent with the hypothesis that facilitation occurred through enrichment of the microbial properties of the soil, especially soil respiration rate and enzyme activity.
Conclusions Our study highlights the importance of soil-mediated plant-plant interactions for the establishment of *C. communis* on heavy metal-contaminated sites. These interactions are important for the restoration of heavy metal wastelands.

Keywords Cu tolerance · Facilitation · Heavy metals · Soil contamination

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Introduction

Plant-plant interactions shape plant community structure, biodiversity, drive community succession and ultimately influence ecosystem functioning (Michalet et al. 2006; McIntire and Fajardo 2014). For many years, competition has dominated mainstream thinking in both theoretical and empirical research in plant community ecology. Over the past two decades, however, the role of positive plant-plant interaction (facilitation) has received more attention (Brooker et al. 2008). Increasing evidence indicates that facilitation, which mostly occurs in severe environments or under high consumer pressure, can promote establishment, growth and recruitment of plants and reduce seedling mortality (Rey et al. 2009; Xu et al. 2010; Michalet et al. 2011; Verwijmeren et al. 2013). Competition and facilitation occur simultaneously, but their relative intensity and significance in determining plant community dynamics remains controversial (Callaway et al. 2002; Maestre et al. 2006; He et al. 2013). Competition is the dominant plant-plant interaction under relatively benign conditions, while facilitation often dominates under extreme conditions, although there is disagreement about very extreme conditions (Brooker et al. 2008; Butterfield 2009; Bowker et al. 2010; Verwijmeren et al. 2013).

Heavy metal wastelands are widespread in China. They are usually characterized by contamination by several metals, low nutrient and water availability, and severely degraded vegetation (Ye et al. 2002). The diversity of plant communities occurring naturally is very low and community succession occurs slowly on these wastelands (Yang et al. 2010). Major efforts have been made to regenerate vegetation using metal tolerant plants or hyperaccumulators (phytoremediation). Even when successful, the resultant communities show very low plant diversity and ecosystem stability (Rey et al. 2009). The role of facilitation in heavy metal or metalloid contaminated ecosystems is becoming widely recognized. There was a positive relationship between biodiversity, productivity and cadmium (Cd) removal efficiency in an aqueous algae community suffering from Cd pollution, and this was attributed to facilitation between Cd tolerant and metal-sensitive algal species (Li et al. 2010, 2012). Selenium (Se) hyperaccumulators facilitated the growth of Se tolerant plants by absorbing Se from soil and reducing herbivory (Mehdawi et al. 2011a), but there were negative effects on the germination and growth of Se sensitive plants, resulting in 10 % lower vegetative coverage (Mehdawi et al. 2011b). The outcome of plant interactions under contamination depends on soil properties and the tolerance of the neighboring plants (Mehdawi et al. 2012).

Elsholtzia splendens Nakai [Lamiaceae] is a highly copper (Cu) tolerant plant, tolerating soils with more than 10^4 mg Cu kg^{-1} DW soil. It is a pioneer species, often dominant at the early stage of secondary succession on Cu mine spoils in China (Yang et al. 2010). Other plant species on these sites include *Commelina communis* L., *Artemisia capillaries* Thunb., *Setaria viridis* (L.) Beauv., *Polygonum hydropiper* L., *Rumex acetosa* L. and *Kummerowia striata* (Thunb.) Schindl., which appear later, often in proximity to *E. splendens* (Fig. 1). It has been hypothesized that vegetation patches on serpentine soils are structured by facilitation (Oviedo et al. 2014). We therefore hypothesized that the Cu tolerant *E. splendens* serves as a nurse plant to facilitate the establishment of other plant species, and that this facilitation is mainly due to mitigation of Cu stress and improvements in other soil physicochemical and biological properties induced by *E. splendens*. A field study was conducted to test this hypothesis with the objectives (1) to verify whether facilitation does exist between *E. splendens* and the coexisting species, (2) if so, to obtain information about the underlying mechanisms of facilitation, and (3) to explore the implications for vegetation restoration and community succession on Cu mine spoils.

Materials and methods

Study site

The field study was conducted on a Cu mine spoil in the Dagong-Fenghuang mountain area in southern Anhui Province, eastern China (30°55′51″N, 118°9′23″E). This area is characterized by a subtropical wet monsoon climate with mean annual temperature, precipitation and evaporation of 15.8 ° C, 1400 mm and 1377 mm, respectively. There is large seasonal variation in temperature and precipitation. The lowest and highest daily mean temperatures are 0 ° C and 33 ° C occur in January and July, respectively. Most precipitation falls from May to July, and the monthly precipitation ranges from 36 mm (December) to 190 mm (June). There is an average of 1935 h sunshine and approximately 237–258 frost-free days per year. The soil is a sandy loam, which is susceptible to water and wind erosion. The spoil has been abandoned for more than 50 years and the surface soil contains a high level of organic matter. The vegetation consists of approximately 40 % herbaceous species, including *E. splendens*, *C. communis*, and *A. capillaries* with scattered shrubs, especially *Ilex aquifolium* L. The study site is located 35 km east of Tongling city (30°45′12″–31°07′56″N, 117°42′00″–118°10′6″E), one of the major Cu mining and smelting centers in eastern China. *C. communis* is common in the area. It is found both growing in clumps (a) alone and (b) mixed with

Fig. 1 Dominant plant species during succession on 6 years old Cu mine spoil in Anhui Province, eastern China: **a** *Elsholtzia splendens*, **b** *Setaria viridis* associated with *E. splendens*, **c** *Commelina communis* (blue flowers) growing together with *E. splendens*; **d** *C. communis* growing in an open gap

E. splendens. Both plants are annuals with very similar phenologies. The local abundance and spatial distribution of *C. communis* growing without *E. splendens* fluctuates greatly from year to year, while populations of *C. communis* growing in proximity to *E. splendens* show more stability over space and time (pers. observation).

Plants and soil

A 3 m×3 m plot was placed in each of four areas on the south slope of a mountain. Within each area the location of the plot was random. The distance between plots ranged from 30 to 50 m. Within each plot we placed four pairs of subplots (1 m×1 m) such that *C. communis* was growing alone in open gaps (labeled "without *Elsholtzia*") in one subplot and together with *E. splendens* (labeled "with *Elsholtzia*") in the other. Subplots within a pair were placed 40 to 60 cm apart: close enough to minimize environmental differences, while not so close that they would influence each other. In each subplot, whole plants of *C. communis* were sampled at the full flowering stage in September 2012. Roots were removed from the soil and shaken, and soil particles adhering to the fine roots were collected and considered rhizospheric soil. About 500 g of this rhizospheric soil from each subplot was pooled and transported immediately to the laboratory. A part of each soil sample was air dried, ground in a ceramic mortar and passed through a 2 mm sieve to determine physicochemical properties. The remaining soil was stored at 4 ° C for further analysis. The plants were washed with tap water to remove adhering soil particles and divided into root and shoot fractions. The shoots and roots were oven dried at 105 ° C for 30 min and subsequently at 80 ° C to a constant weight. A 1 m×1 m stainless frame was used to visually estimate the ground cover of *C. communis* in the subplots when sampling soil and plants. The biomass of *C. communis* was expressed as dry weight per individual plant and per square meter.

Measurements

Soil temperature and relative humidity

Soil temperature and relative humidity in the rhizosphere of *C. communis* were recorded every 15 min in both the open gaps and the subplots in which *C. communis* coexisted with *E. splendens* using a real time temperature and humidity recorder (JL01, HanDan Qingsheng Electronic Science Technology Co., Ltd, China). The digital sensors for temperature and relative humidity were placed in the soil at the depth of 5.0 cm and 15.0 cm, respectively. Data from16 consecutive clear days from 16 to 31 in July of 2012 were used for the analysis.

Soil physicochemical properties

Soil granulometry was determined using a laser diffraction particle size analyzer (MS-2000, Malvern Instruments Ltd. UK) after the removal of organic matter

(Nayar et al. 2007). Soil pH was measured in a suspension with 1.0 mol l^{-1} KCl (2.5:1, KCl aqueous soil solution). Organic matter content was determined using $K_2Cr_2O_7$-H_2SO_4 (Lu 2000). Total and Olsen P were quantified using H_2SO_4-$HClO_4$ digestion and HCl-H_2SO_4 extraction, respectively (Olsen and Sommers 1982). Extractable N (NO_3^--N and NH_4^+-N) was measured using phenol disulfonic acid and indophenol blue colorimetric method (Lu 2000). Total and extractable Cu and zinc (Zn) concentrations were extracted with HF-$HClO_4$-HNO_3 and 0.1 mol l^{-1} HCl, respectively (Lu 2000), and subsequently analyzed by flame atomic absorption spectrophotometry (AA-6650, Shimadzu, Japan).

Soil active organic matter (AOM)

Soil active organic matter was determined using the $KMnO_4$ oxidation method described by Blair et al. (1995). Briefly, air-dried soil containing about 15 mg C, calculated from the known total C content, were weighed into screw-cap centrifuge tubes containing 25 ml 333 mmol l^{-1} $KMnO_4$ solution. The suspensions were shaken for 1 h at 25 ° C and then centrifuged at 2000 rpm for 5 min. The supernatant was diluted 1:250 with deionized water and the absorbance was measured at 565 nm using a spectrophotometer (UV757CRT, Shanghai Precision and Scientific Instrument Co., Ltd. Shanghai, China). Changes in concentrations of $KMnO_4$ were used to estimate the amount of oxidized C.

Soil microbial biomass carbon (MBC)

Microbial biomass carbon was determined using the fumigation-extraction method (Vance et al. 1987). Moisture contents of the fresh soils were determined before microbial biomass carbon measurement. Fresh soil (10 g dry weight equivalent) was extracted with 40 ml 0.5 mol l^{-1} K_2SO_4, shaken for 30 min and filtered through 0.45 μm micro-pore film on a vacuum extraction set. Another fresh soil sample of the same weight was fumigated with ethanol free chloroform for 48 h and extracted with the same agent as above. Total organic carbon (TOC) was measured using a TOC-Vwp analyzer (Shimadzu, Japan). MBC was calculated from the total dissolved organic carbon divided by a kec-factor of 0.38 (Lu 2000).

Soil basal respiration(BR) and metabolic quotient (qCO_2)

Soil basal respiration was determined using the alkali absorption method with modifications (Alef 1995). To be specific, 100 g of moist soil was incubated in an airtight chamber (9.5 cm in diameter, 12.5 cm in height) at 25 °C for 24 h in the dark. A container (5 cm in diameter, 6 cm in height) with 20 ml of 0.1 mol l^{-1} NaOH solution was placed on a bracket 2 cm above the soil surface. The CO_2 was captured with NaOH and precipitated with 1 mol l^{-1} $BaCl_2$. The excess alkali was titrated with 0.1 mol l^{-1} HCl using phenolphthalein as an indicator. An identical chamber without soil was used as blank control. Soil respiration was determined by quantifying the CO_2released in the process of microbial respiration during 24-h-incubation and expressed as μg CO_2-C released g^{-1} soil h^{-1}. The water content of the fresh soil was determined independently. The microbial metabolic quotient (qCO_2) was calculated by dividing respiration rates by microbial biomass C.

Soil enzyme activity assays

Sucrase and urease activities were measured using the method of Ge et al. (2010). Alkaline and acid phosphatase activities were determined using the procedures described by Guan (1986). Control tests without soils or substrates were carried out to evaluate the spontaneous or abiotic transformation of substrates.

Plant Cu concentration

Plant shoots and roots were ground into fine powder and incinerated into ash at 600 ° C for 2 h. The ash was dissolved in 1:1 (v/v) nitric acid (Lu 2000). The Cu concentration was measured using atomic absorption spectroscopy (AA6650, Shimadzu, Japan).

Relative species interaction intensity

Relative interaction intensity (RII) of *C. communis* was calculated to quantify the responses of *C. communis* to the presence of *E. splendens* following the formula proposed by Armas et al. (2004):

$$RII = \frac{(X_{T+N} - X_{T-N})}{(X_{T+N} + X_{T-N})}$$

where $X_{T\text{-}N}$ and X_{T+N} are the biomass of target species, *C. communis*, in the absence and presence of *E. splendens*. The index is symmetrical around zero (no interaction), and is constrained by +1 (facilitation) and-1 (competition).

Data analysis

All data, including soil physicochemical and biological properties, plant biomass and heavy metal uptake, were inspected for homogeneity of variance and analyzed with two-way ANOVA with Cu concentration (4 Cu levels) and treatment (with or without *E. splendens*) as factors. More complicated nested models gave similar results, so we present the simpler, conservative analyses here. A single sample *t*-test was used to analyze the significant deviation of RII values from zero. A two-tailed Pearson correlation analysis was conducted between soil physicochemical and biological properties. Analyses were performed with SPSS Statistics V.20.0 software (SPSS, Inc., Chicago, IL) and JMP 11 (SAS Institute).

Results

Soil physicochemical properties

The presence of *E. splendens* was significantly associated with a lower percentage of sand in the rhizospheric soil of *C. communis*. The soil was highly acidic, with characteristics of high nutrients and combined contamination of Cu and Zn (Table 1). A relatively high homogeneity of most soil basic properties was observed in subplots with and without *E. splendens*. There was no significant effect of the presence of *E. splendens* on soil pH, total Cu, available Cu, total Zn, available Zn or total P. There was a marginally significant positive effect of presence of *E. splendens* on soil organic matter content (F=3.91, P<0.058) and extractable N (F=4.45, P= 0.046). Subplots with versus without *E. splendens* did not separate clearly in several multivariate analyses of soil properties (results not shown).

Biomass and RII values of *C. communis*

Total biomass (log transformed to homogenize variation) was significantly higher in the plots with *Elsholtzia* (F=9.68, P=0.004) but there was no effect on log-transformed individual biomass.

Table 1 Soil physicochemical properties in the rhizosphere of *Commelina communis* plants

Properties	Treatment	
	With *Elsholtzia*	Without *Elsholtzia*
Clay (%)	5.46±0.44a	4.86±0.29a
Silt (%)	86.63±0.90a	84.35±1.24a
Sand (%)	7.88±0.75b	10.74±1.14a
pH	4.09±0.06a	4.09±0.09a
Soil organic matter (%)	7.89±1.23a	5.79±0.77a
Total P (g kg^{-1})	0.98±0.06a	1.06±0.10a
Olsen P (mg kg^{-1})	17.25±1.51a	17.24±1.60a
Extractable N (g kg^{-1})	0.85±0.05a	0.69±0.06b
Total Cu (g kg^{-1})	6.12±0.68a	6.88±0.69a
Available Cu (g kg^{-1})	1.23±0.17a	1.40±0.18a
Total Zn (mg kg^{-1})	333.59±13.53a	333.76±11.73a
Available Zn (mg kg^{-1})	24.64±1.28a	23.64±1.18a

The data were analyzed with two-way ANOVA with treatment (n= 16, df=1) and plot (n=8, df=3) as factors. Data are means±S.E. The data marked by different letters indicate significant difference at P<0.05

Two different types of *C. communis* individuals are observed on the Cu spoil: growing alone in open gaps and growing mixed with *E. splendens*. The population density and total biomass of *C. communis* per square meter coexisting with *E. splendens* were higher than when growing in open gaps (Figs. 1c, d and 2a), especially in high Cu plots. In contrast, *C. communis* growing alone had a higher individual biomass, especially in low Cu plots (Fig. 2b). However, this individual size-advantage was reduced and then reversed with increasing Cu concentration.

The positive effect of *E. splendens* on *C. communis*, expressed as RII, increased with total soil Cu. There was a marginally significant positive relationship between RII of total biomass and total Cu (F=4.31, P=0.057, Fig. 3a), and a significant positive relationship between RII of individual biomass and total Cu (F=5.87, P=0.03, Fig. 3b).

Soil temperature and relative humidity

There was a very pronounced diurnal pattern of surface soil temperature (Fig. 4a). The maximum temperature occurred at 15:00 was 38.93 °C for subplots without *Elsholtzia* and 39.98 ° C for subplots with *Elsholtzia*. Temperature changes occurred a bit sooner and were

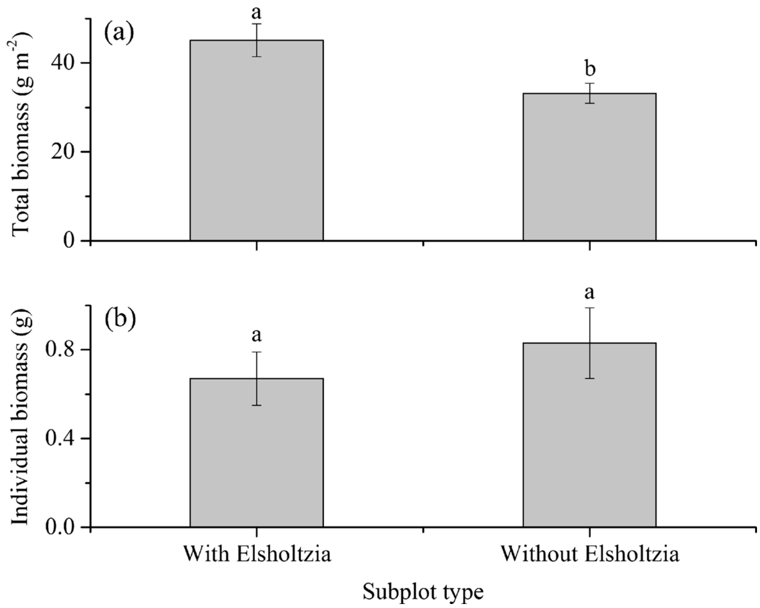

Fig. 2 Total **a** and individual **b** biomass of *Commelina communis* growing with *Elsholtzia splendens* or in open gaps. The columns marked by different *letters* indicate significant difference at $P<0.05$. *Error bars* represent S.E

slightly greater in the presence of *E. splendens*. The effects of *E. splendens* on soil temperature could be divided into two periods: significant differences in soil temperature between subplots with and without *Elsholtzia* were observed from 9:00 to 24:00, with temperature with higher than without *Elsholtzia* from 9:00 to 16:00, and lower from 17:00 to 24:00 ($n=64$, $P<0.05$).

The shape of diurnal soil relative humidity was similar to that of soil temperature (Fig. 4b). The maximum relative humidity appeared at 12:00, which were higher than 52 % for both subplots with and without *Elsholtzia. E. splendens* significantly increased the soil relative humidity from 5:00 to 11:00 ($n=64$, $P<0.05$). The changing trends of soil relative humidity were almost the same besides the time from 1:00 to 6:00, when relative humidity over subplots without *Elsholtzia* continued to decline, while that above subplots with *Elsholtzia* remained unchanged.

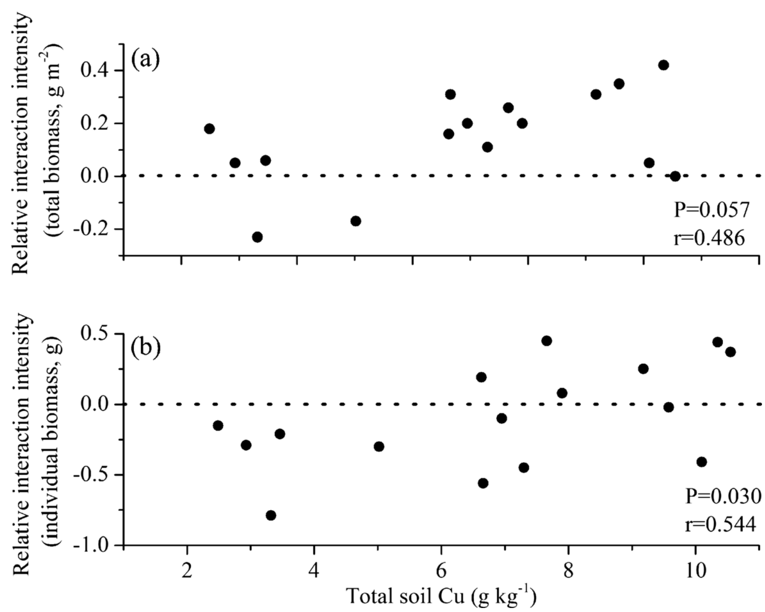

Fig. 3 The relationship between relative interaction intensity (RII) of total biomass **a**, individual biomass **b** of *Commelina communis* and total soil Cu. The *dotted horizontal line* at the 0 point indicates no effect between *Elsholtzia splendens* and *C. communis*

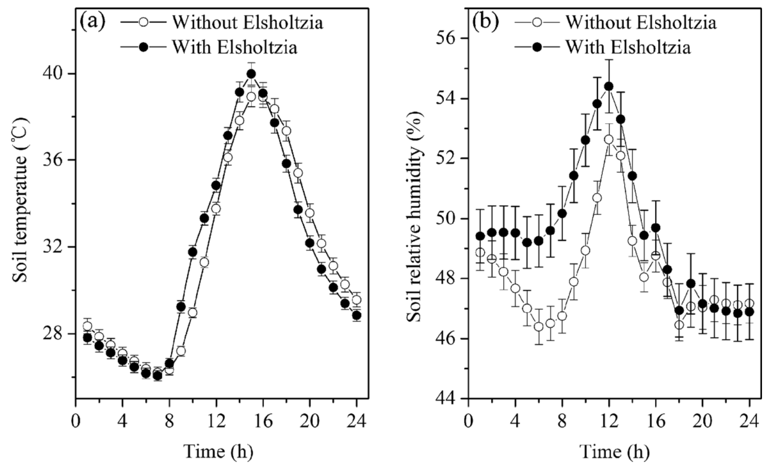

Fig. 4 Diurnal patterns of soil temperature **a** and relative humidity **b** in the rhizosphere of *Commelina communis* growing alone or with *Elsholtzia splendens*. *Error bars* represent S.E

Soil biological properties

The presence of *E. splendens* had large and significant positive effects on the soil respiration rate (F=53.41, P<0.0001, Fig. 5a), active organic matter (F=8.22, P<0.01, Fig. 5b), qCO_2 (F=10.99, P<0.03, Fig. 5c), sucrase activity (F=41.62, P<0.0001, Fig. 6a), urease activity (F=23.33, P<0.0001, Fig. 6b), alkaline phosphatase activity (F=17.06, P=0.0004, Fig. 6c) and acid phosphatase activity (F=39.22, P<0.0001, Fig. 6d).

There was a marginally significant positive effect of *E. splendens* on soil microbial carbon (F=3.5, P=0.07, Fig. 5d). Microbial biomass carbon was positively correlated with soil organic matter (r=0.496, n=32, P<0.004).

Soil active organic matter was always higher in the presence of *E. splendens* and the overall effect was

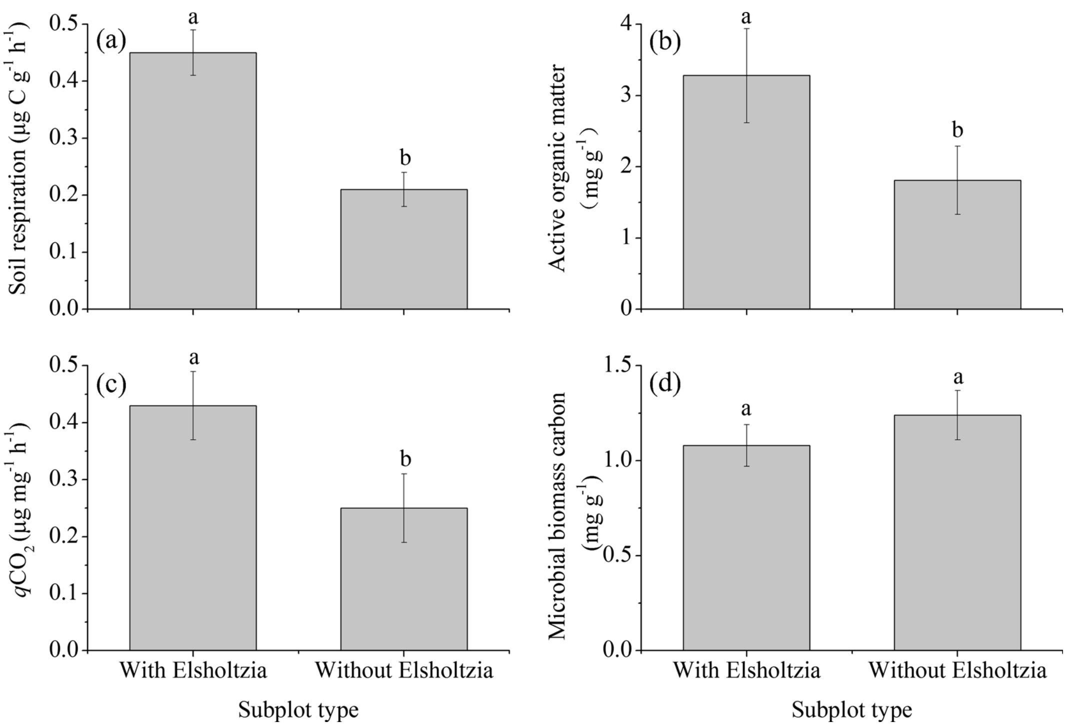

Fig. 5 Basal respiration **a**, active organic carbon **b**, qCO_2 **c** and microbial biomass carbon **d** of the rhizospheric soils collected from *Commelina communis* growing with *Elsholtzia splendens* or in open gaps. *Error bars* represent S.E. The columns marked by different *letters* indicate significant difference (P<0.05)

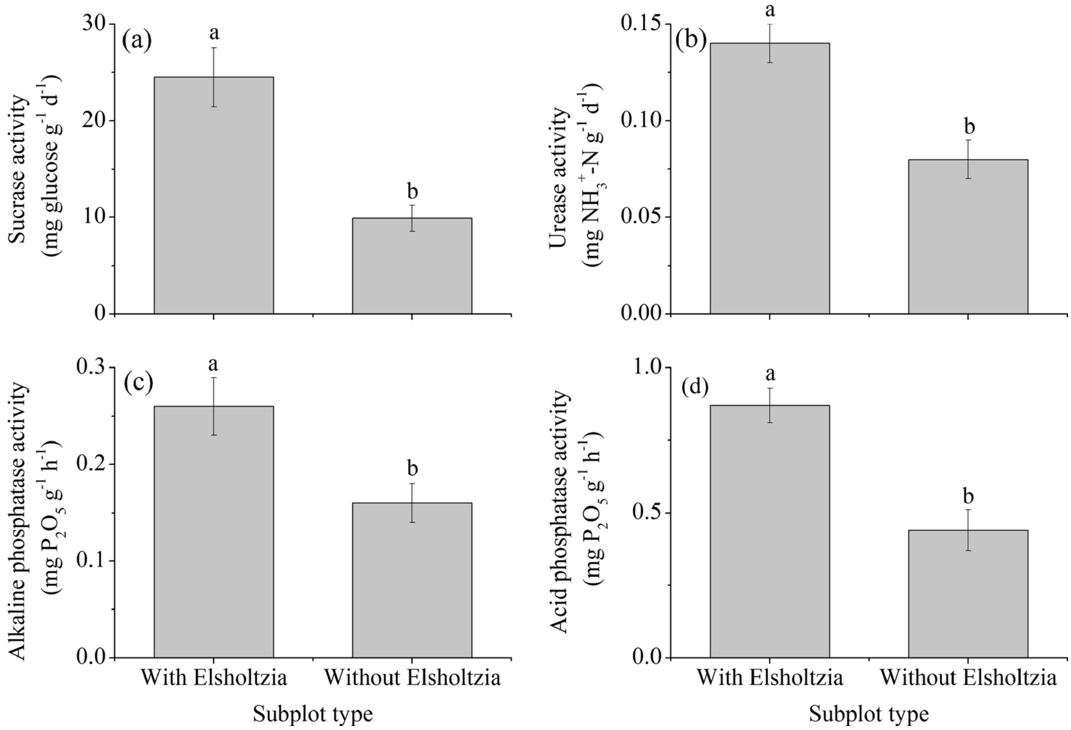

Fig. 6 Soil enzyme activities in the rhizosphere of *Commelina communis* with *Elsholtzia splendens* or in open gaps. **a** Sucrase activity; **b** Urease activity; **c** Alkaline phosphatase activity; **d** Acid phosphatase activity. *Error bars* represent S.E. The columns marked by different *letters* indicate significant difference ($P<0.05$)

significant (see above). Soil active organic matter showed positive correlations with both microbial biomass carbon ($r=0.547$, $n=32$, $P<0.01$) and total soil organic matter ($r=0.306$, $n=32$, $P>0.05$). All enzyme activities were positively correlated with soil organic matter ($r=0.769$, 0.659, 0.753 and 0.730 for sucrase, urease, alkaline phosphatase and acid phosphatase activities, respectively, $n=32$, all $P<0.01$). However, urease and phosphatase activities showed no significant relationship with soil N or P concentrations.

Cu and Zn concentrations in *C. communis*

C. communis growing with *E. splendens* accumulated less Cu and Zn in roots and shoots than in open gaps, although the difference was only significant for Cu in shoots (Table 2).

Discussion

Both negative and positive effects of the presence of *E. splendens* on *C. communis* were detected in our study. The effects of *E. splendens* on *C. communis* tended to be negative when Cu levels were low, and positive at higher Cu levels (Fig. 3), consistent with our hypothesis that *E. splendens* mitigates the negative effects of Cu stress. Positive interactions can be highly species-specific and determined by the traits of plants as well as the stress factor involved (Callaway 1998; Maestre et al. 2009; Mehdawi et al. 2012). *C. communis* has considerable Cu tolerance (Tang et al. 1999). Our results indicate that *C. communis* performs better when growing alone under lower Cu concentrations but tends to be found with *E. splendens* when the soil Cu level is near or above its physiological limit. There was no direct effect of the presence of *E. splendens* on log total *C. communis* biomass, but there was a significant *E. splendens*×Cu level interaction ($F=5.16$, $P=0.03$). Facilitation is often observed at the extreme ends of a species' tolerance range (Liancourt et al. 2005), and can expand the habitat range of the facilitated species (Bruno et al. 2003). Our results highlight the importance of belowground plant-soil interactions, especially the enrichment of soil basal respiration and enzyme activity, on the establishment of *C. communis*.

Table 2 Shoot and root concentrations of Cu and Zn in *Commelina communis* (n=16)

Treatment	Shoot Cu (mg kg^{-1})	Root Cu (mg kg^{-1})	Shoot Zn (mg kg^{-1})	Root Zn (mg kg^{-1})
With *Elsholtzia*	134.40±19.90b	263.80±22.78a	60.99±3.26a	164.40±18.51a
Without *Elsholtzia*	190.00±14.70a	304.40±21.92a	69.93±4.47a	195.00±17.89a

Data are means±S.E. Different letters indicate significant difference at $P<0.05$

According to classical population ecology theory, the relationship between overall density and individual fitness is generally negative. However, recent studies have highlighted the benefits of living at high density in harsh environments (Bruno et al. 2003; Fajardo and Mclntire 2011). Here, the presence of another species increased the overall plant density, and this had positive effects on the target species at high soil Cu levels (Fig. 3).

Of course it is not possible to completely exclude alternative hypotheses in a non-experimental field study like this one. It could be that *E. splendens* establishes preferentially in patches with particular conditions, and *C. communis* growing in such patches will also experience these conditions. While experimental studies are necessary to provide stronger inferences, our knowledge of these communities and soils points toward a causal role for *E. splendens* in the establishment and growth of *C. communis* observed here.

Facilitation associated with increased microbial activity

C. communis prefers highly humid habitats, so we selected a hot and humid growth period to investigate the effects of *E. splendens* on soil temperature and moisture. Compared with shrubs in arid environments that protect understory plants from strong irradiation and high temperature (Xu et al. 2010), *E. splendens* is an herbaceous plant with a restricted canopy, which does not provide a strong temperature buffer (Fig. 4a).

The study site is located in a subtropical area and has undergone a long-term natural succession. Unlike many heavy metal wastelands, the combined contamination of Cu and Zn and their relatively high bioavailability, rather than moisture limitation or nutrient status, (Table 1 and Fig. 4b) are likely to be the critical factors impeding plant colonization (Yang et al. 2010). The sand content of the soil appears to be lower near *E. splendens*, which would improve the water retention of the soil. The significant increase of soil humidity in subplots with *E. splendens* may also have contributed to the improved performance of *C. communis* (Fig. 4b). Although no consistently significant differences could be found in most soil physicochemical properties, our results suggest that *E. splendens* ameliorates the effects of heavy metal contamination on *C. communis* compared with plants growing in open gaps. This could explain why *C. communis* accumulated less Cu and Zn when growing with *E. splenden*s (Table 2).

Except for direct mitigation of biotic or abiotic stress, indirect facilitation mediated by a third participant from the same or a different trophic level has received increasing attention in recent years (Callaway 2007; Brooker et al. 2008; van der Putten 2009; Duponnois et al. 2011). Brooker et al. (2008) proposed that indirect facilitation should be more common in communities where several limiting factors co-occur with a similar strength. This was not the case in the present study, in which Cu and Zn represent the most dominant stress factors and varied in strength. In this study, the facilitation between *E. splendens* and *C. communis* likely resulted from the enrichment of microbial activity rather than amelioration of soil physicochemical properties or the heavy metal uptake of the plants. These results are in accordance with a previous study in which the establishment of *Cypresses* seedlings in an arid Mediterranean ecosystem was increased by nurse shrubs enhancing the mycorrhizal associations and the microbial activity in the soil (Duponnois et al. 2011). Similarly, seedlings growing in serpentine soils in Cuba tend to be aggregated in patches, which may be a result of direct and indirect facilitation (Oviedo et al. 2014).

The results suggest that the rhizospheric microbes in the present study were highly adaptive to heavy metals, since no significant inhibition of microbial processes was observed. Although *E. splendens* did not increase microbial biomass, it enhanced microbial metabolic and enzyme activity, likely accelerating soil processes such as mineralization of organic matter and nutrient cycling, which benefit plant growth. It is well known that there can be a positive or negative feedback between plant and soil microbial community depending on the features of both and the abiotic environment (Hortal et al. 2013; van der Putten et al. 2013). In our study, significant correlation between the biological properties of the soil and soil organic matter suggest that the improved microbial performance resulted from an increased quantity of plant litter and diversity of root exudates (Stefanowicz et al. 2012; Hortal et al. 2013).

Facilitation-based vegetation restoration of Cu mine spoils

The landmarks of successful restoration include not only the colonization of barren land but also the recovery of plant community behavior and processes. Traditional restoration practices, which focus on the transplanting of individual species, seldom consider the species diversity and composition of the native community and are usually constrained by high seedling mortality (Rey et al. 2009). A growing body of evidence has demonstrated the significance of facilitation on plant community diversity, assembly, stability and dynamics (Butterfield 2009; Xiao et al. 2013; Mclntire and Fajardo 2014). One-third of the species in subalpine grassland were facilitated by nurse shrubs, which decreased extreme temperatures, suggesting a role for facilitation in community restoration (Xu et al. 2010). Nurse plant-based community restoration can accelerate the recovery of community structure and properties (Rey et al. 2009). In our study, the spatial distribution pattern of the co-occurring plants and facilitation of *C. communis* by *E. splendens* under high Cu stress suggests that community level facilitation may also occur on Cu mine spoils. This can serve as a basis for incorporating facilitation in vegetation restoration programs.

Conclusions

Our results are consistent with the hypothesis that the growth of *C. communis*is facilitated by the presence of *E. splendens* at high Cu stress through indirect enrichment of soil respiration and enzyme activity, which are positively correlated with soil organic matter. Our findings suggest that facilitation-based community level approaches show great potential for vegetation restoration on heavy metal wastelands.

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