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New Phytologist

Research

A novel proxy to examine interspecific phosphorus facilitation between plant species

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Summary

- Resource complementarity can contribute to enhanced ecosystem functioning in diverse plant communities, but the role of facilitation in the enhanced complementarity is poorly understood.
- Here, we use leaf manganese concentration ([Mn]) as a proxy for rhizosheath carboxylate concentration to explore novel mechanisms of complementarity mediated by phosphorus (P) facilitation.
- In pot experiments, we showed that mixtures involving *Carex korshinskyi*, an efficient P-mobilizing species, exhibited greater biomass and relative complementarity effect than combinations without *C. korshinskyi* on P-deficient soils. Compared with monocultures, leaf [Mn] and [P] of species that are inefficient at P mobilization increased by 27% and 21% when grown with *C. korshinskyi* (i.e. interspecific P facilitation via carboxylates) rather than next to another inefficient P-mobilizing species. This experimental result was supported by a meta-analysis including a range of efficient P-mobilizing species. Phosphorus facilitation enhanced the relative complementarity effect in low-P environments, related to a greater change in several facilitated species of their root morphological traits relative to those in monoculture.
- Using leaf [Mn] as a proxy, we highlight a vital mechanism of interspecific P facilitation via belowground processes and provide evidence for the pivotal role of P facilitation mediated by the plasticity of root traits in biodiversity research.

Introduction

Plant diversity is usually positively correlated with ecosystem functioning, for example, enhanced productivity and nutrient uptake (Tilman *et al.*, 2014; Barry *et al.*, 2019a; Brooker *et al.*, 2021). The positive biodiversity–ecosystem functioning (BEF) relationships in diverse communities may occur because species with large biomass in monoculture can also win in competition in mixtures, coined a positive selection effect (Loreau & Hector, 2001). Increased ecosystem functioning in communities may also result from a positive complementarity effect, which occurs when species generally perform better in mixtures, regardless of their monoculture biomass. This effect may arise from processes including niche differentiation (e.g. temporal and spatial resource partitioning), direct abiotic facilitation (e.g. nutrient enrichment), and indirect biotic facilitation (e.g. disease suppression, Loreau & Hector, 2001; Wright *et al.*, 2017; Barry *et al.*, 2019a). A positive complementarity effect, rather than a positive selection effect, contributes more to greater ecosystem productivity in diverse plant communities (van Ruijven & Berendse, 2005; Cardinale *et al.*, 2007), while spatial resource partitioning alone does not always provide a full explanation for a positive complementarity effect in grasslands (Jesch *et al.*, 2018; Barry *et al.*, 2019b). Therefore, facilitation is receiving increasing attention as a mechanism enhancing ecosystem functioning. This occurs when one or more facilitated species in diverse plant communities increase their productivity in the presence of a facilitating species (Wright *et al.*, 2017; Yu *et al.*, 2021). Even if the enhanced performance of facilitated species is usually regarded as interspecific facilitation, there are few proxies to study mechanisms of facilitation in species-diverse communities.

It is widely accepted that legumes may facilitate neighbors via belowground facilitation of nitrogen (N) acquisition driven by increased soil N availability via dinitrogen (N_2) fixation in symbiosis with soil microorganisms (Wright *et al.*, 2017; Barry *et al.*, 2019a). However, beyond N facilitation, belowground phosphorus (P) facilitation has received little attention in BEF studies (Yu *et al.*, 2021). Carboxylates and protons in root exudates mobilize sorbed P, and phosphatases hydrolyze soil organic P and convert it to plant-available inorganic P (Lambers *et al.*, 2006). Our previous findings showed that grassland species vary in their capacity to mobilize sorbed or organic soil P. For example, some sedges (e.g. *Carex korshinskyi* and *Carex duriuscula*) and forbs (e.g. *Potentilla tanacetifolia* and *Artemisia frigida*) exhibit relatively greater release of carboxylates or phosphatases than grass species do (e.g. *Stipa grandis* and *Cleistogenes squarrosa*) (Yu *et al.*, 2020a,b). Species that exude phosphatases, carboxylates, or protons into rhizosheath soil, mobilizing and converting organic or sorbed soil P to plant-available forms for plant uptake, are defined as efficient P-mobilizing species. Conversely, species with a limited capacity to mobilize sorbed or organic P are considered species that are inefficient at P mobilization (Horst *et al.*, 2001; Li *et al.*, 2014).

When growing species with divergent P-mobilizing capacities together, interspecific P facilitation by efficient P-mobilizing species may enhance leaf P concentration ([P]), productivity, and/or shoot P content of neighboring species that are inefficient at P mobilization (Karanika *et al.*, 2007; Li *et al.*, 2007, 2014). Plant species can integrate information relating to neighbor identity and nutrient availability in mixtures and coordinate root growth based on nutrient status and signals (Cahill *et al.*, 2010). Species that are inefficient at P mobilization usually exhibit greater specific root length, root branching intensity, or root hair length than efficient P-mobilizing species (Lambers *et al.*, 2006; Wen *et al.*, 2019). These root morphological traits may make them more efficient at acquiring P when soil P availability is higher (Zhang *et al.*, 2016). Our previous study showed that compared with monocultures, species that are inefficient at P mobilization but exhibit a greater increase of root morphological traits (e.g. total root length and proportion of fine roots) in response to a facilitator may have an enhanced opportunity to be facilitated compared with other species that exhibit a smaller response of root traits to a facilitator (Yu *et al.*, 2020a). However, little is known about the pathways involved in P facilitation based on measurements of root morphological traits. In addition, because roots intermingle in mixtures, it is difficult to precisely test how efficient P-mobilizing species facilitate their neighbors by measuring rhizosphere processes *in situ*.

Carboxylates not only mobilize sorbed soil inorganic P (Pi) to unsorbed Pi in the soil solution, but also some metal cations, for example, manganese (Mn). Then, soil Mn^{2+} and Pi can be taken up by roots from the rhizosphere; Mn availability is also increased by root-released carboxylates, which chelate Mn and reduce Mn^{4+} to Mn^{2+} (Lambers *et al.*, 2015). Recent studies show that leaf Mn concentration ([Mn]) is correlated with carboxylate concentration in the rhizosheath at various soil P levels; leaf P content is also positively correlated with rhizosheath carboxylates and root morphological traits of chickpea (Huang *et al.*, 2017; Pang *et al.*, 2018; Wen *et al.*, 2021). When soil P availability is very low, facilitated plants may not show increased leaf [P], and hence having a proxy is very useful, especially in field observations where increased productivity may be hard to measure (Shen *et al.*, 2023). Therefore, leaf [Mn] can be used as an easily-measurable proxy that reflects the mobilization of sorbed soil P for a given species (Pang *et al.*, 2018; Wen *et al.*, 2021) or a range of species (Lambers *et al.*, 2015, 2021, 2022; Lambers, 2022).

Although previous studies have shown that leaf [Mn] of species that are inefficient at P mobilization may increase in biculture (Gardner & Boundy, 1983; Muler *et al.*, 2014), to our knowledge, no study has unequivocally demonstrated whether leaf [Mn] can be used as a proxy to test for interspecific P facilitation by carboxylates. The roles of interspecific P facilitation and relative changes of functional traits between mixtures and monocultures in a complementarity effect have received little attention.

Based on previous advances and knowledge gaps, we present three hypotheses. (1) Species combinations that include efficient P-mobilizing species will exhibit a greater productivity and complementarity effect, especially on P-deficient soils. (2) Leaf [Mn] of species that are inefficient at P mobilization is greater when grown with efficient P-mobilizing species rather than next to another inefficient P-mobilizing species, implying the existence of interspecific P facilitation via carboxylates. Such P facilitation by efficient P-mobilizing species occurs in species-diverse plant communities in both natural ecosystems and agroecosystems. (3) An enhanced complementarity effect via interspecific P facilitation is partly mediated by greater relative changes of root traits between mixtures and monocultures of facilitated species.

To test these hypotheses, we applied a three-step approach: first, we grew five dominant and subdominant species from a typical steppe that differ in their capacity to mobilize P at two soil P levels in monoculture. We classified the species into efficient P-mobilizing species and inefficient ones based on P-mobilization traits and tested the relationship between leaf [Mn] and rhizosheath carboxylate concentration among these species. Then, we grew 10 two-species mixtures, using the additive partitioning method to calculate the relative selection effect and relative complementarity effect to allow comparison among combinations with varied biomass (Loreau & Hector, 2001; Craven *et al.*, 2016). We compared the differences between combinations that included efficient P-mobilizing species and combinations without efficient P-mobilizing species. Second, we used leaf [Mn] and [P] to examine whether leaf [Mn] can be used as a proxy for interspecific P facilitation by carboxylates. Then, we tested whether the strength of P facilitation increases with enhanced dominance of the facilitator in our glasshouse study. We also examined whether the enhanced leaf [Mn] in bicultures including efficient P-mobilizing species was a general pattern by using a meta-analysis. Finally, we analyzed the relative change of root traits in mixtures than the corresponding monoculture of each facilitated species. We further tested the role of relative changes of root traits in interspecific P facilitation (proxied by leaf [Mn]) and relative complementarity effect.

Materials and Methods

Glasshouse study

Experimental setup Five dominant and subdominant species of a typical steppe were selected, including *Stipa grandis* P. Smirn. (perennial bunchgrass, dominant species), *Leymus chinensis*

(Trin.) Tzvel. (perennial rhizome grass, dominant species), *Cleistogenes squarrosa* (Trin.) Keng (perennial bunchgrass, subdominant species), *Carex korshinskyi* Kom. (sedge, subdominant species), and *Artemisia frigida* Willd. (forb, subdominant species) (Yu *et al*., 2020b). Seeds of each species were collected from multiple individuals in a typical steppe in Inner Mongolia (43.55°N, 116.70°E) and thoroughly mixed to avoid any genotype dominating in any pots (Yu *et al*., 2020a). Seeds were surface-sterilized with hydrogen peroxide (15% v/v) for 15 min and then washed three times in deionized water.

Soils from the top 20-cm layer were collected in a typical steppe in Inner Mongolia. The soil was air-dried, passed through a 2-mm sieve and evenly mixed. The soil properties were as follows: bulk density, 1.52 $\mathrm{g\,cm^{-3}}$; soil texture: sand 84%, silt 10%, clay 6%; organic matter, 22.5 $\mathrm{g\,kg^{-1}}$; total N, 1.16 $\mathrm{g\,kg^{-1}}$; total P, 0.28 $\mathrm{g\,kg^{-1}}$; Olsen P, 2.85 $\mathrm{mg\,kg^{-1}}$; pH (soil : water ratio = 1 : 2.5), 7.44. We used a P addition with 0 $\mathrm{mg\,kg^{-1}}$ as low-P treatment (LP), and 60 $\mathrm{mg\,kg^{-1}}$ as high-P treatment (HP), supplied as KH_2PO_4. The bulk soil Olsen P concentration after P fertilization was 22.4 $\mathrm{mg\,kg^{-1}}$. As the soils were collected in a typical steppe, which is the natural condition for plant growth, no additional basal nutrients were supplied. Pots with a base diameter of 12 cm, a top diameter of 18 cm, and a height of 14 cm were filled with 1.5 kg of air-dried soil. Pots were arranged as randomized complete block design with four replicates for each species in monoculture and for each combination in the mixture in each of the P treatments (LP and HP).

Plant growth conditions The experiment was carried out in a glasshouse with natural light at China Agricultural University, Beijing. The temperature was 22–30°C during the day. Seeds of experimental species with uniform seed mass were sown in pots. For information of the experiment and the tested species, see Supporting Information Table S1. For information of the species combinations, see Table S2. We standardized the density to six uniform individuals in monoculture and three individuals of each species in a mixture 30 d after the emergence of all species. Two species in this study had rhizomes, that is, *L. chinensis* and *C. korshinskyi*. We controlled their density on the 30th day. No additional density control was further conducted, although these species might produce more individuals by rhizome during the experiment. During the glasshouse experiment, any other species emerging in pots with a given combination were removed. Each pot had several small holes at the bottom, through which water could be taken up. Before planting, all pots were irrigated through the holes at the bottom, reaching pot capacity. After 15 d of growth, the pots were maintained at 75% of pot capacity (15% water content) to support the optimal growth measured by weight, until the final harvest. Plants were harvested 100 d after sowing.

Harvest and measurements We gently lifted the entire root systems from the pots and separated these from the soil. Soil clumps and excess soil were removed, and the soil that tightly adhered to roots was considered rhizosheath soil (Pang *et al*., 2017). After that, shoots were harvested at the base, and we separated the root samples of each species in monoculture into two even parts, that is, *subsample A* and *subsample B*, for further physiological and morphological trait measurements.

We carefully lifted the entire root system from its pot and sorted it into species in the mixtures; the roots attached to the stem base were the roots of the target species. Then, shoots of each species in the mixture were harvested at the base. Roots of each species in the mixture were separated into two even parts as *subsample A* and *subsample B*, that is, *subsample A* comprised half of the root samples of species 1 (e.g. *S. grandis*) and species 2 (e.g. *L. chinensis*) in the *S. grandis*/*L. chinensis* combination; the root samples of these two species were stored separately for further root morphology measurements. Most roots were sorted into species; the remaining mixed roots (*subsample C*) were carefully collected without separating them into species. Since roots were twined in mixture, we did not measure physiological traits in mixture, but root morphological traits of each species were measured.

P-mobilization traits in monoculture. A subsample (*subsample A*) in monoculture was transferred into a beaker with 100 ml of 0.2 mM $CaCl_2$ (Pearse *et al*., 2007). Roots were gently dunked for 60 s to remove the rhizosheath soil as much as possible and minimize root damage. A subsample of 10 ml supernatant was transferred into a centrifuge tube with two drops of microbial inhibitor Micropur (Sicheres Trinkwasser, Munich, Germany) at 0.01 $\mathrm{g\,l^{-1}}$ and three drops of concentrated phosphoric acid. The samples were stored at −20°C until analysis of carboxylates by HPLC–MS/MS after passing through a 0.22 μm filter. The measurement of carboxylates followed the method of Fiori *et al.* (2018). The remaining soil in the beaker was air-dried and weighed to calculate carboxylate concentrations based on the weight of rhizosheath soil.

We used a brush to remove the rhizosheath soil from another subsample (*subsample B*) of the root systems in monoculture to measure rhizosheath acid phosphatase activity (Apase) and pH. Apase was measured according to Tabatabai & Bremner (1969): to 1.00 g fresh soil, 0.80 ml acetate buffer at pH 5.2 and 0.20 ml *p*-nitrophenyl phosphate (*p*-NPP) substrate was added to be incubated at 30°C for 1 h; 1.00 ml of 0.5 M NaOH was added to terminate the reaction. Absorption was measured spectrophotometrically at 405 nm (Uvmini-1240; Shimadzu Corp., Kyoto, Japan). Rhizosheath pH was measured with a soil : water ratio = 1 : 2.5 with a pH meter (UB-7; Denver Instrument Co., Arvada, CO, USA).

Root morphological traits in monoculture and mixture. The *subsample A* in monoculture and mixture was washed to remove the remaining soil and intact root segments were selected and stored in 50% (v/v) ethanol at 4°C for root hair measurement (Haling *et al*., 2016). Root hairs were photographed using a SZX16 Wide Zoom Versatile Stereo Microscope (Olympus Corp., Tokyo, Japan). The length of 10 root hairs (2–8 cm from the root tip) for each species per replicate was measured using IMAGEJ (NIH Image, Bethesda, MD, USA), and the number of root hairs was counted as root hair density (Haling *et al*., 2016).

The *subsample B* in monoculture and mixture was washed several times to remove remaining soil and scanned at 600 dpi (Perfection V750 Pro; Epson, Suwa, Japan). Root length and average root diameter were analyzed using the WinRhizo scanner-based system (WinRhizo system; Regent Instruments Inc., Quebec, QC, Canada). Fourier-transform infrared (FTIR) spectroscopy was used to determine the proportion of roots of a given species in mixed root samples (*subsample C*) (Streit *et al*., 2019), as detailed in Methods S1; Tables S3 and S4. Roots of *subsamples A*, *B*, and *C* were dried at 70°C for 48 h, and then root biomass of each species and total root biomass were calculated based on *subsamples A* and *B* in monocultures, based on all subsamples in mixtures.

Harvested shoot samples were oven-dried at 70°C for 48 h, and weighed and ground for nutrient analyses. Leaf material was digested and inductively coupled plasma optical emission spectroscopy (ICP-OES, OPTIMA 3300 DV; Perkin-Elmer, Waltham, MA, USA) was used for leaf [Mn] and [P] measurement.

Calculation As the above- and belowground biomass differed among the 10 species combinations, for each species combination, we standardized the complementarity effect and selection effect by the mean biomass of two species in monoculture of the corresponding treatment (i.e. relative complementarity and selection effect; Craven *et al*., 2016). Then, the strengths of mixtures across species combinations can be compared. The relative complementarity and selection effects were calculated following the additive partitioning approach (Loreau & Hector, 2001; Craven *et al*., 2016):

$$\Delta \mathrm{RB} = \mathrm{RB_O} - \mathrm{RB_E} = \mathrm{B_{mixture}}/\mathrm{B_{monoculture}} - 0.5$$

In this equation, RB indicates relative biomass; the observed RB ($\mathrm{RB_O}$) is the biomass of a given species in the mixture divided by that in monoculture. The expected RB ($\mathrm{RB_E}$) is the proportion of a given species planted in the mixture, which was 0.5 in this study. The relative complementarity effect (rCE) was calculated as:

$$\begin{aligned}\mathrm{rCE} &= n \times \mathrm{mean}(\Delta \mathrm{RB}) \times \mathrm{mean}(M)/\mathrm{mean}(M)\\ &= n \times \mathrm{mean}(\Delta \mathrm{RB})\end{aligned}$$

In this calculation, n is the number of species in the mixture which was always two in this study; M is the monoculture biomass of the given species. The relative selection effect (rSE) is calculated as:

$$\mathrm{rSE} = n \times \mathrm{covariance}(\Delta \mathrm{RB}, M)/\mathrm{mean}(M)$$

A positive relative complementarity effect reflects that, on average, species perform better in the mixture than in monoculture. A positive relative selection effect indicates that the enhanced biomass is controlled by the species with greater performance in monoculture, which also dominates the biomass in the mixture (Loreau & Hector, 2001).

The calculation of root morphological traits in monoculture and mixture was based on *subsample B*. Specific root length was calculated as total root length divided by root biomass (dry weight). The proportion of fine roots was calculated as the root length of fine roots with a diameter < 0.2 mm divided by total root length (Bergmann *et al*., 2017). Root branching intensity was calculated as the number of root tips divided by the root length of the subsample (Kramer-Walter *et al*., 2016).

The relative change of root traits in the mixture from monoculture was calculated as:

$$\text{Relative change of a given trait} = (\mathrm{trait}_{i\ \mathrm{mix}} - \mathrm{trait}_{i\ \mathrm{mono}})/\mathrm{trait}_{i\ \mathrm{mono}}$$

where $\mathrm{trait}_{i\ \mathrm{mix}}$ and $\mathrm{trait}_{i\ \mathrm{mono}}$ were the targeted trait of a given species in mixture and monoculture, respectively. The dominance of efficient P-mobilizing species in the mixture was calculated as the biomass of the given species divided by the total biomass of the mixture.

Statistical analyses

Monoculture All statistical analyses were conducted in R v.4.1.3 (R Core Team, 2022). We conducted ANOVAs with block as a factor. First, we used one-way ANOVAs to examine the effect of P addition on the biomass, leaf [P] and P content of each species in monoculture. One-way ANOVAs were used because species varied in their biomass; we mainly focused on the effect of P addition on the performance of a given species in this study. Then, two-way ANOVAs were performed to test the effects of P addition and species identity on P-mobilization traits (i.e. rhizosheath Apase, pH and carboxylates) and leaf [Mn]. Tukey's *post-hoc* HSD test was performed at the 5% probability level in ANOVAs. To test the differences in P mobilization capacity among species, we carried out a principal component analysis using P-mobilization traits in monoculture, using the VEGAN package (Oksanen *et al*., 2022). Correlations were then conducted to examine the relationship between leaf [Mn] and rhizosheath carboxylate concentration, pH, and Apase.

In addition, we conducted a cluster analysis using P-mobilization traits to group species into efficient P-mobilizing species and species that are inefficient at P mobilization in monoculture in P-deficient soils and P-sufficient soils, following Ward's method (Borcard *et al*., 2018). We partitioned species into two groups according to the average silhouette width (Borcard *et al*., 2018). The package 'CLUSTER' was used for cluster analysis (Maechler *et al*., 2022).

Mixture Linear mixed-effect models were performed to test the effect of P addition and group (i.e. mixtures that included efficient P-mobilizing species and mixtures without efficient P-mobilizing species) on biomass, P content, relative diversity effect (i.e. relative selection effect and complementarity effect), and relative biomass. Phosphorus addition and group were treated as fixed effects, block and combination identity were treated as random effects.

Two-way ANOVAs were used to examine the effects of P addition and neighbor identity (four different species that grew next to the focal species) on relative biomass of each species. The effect of group on leaf [Mn], leaf [P] and relative changes of trait from monoculture on low-P soils, and the differences in relative complementarity effect among 10 species combinations on P-deficient soils were tested, using one-way ANOVAs.

Two-sided *t*-tests were conducted to examine whether the relative selection and complementarity effect significantly differed from zero, that is, expected performance (Loreau & Hector, 2001). If the relative selection effect was significantly greater than zero, this indicates that the contribution to enhanced performance of mixtures was due to increased biomass in mixtures by the species that also had the highest biomass in monoculture. If the relative complementarity effect was positive, this reflects interspecific facilitation and niche differentiation. We also used two-sided *t*-tests to test whether the observed relative biomass of a given species significantly differed from 0.5, that is, expected relative biomass. When the observed relative biomass was > 0.5, the given species was overperforming in mixtures.

Correlations between relative changes of leaf [Mn] and leaf [P], between relative changes of leaf [Mn] of neighbor species that are inefficient at P mobilization and the dominance of efficient P-mobilizing species, and between relative complementarity effect and relative changes of trait of species that are inefficient at P mobilization were performed. To test the differences in relative changes of root trait and leaf [Mn] of species that are inefficient at P mobilization in response to neighbor identity, we also carried out a principal component analysis using five root morphological traits and leaf [Mn].

Meta-analysis Literature including the difference in leaf [Mn] in monoculture and mixture was collected through Web of Science and China National Knowledge Infrastructure. We further extracted leaf [P] data in the selected literature. A weighted response ratio approach was used to conduct the meta-analysis (Hedges *et al.*, 1999; Luo *et al.*, 2006). For full details of data compilation and statistical analyses, see Methods S2. Literature included in this meta-analysis is given in Dataset S1.

Results

Species vary in root traits to mobilize P in monoculture

Phosphorus addition increased the aboveground biomass of all tested species, except for *C. korshinskyi*, but only increased the belowground biomass of *S. grandis* (Table 1). Phosphorus addition increased aboveground [P] and P content of most species (Table S5). Species varied in root traits that relate to mobilization of sorbed P, while P addition did not affect root traits. *Carex korshinskyi* exhibited greater rhizosheath carboxylate concentration and lower rhizosheath pH than other species did (Figs 1a, S1a). Species varied significantly in their capacity to mobilize sorbed soil P, and *C. korshinskyi* exhibited significantly greater P-mobilization traits than other species did (PERMANOVA test $P < 0.01$, Fig. 1c; Table S6). We further conducted a cluster analysis using these P-related traits to classify the five species into efficient P-mobilizing species and species that are inefficient at P mobilization. The results showed that *C. korshinskyi* was an efficient P-mobilizing species on both P-deficient and P-sufficient soils (Fig. 1d). Others were considered species that are inefficient at P mobilization.

Table 1 Above- and belowground biomass in monoculture and mixture at two phosphorus (P) levels.

Biomass (g)	Species/group	P level LP	 HP
Aboveground biomass in monoculture	Sg ($n = 4$)	0.84b	1.47a
	Lc ($n = 4$)	3.43b	4.26a
	Ck ($n = 4$)	1.87a	2.02a
	Cs ($n = 4$)	5.21b	7.07a
	Af ($n = 4$)	3.15b	5.57a
Belowground biomass in monoculture	Sg ($n = 4$)	0.25b	0.43a
	Lc ($n = 4$)	1.06a	1.66a
	Ck ($n = 4$)	0.41a	0.58a
	Cs ($n = 4$)	0.61a	0.71a
	Af ($n = 4$)	0.45a	0.65a
Aboveground biomass in monoculture and mixture	Monoculture ($n = 20$)	2.90bB	4.08aA
	With ($n = 16$)	4.26aA	4.09aA
	Without ($n = 24$)	3.86bAB	5.10aA
Belowground biomass in monoculture and mixture	Monoculture ($n = 20$)	0.56bB	0.81aA
	With ($n = 16$)	1.18aA	1.16aA
	Without ($n = 24$)	0.80bAB	1.19aA

n is the number of data in the analysis. Lowercase letters indicate differences between P levels, uppercase letters represent differences among monoculture, mixtures that include (with) and do not include an efficient P-mobilizing species (without) at a given P level. The same letter means no significant difference (Tukey HSD). Af, *Artemisia frigida*; Ck, *Carex korshinskyi*; Cs, *Cleistogenes squarrosa*; HP, P-sufficient soil; Lc, *Leymus chinensis*; LP, P-deficient soil; Sg, *Stipa grandis*.

Artemisia frigida and *C. korshinskyi* also exhibited a relatively greater leaf [Mn] than other species did (Fig. 1b), and leaf [Mn] was positively correlated with main and total carboxylate concentration, negatively with rhizosheath pH, and did not correlate with Apase in both P-deficient and P-enriched soils (Figs 1e,f, S2, S3).

Combinations that included *C. korshinskyi* exhibited a greater relative complementarity effect

To explore whether mixtures differed in productivity and diversity effects, we partitioned 10 species combinations into combinations that involved an efficient P-mobilizing species (i.e. *C. korshinskyi* in this study) and combinations that did not include an efficient P-mobilizing species based on the cluster analysis. Combinations with *C. korshinskyi* exhibited a greater above- and belowground biomass but not aboveground P content than monocultures and mixtures without an efficient P-mobilizing species in low-P soils (Tables 1, S5).

Relative selection effect was not affected by P addition and group (i.e. mixtures that included *C. korshinskyi* and mixtures without *C. korshinskyi*) (Fig. 2a,d). Aboveground relative complementarity effects were significantly positive and greater in combinations including an efficient P-mobilizing species than those in

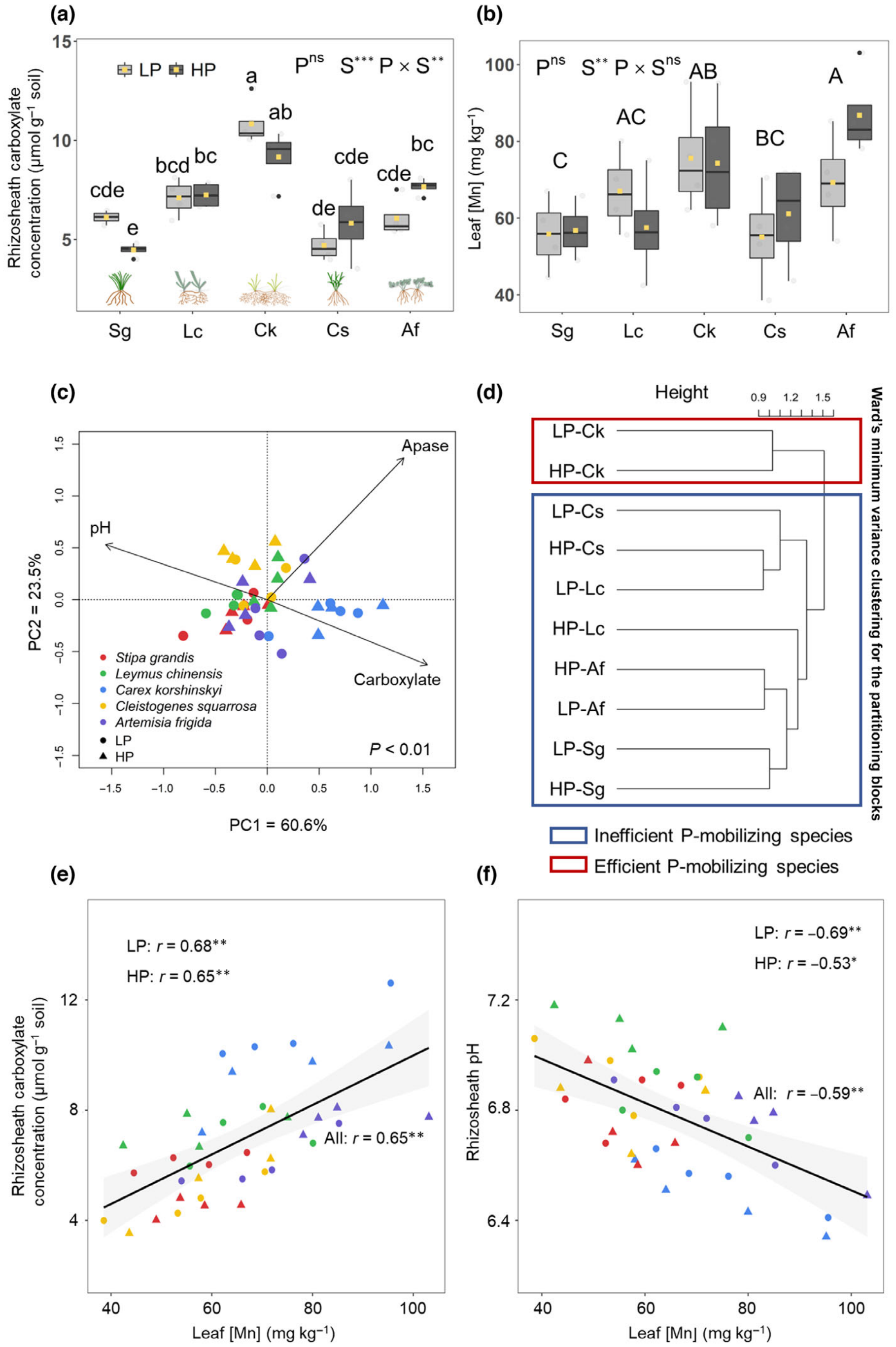

Fig. 1 Variations in phosphorus (P)-mobilization traits among species in monoculture. Effects of P addition on (a) rhizosheath carboxylate concentration and (b) leaf manganese concentration ([Mn]) among species (S) in monoculture. The yellow square indicates the mean value, the central line represents the median, the bottom and top of the box indicate the 25th and 75th percentiles, respectively. Whiskers are the smallest and largest value within the 1.5 times interquartile range below and above the 25th and 75th percentiles. $n = 4$. Lowercase letters indicate differences between treatments if the interaction effect was significant. Uppercase letters mean differences among species. The same letter means there was no significant difference (Tukey HSD). (c) Principal component analysis (PCA) for root traits associated with P mobilization in monoculture of five species in monoculture. PC1 represents the first axis, PC2 represents the second axis, and the percentage number represents the proportion of variation the axis explained. A PERMANOVA test showed that species exhibited significant differences based on P-mobilization traits ($P < 0.01$). (d) Cluster analysis across species monocultures based on root physiological traits, that is, rhizosheath acid phosphatase activity (Apase), pH, and total carboxylate concentration using Ward's method. Species shown in blue and red boxes represent inefficient and efficient P-mobilizing species, respectively. (e, f) Correlations between total carboxylate concentration and pH in rhizosheath soil and leaf [Mn] in species monoculture. Light grey bands represent 95% confidence intervals. Af, *Artemisia frigida*; Ck, *Carex korshinskyi*; Cs, *Cleistogenes squarrosa*; HP, P-sufficient soil; Lc, *Leymus chinensis*; LP, P-deficient soil; Sg, *Stipa grandis*. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

the absence of *C. korshinskyi* in P-deficient soils, but not in high-P soils (Fig. 2b). Furthermore, species that are inefficient at P mobilization usually exhibited greater relative aboveground biomass when grown with *C. korshinskyi* than when grown next to another inefficient P-mobilizing species, particularly on low-P soils (Fig. 2c; Table 2). Our evidence also shows that combinations that included *C. korshinskyi* exhibited a greater belowground complementarity effect and relative biomass of species that are inefficient at P mobilization than those that did not include *C. korshinskyi*, independent of P-addition treatment (Fig. 2e,f).

Leaf [Mn] can be used as a proxy for interspecific P facilitation via carboxylates

We aimed to examine whether leaf [Mn] can be used to detect interspecific facilitation of P acquisition. We compared leaf [Mn] and [P] in monocultures and mixtures with or without *C. korshinskyi*. We show that species that are inefficient at P mobilization that grew with *C. korshinskyi* exhibited a significantly greater leaf [Mn] and [P] than those in monoculture or in mixtures without an efficient P-mobilizing neighbor in P-deficient soils, but not in high-P soils (Fig. 3a,b). The changes in leaf [Mn] were

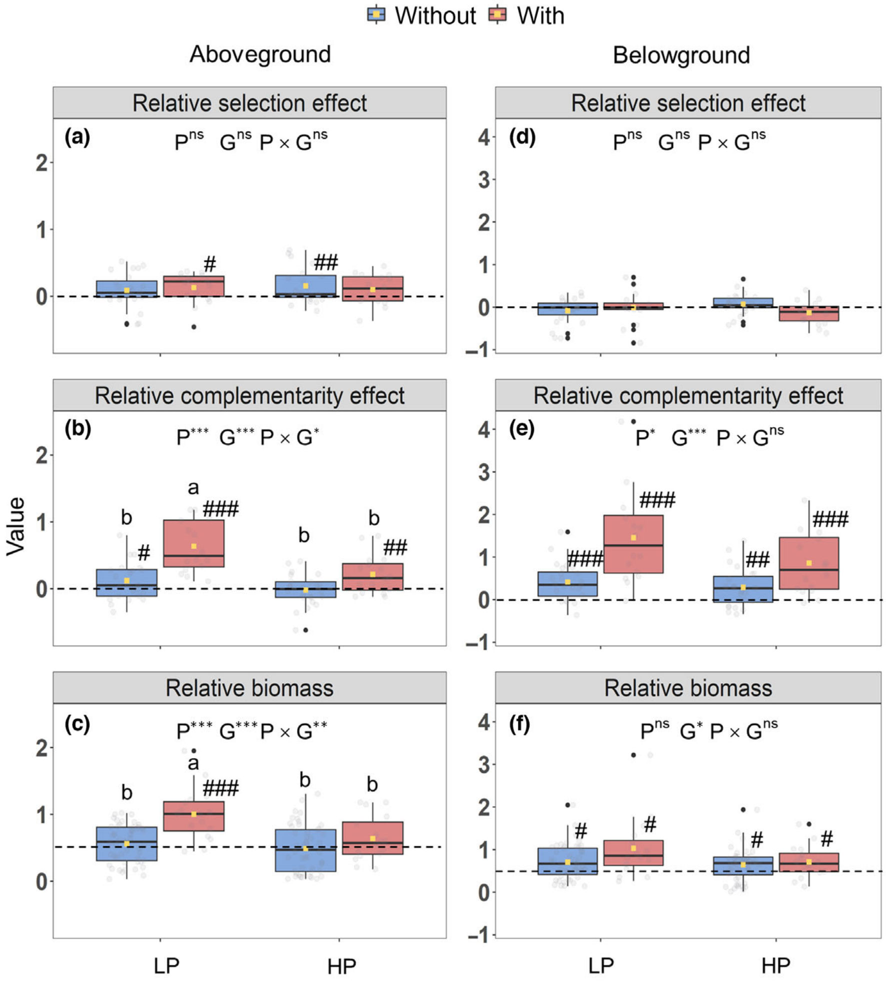

Fig. 2 Aboveground and belowground relative diversity effects and relative biomass in mixtures. Effects of phosphorus addition (P) and group (G) on relative selection effect, relative complementarity effect, and relative biomass of (a–c) aboveground biomass and (d–f) belowground biomass. The group includes mixture with *Carex korshinskyi* (With, $n = 16$) and without *C. korshinskyi* (Without, $n = 24$). The yellow square indicates the mean value, the central line represents the median, the bottom and top of the box indicate the 25th and 75th percentiles, respectively. Whiskers are the smallest and largest value within the 1.5 times interquartile range below and above the 25th and 75th percentiles. Lowercase letters indicate differences across treatments if the interaction effect was significant. The same letter means there was no significant difference (Tukey HSD). No lowercase letters were used if the interaction effect was not significant. HP, P-sufficient soil; LP, P-deficient soil. # in bars indicate a significant difference between relative selection effect and complementarity effect from zero, and between relative biomass from 0.5. #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$. Asterisks indicate a significant difference in P addition, group, and their interaction effects. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

positively correlated with that of leaf [P] (Fig. 3c), indicating soil P mobilized by *C. korshinskyi* facilitated P uptake of neighboring inefficient species, implying the existence of interspecific P facilitation via carboxylate release. We further showed that changes in leaf [Mn] of inefficient species were positively correlated with the above- and belowground dominance of *C. korshinskyi* in mixtures (Fig. 3e,f).

To examine whether the observed results in our glasshouse experiment were similar to those reported in the literature, we performed a meta-analysis testing the effect of species mixtures on leaf [Mn] and [P]. Species included in this meta-analysis were grouped into efficient P-mobilizing species and inefficient ones based on references (Table S7). No publication bias of leaf [Mn] and [P] was found in this meta-analysis (Fig. S4).

Our analysis showed that compared with monoculture, grown with efficient P-mobilizing species, leaf [Mn] and [P] of inefficient species were 9% and 28% greater without P addition, and 16% and 7% under P addition, respectively (Fig. 3d). However, compared with monocultures, the leaf [Mn] and [P] did not differ when two species that are inefficient at P mobilization were grown together, supporting the results of the present study.

Interspecific P facilitation enhanced complementarity and required greater changes in traits of facilitated species in mixtures than monocultures

To explore the role of root traits of facilitated species in interspecific P facilitation via carboxylates (proxied by leaf [Mn]), we further calculated the change in traits of species that are inefficient at P mobilization relative to that in corresponding monocultures. The results showed that species that are inefficient at P mobilization exhibited greater relative changes of leaf [Mn], proportion of fine roots, root branching intensity, and root hair length when growing with *C. korshinskyi* than when grown with another inefficient P-mobilizing species on P-deficient soils (Fig. 4a). When grown with *C. korshinskyi*, *S. grandis* exhibited greater relative change of root morphological traits than other species did (Fig. 4b). *Artemisia frigida* exhibited a greater relative change of leaf [Mn] when grown with *C. korshinskyi*, while the relative change of root morphological traits was more negative in mixtures than in monocultures (Fig. 4b).

The changes in leaf [Mn] of *S. grandis*, *L. chinensis* and *A. frigida* were positively associated with the relative complementarity effect (Fig. 4c). By contrast, the relative change of leaf [P] did not show a significant correlation with the relative complementarity

Table 2 Above- and belowground relative biomass of each species when grown with different neighbors in phosphorus (P)-deficient soils (LP) and P-sufficient soils (HP).

		Aboveground				Belowground			
Species	Neighbor	LP	HP	Variable	*P* value	LP	HP	Variable	*P* value
Sg	Lc	0.61	0.13	P	<0.001	1.27	0.53	P	<0.05
	Ck	0.95	0.41	N	<0.001	0.77	0.45	N	<0.01
	Cs	0.21	0.06	P × N	0.14	0.33	0.15	P × N	0.24
	Af	0.59	0.45			0.78	0.77		
Lc	Sg	0.77	0.87	P	0.20	0.53	0.74	P	0.37
	Ck	1.39	1.02	N	<0.001	1.88	1.05	N	<0.05
	Cs	0.74	0.63	P × N	0.27	0.89	0.71	P × N	0.20
	Af	0.63	0.58			1.00	1.14		
Ck	Sg	1.12	1.10	P	0.56	2.56	1.99	P	0.38
	Lc	0.30	0.32	N	<0.001	0.92	0.85	N	<0.01
	Cs	0.36	0.22	P × N	0.94	1.00	0.40	P × N	0.79
	Af	0.75	0.66			1.21	1.35		
Cs	Sg	0.85	1.02	P	0.54	0.72	0.88	P	0.72
	Lc	0.73	0.59	N	<0.01	0.80	0.80	N	0.71
	Ck	1.04	0.77	P × N	<0.05	0.80	0.78	P × N	0.65
	Af	0.82	0.93			0.91	0.85		
Af	Sg	0.26	0.35	P	<0.05	0.27	0.51	P	0.53
	Lc	0.37	0.21	N	<0.001	0.72	0.45	N	<0.01
	Ck	0.63	0.35	P × N	<0.05	0.69	0.57	P × N	0.21
	Cs	0.15	0.06			0.27	0.21		

$n = 4$. Neighbor identity effect refers to the effects of four different species grown next to the focal species on above- and belowground relative biomass of each species. Af, *Artemisia frigida*; Ck, *Carex korshinskyi*; Cs, *Cleistogenes squarrosa*; Lc, *Leymus chinensis*; N, neighbor identity effect; P, P addition effect; Sg, *Stipa grandis*.

effect, except for *S. grandis* of aboveground complementarity (Fig. S5). Relative changes of leaf [Mn] of *S. grandis* also exhibited a positive correlation with changes in proportion of fine roots and root branching intensity when grown with different species (Fig. 4c). However, the relative change of root traits from the monoculture of other species did not exhibit a positive correlation with that of leaf [Mn], except for proportion of fine roots and root hair length of *A. frigida* (Figs 4c, S6).

In addition, *C. korshinskyi* and *S. grandis* exhibited greater relative biomass in a *S. grandis*/*C. korshinskyi* combination (Table 2), therefore exhibiting a greater above- and belowground relative complementarity effect than other combinations on P-deficient soils (Fig. 4d).

Discussion

Plant growth was limited by P in this study (Table 1). *Carex korshinskyi* was grouped as an efficient P-mobilizing species, with greater carboxylate and proton release, had similar biomass on low-P and high-P soils, due to its greater capacity to mobilize sorbed P than other species (Fig. 1c,d). Most biodiversity research focuses on the effects of species richness on aboveground productivity, while belowground overperformance and complementarity effects have received far less attention, probably due to method limitations. By calculating the root biomass of each species via FTIR spectroscopy, we quantified belowground complementarity effect in mixtures. We found that combinations involving *C. korshinskyi* usually exhibited greater above- and belowground biomass and relative complementarity effects than those in the absence of *C. korshinskyi* on P-deficient soils (Table 1; Fig. 2), which supports the first hypothesis. Recent studies also show that plant mixtures enhance productivity and complementarity effects via increased soil P availability in diverse ecosystems, in which interspecific P facilitation is a key mechanism to drive overperformance in species mixtures (Yu *et al.*, 2020a; Chen *et al.*, 2022). We found that aboveground relative biomass of *S. grandis*, *L. chinensis*, and *C. squarrosa* was near or > 1 when neighbored with *C. korshinskyi* on P-deficient soils (Table 2), which probably reflects interspecific facilitation (Wagg *et al.*, 2019). We further explore the potential proxies to study mechanisms of facilitation in species-diverse communities.

We found that leaf [Mn] was positively correlated with total and main carboxylate concentrations in the rhizosheath on P-deficient and P-sufficient soils (Figs 1e, S2b). Oxalate, fumarate, and malonate accounted for 96% of total carboxylates (Fig. S2a), where oxalate and malonate have a similar efficiency in mobilizing sorbed P to citrate (Ström *et al.*, 2005; Playsted *et al.*, 2006; Pang *et al.*, 2018). Then, we showed that greater leaf [Mn] and [P] of species that are inefficient at P mobilization were observed only in the presence of *C. korshinskyi*, an efficient P-mobilizing species, on P-deficient soils (Fig. 3a,b). The mechanism underpinning this species-specific interaction presumably is the chelation of Mn by carboxylates and the decrease in rhizosheath pH by efficient P-mobilizing species, as Mn reduction in the soil is enhanced at low pH (Gardner *et al.*, 1982). The decrease in rhizosheath pH by efficient P-mobilizing species also enhances soil P availability through the dissolution of P-containing minerals, facilitating P uptake of neighboring species that are inefficient at

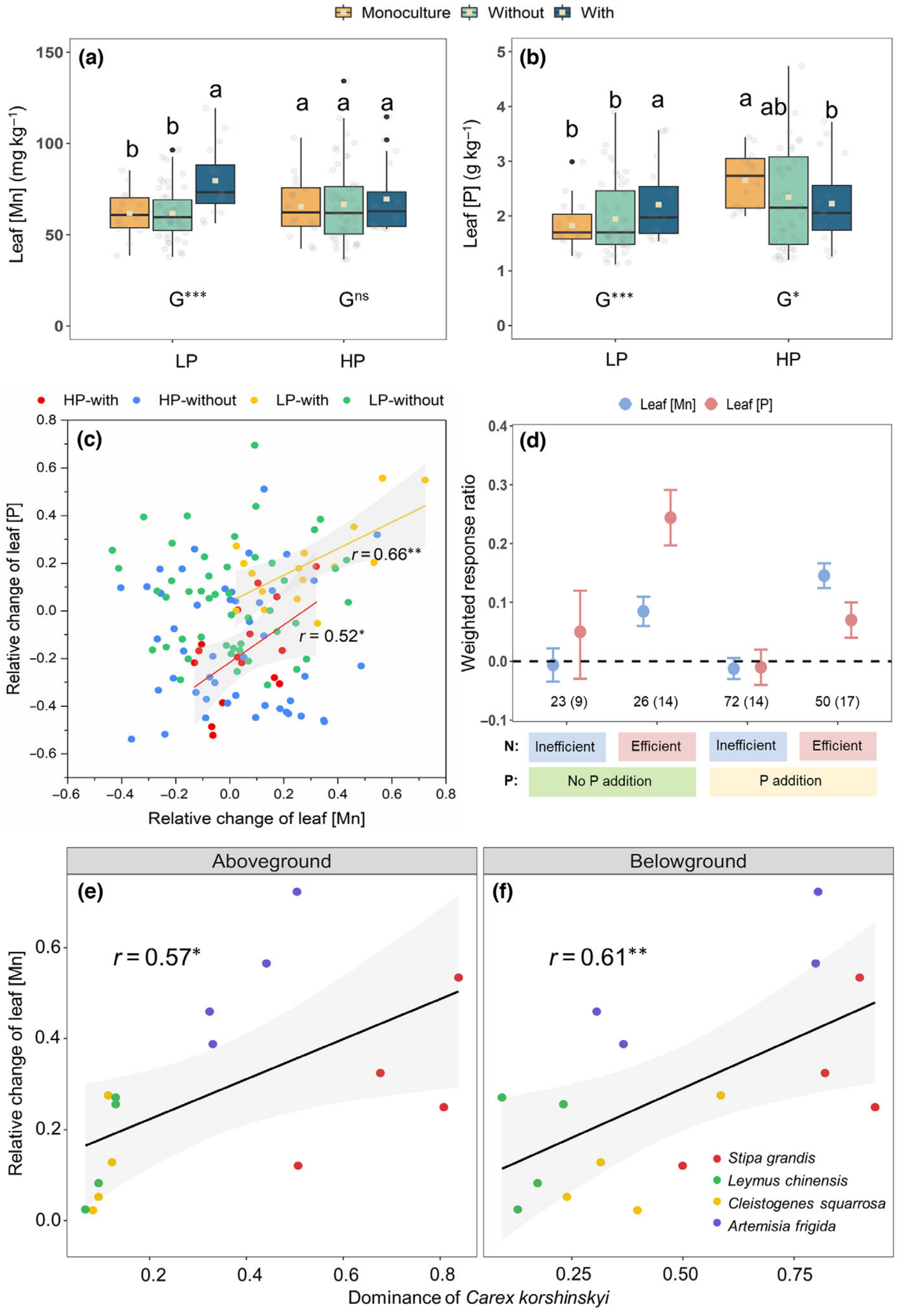

Fig. 3 Leaf manganese concentration ([Mn]), leaf phosphorus concentration ([P]), and their relative change between mixtures and monocultures in the glasshouse experiment and meta-analysis. Effects of group (G) on (a) leaf [Mn] and (b) leaf [P] of species that are inefficient at P mobilization. The group includes monoculture ($n = 16$), neighbored with *Carex korshinskyi* (With, $n = 16$) and grown without *C. korshinskyi* (Without, $n = 48$). The yellow square indicates the mean value, the central line represents the median, the bottom and top of the box indicate the 25th and 75th percentiles, respectively. Whiskers are the smallest and largest value within the 1.5 times interquartile range below and above the 25th and 75th percentiles. Lowercase letters indicate differences among the group on P-deficient soils (LP) and P-sufficient soils (HP). The same letter means there was no significant difference (Tukey HSD). (c) Correlations between the relative change of leaf [P] and that of leaf [Mn] of species that are inefficient at P mobilization in combination with and without *C. korshinskyi* at two soil P-levels. (d) The weighted response ratio (RR_{++}) of leaf [Mn] and leaf [P] of inefficient grown with different neighbors (N) in the meta-analysis. The list of inefficient and efficient P-mobilizing species is shown in Supporting Information Table S7. Bars represent 95% confidence intervals of RR_{++}. The numbers outside and inside the brackets indicate the sample size of leaf [Mn] and leaf [P] data, respectively. (e, f) Correlations between relative change of leaf [Mn] of neighboring species from corresponding monocultures and the dominance of *C. korshinskyi* on low-P soils. Panels (a–c, e, f) showed the results at present glasshouse experiment, panel (d) showed the results from the meta-analysis. Light grey bands represent 95% confidence intervals. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

P mobilization (Li *et al.*, 2007, 2014). The changes in leaf [Mn] were positively correlated with leaf [P], implying the neighboring inefficient species took up the Mn^{2+} and soluble P mobilized by the facilitator (Lambers *et al.*, 2015). In support of this, our meta-analysis also shows that greater leaf [Mn] and [P] of species only occurred when grown with an efficient P-mobilizing species, rather than with another inefficient species in both agroecosystems and natural systems, especially without P addition (Fig. 3d). Leaf [P] of species increased under P addition, indicating the amelioration of P status, while leaf [Mn] did not change in HP treatment (Fig. 3a,b). Conversely, leaf [Mn] of facilitated species increased when grown next to efficient P-mobilizing species in both the glasshouse experiment and a meta-analysis. The changes in leaf [P] are affected by multiple factors, for example, plant P-acquisition strategy, neighbor identity, and soil P availability which do not specifically reflect interspecific P facilitation (Lambers, 2022). Increased leaf [Mn] of facilitated species is likely related to the enhanced soil P availability as a result of rhizosphere processes of the facilitator (Lambers *et al.*, 2015; Lambers, 2022). Therefore, we highlight that leaf [Mn], rather than leaf [P], is a good proxy to reflect interspecific P facilitation.

We only found a single efficient P-mobilizing species, that is, *C. korshinskyi*, in the present study. However, mycorrhizal species *Melissilus ruthenicus*, *P. tanacetifolia* and *Filifolium sibiricum* in our previous research (Yu *et al.*, 2020a), and non-mycorrhizal species such as *Lupinus albus* (Dissanayaka *et al.*, 2015) and

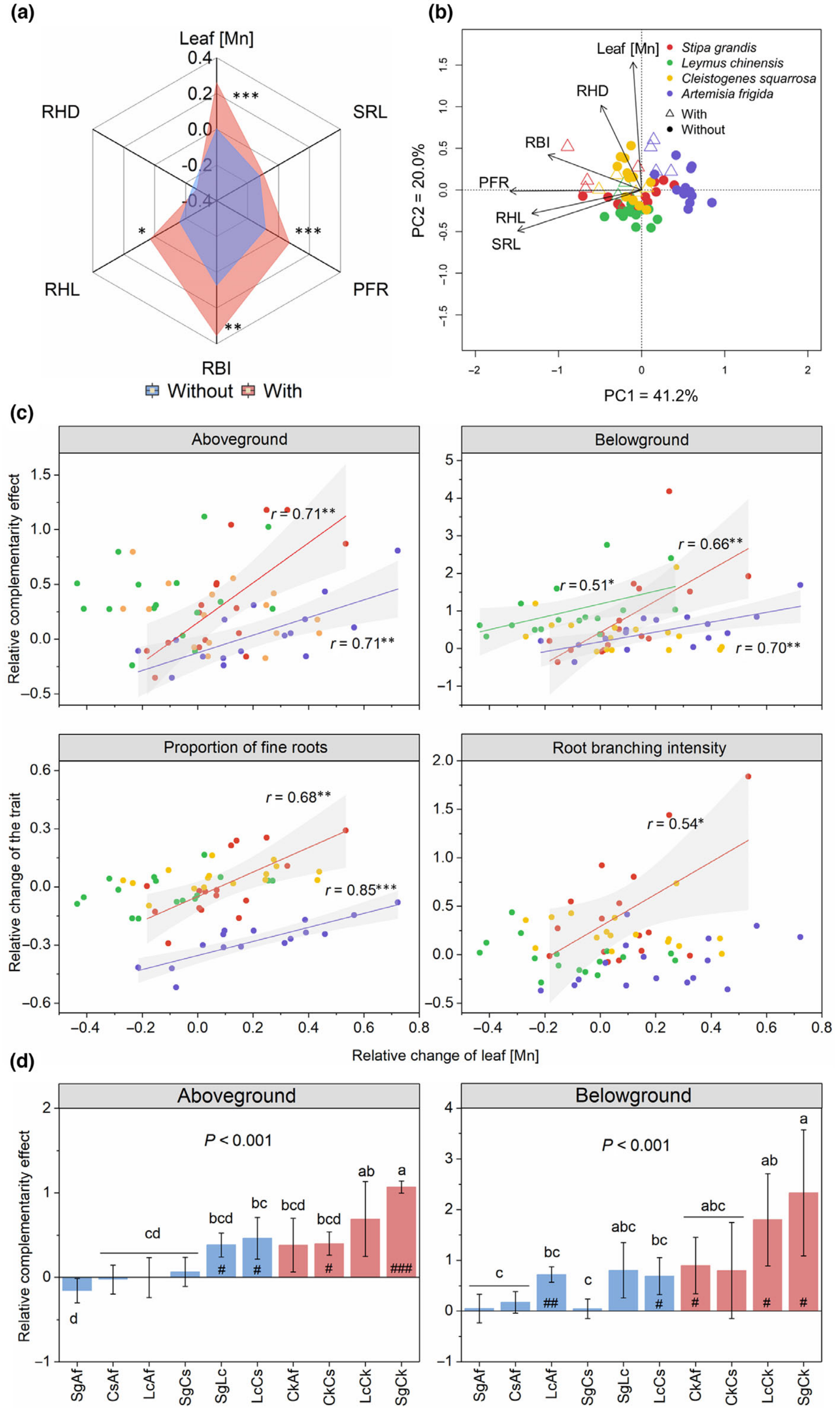

Fig. 4 The role of relative change of leaf manganese concentration ([Mn]) and root morphological traits between mixtures and monocultures of species that are inefficient at phosphorus (P) mobilization in complementarity. (a) Relative change of functional traits of species that are inefficient at P mobilization in response to the group includes a mixture with *Carex korshinskyi* (With, red polygon, $n = 16$) and without *C. korshinskyi* (Without, blue polygon, $n = 48$) on low-P soils. Asterisks indicate a significant difference between groups. (b) Principal component analysis (PCA) for relative change of traits of species grown with or without *C. korshinskyi* on P-deficient soils. PC1 represents the first axis, PC2 represents the second axis, and the percentage number represents the proportion of variation the axis explained. (c) Correlations between relative complementarity effect and the relative change of leaf manganese concentration ([Mn]) of each species that are inefficient at P mobilization, and between the relative change of proportion of fine roots (PFR) and root branching intensity (RBI) and that of leaf [Mn] of each species that is inefficient at P mobilization grown with one of four species ($n = 16$) on low-P soils. Light grey bands represent 95% confidence intervals. (d) Relative complementarity effect of above- and belowground biomass across combinations on P-deficient soils ($n = 4$). Bars are means ± SE. # indicates a significant difference between the relative complementarity effect from zero. #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$. Lowercase letters indicate differences among combinations. The same letter means that there was no significant difference (Tukey HSD). Af, *Artemisia frigida*; Ck, *Carex korshinskyi*; Cs, *Cleistogenes squarrosa*; Lc, *Leymus chinensis*; RHD, root hair density; RHL, root hair length; Sg, *Stipa grandis*; SRL, specific root length. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Banksia attenuata (Muler *et al.*, 2014) in other studies are also considered efficient P-mobilizing species. Therefore, we surmise that species that are inefficient at P mobilization might exhibit greater productivity when grown with an efficient P-mobilizing species by P facilitation on low-P soils (Li *et al.*, 2014). This implies that the effects of interspecific P facilitation associated with a positive BEF relationship by facilitators are common, rather than species specific. Therefore, the results from our glasshouse experiment and the meta-analysis provide further support that leaf [Mn] can be used as a proxy of interspecific P facilitation via carboxylate release in various ecosystems, supporting the second hypothesis.

Grown with *C. korshinskyi*, the neighboring species usually exhibited greater relative changes of a range of traits (i.e. leaf [Mn], proportion of fine roots, root branching intensity, and root hair length) than when grown with another species that was inefficient at P mobilization on low-P soils (Fig. 4a). We further showed that the roots of grass species were thinner than those of *A. frigida* when grown with *C. korshinskyi* (Fig. 4b); *S. grandis* exhibited greater changes of morphological traits than other species did, and interspecific P facilitation (proxied by leaf [Mn]) enhanced relative complementarity effect via changes of root morphological traits (Fig. 4b,c). In the presence of *C. korshinskyi*, the *S. grandis*/*C. korshinskyi* combination also exhibited a greater relative complementarity effect than other species combinations (Fig. 4d). This is probably because the root growth of facilitated species can change in response to increased P availability in the rhizosheath soil of the facilitator (Zhang *et al.*, 2016, 2020). Our previous results showed that grass species exhibit greater inherent root morphological traits and relative changes of those traits than forbs do; this may allow grasses to obtain greater benefits from efficient P-mobilizing neighbors than forbs do (Yu *et al.*, 2020a). This is probably also related to contrasting P-acquisition strategies and trade-offs among root traits between grasses and forbs (Wen *et al.*, 2019). The morphological traits examined in this study are important for P acquisition (Lambers *et al.*, 2006), while our evidence shows that specific root length, root hair length and density did not exhibit a positive correlation with leaf [Mn] of the tested species, indicating that the changes in the appropriate trait (e.g. the proportion of fine roots in the present study) may be required in interspecific P facilitation. In addition, *C. korshinskyi* exhibited greater relative biomass when grown with *S. grandis* which may be related to the lower competition by *S. grandis*; and the strength of interspecific P facilitation may increase with enhanced dominance of *C. korshinskyi* (Table 2; Fig. 3e,f). Therefore, *S. grandis* may obtain more benefit via both increased dominance of the facilitator to mobilize more sorbed P into the soil solution, and *S. grandis* exhibits greater relative changes of a range of traits in response to the facilitator, supporting our third hypothesis.

By contrast, although the relative change of leaf [Mn] of *A. frigida* was positively correlated with the relative complementarity effect, the inherently coarse roots and negative response of root morphological traits to *C. korshinskyi* may offset the benefits via carboxylates, resulting in a lower relative complementarity effect than that in other mixtures that include *C. korshinskyi* (Fig. 4b, d). The *L. chinensis*/*C. korshinskyi* combination exhibited a relatively greater complementarity than that of other mixtures, and enhanced interspecific P facilitation may increase belowground complementarity in the mixture (Fig. 4c,d). However, we did not observe a positive correlation between root morphological traits of *L. chinensis* and interspecific P facilitation (Figs 4c, S6). Complementarity effects may be driven by multiple mechanisms, and as such, variations in another mechanism may obscure clear correlations between complementarity effects and one particular mechanism (e.g. P facilitation) in the *L. chinensis*/*C. korshinskyi* combination. *Leymus chinensis* has greater stoichiometric stability than other species in a typical steppe, that is, a greater ability to maintain plant nutrient status despite variations in soil nutrient availability (Yu *et al.*, 2010). We surmise that the inherently thin roots and the rhizome of *L. chinensis* facilitate the growth and limited response to soil nutrient availability via changes in root traits. Although greater relative biomass of some species that are inefficient at P mobilization is observed when neighbored with *C. korshinskyi* on high-P soils (Table 2), the results likely reflect stronger competition of focal species than *C. korshinskyi*, rather than interspecific P facilitation (Figs 2b, 3a,b).

To our knowledge, this is the first time to comprehensively use leaf [Mn] as a proxy to examine interspecific P facilitation via carboxylates in biodiversity research. Importantly, using leaf [Mn] as proxy, we highlight that direct interspecific facilitation of P acquisition via belowground processes is a vital mechanism that can enhance complementarity and productivity. We can examine P facilitation by measuring a straightforward leaf trait rather than collecting complicated root traits in species-diverse plant communities. What is less clear is how much of the biodiversity effects in bicultures containing *C. korshinskyi* is due to facilitation as opposed to niche differentiation. This question may be addressed directly by including single plant controls, as, for example, in Schöb *et al.* (2018). Future research would also consider more species combinations with divergent P strategies to explore the general role of interspecific P facilitation in BEF relationships.

Leaf [Mn] exhibits a positive correlation with rhizosheath carboxylates, whereas soil [Mn] is intercepted by arbuscular mycorrhizal fungi (AMF), and plant Mn uptake is affected by the soil microbiome (Kothari *et al.*, 1991; Lehmann & Rillig, 2015), soil Mn availability and plant strategy (Tian *et al.*, 2016; Lambers *et al.*, 2021). The roots of species that were included in this study are colonized by AMF except for *C. korshinskyi*; the colonization rates range from 16% to 39% in tested species (Tian *et al.*, 2009). Therefore, further studies should explore the role of the AMF, soil microbiome and Mn-acquisition strategies in interspecific P facilitation in species-diverse communities.

In conclusion, we show that leaf [Mn] is a proxy for interspecific P facilitation via rhizosheath carboxylates in grassland communities, as well as in shrub and crop species through a meta-analysis. There is a potential application using leaf [Mn] as a trait to examine P facilitation in other ecosystems such as forests and intercrops. Furthermore, combinations that include an efficient P-mobilizing species tend to exhibit a greater relative above- and belowground complementarity effect on P-impoverished soils which is related to direct P facilitation via carboxylate release. Neighboring species that exhibit a greater response of specific traits obtain greater benefits from the facilitator, in which *S. grandis* may be better matched with *C. korshinskyi*. This is the first report demonstrating how interspecific P facilitation via carboxylate release increases above- and belowground complementarity, enhancing our understanding of BEF relationship and species coexistence.

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Competing interests

None declared.

Author contributions

LL and R-PY designed the experiment. R-PY, YS, RA, HY, X-TY, and YX collected data and performed analyses. HL provided the idea to focus on leaf [Mn] as a proxy to examine interspecific phosphorus facilitation via carboxylate release in mixtures. R-PY drafted the paper, and HL, JR, W-PZ, and LL contributed substantially to revisions.

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Data availability

Data are available in Zenodo at doi: 10.5281/zenodo.7919182.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 List of selected literature included in the meta-analysis.

Fig. S1 Effects of phosphorus addition on rhizosheath pH and acid phosphatase activity among species in monoculture.

Fig. S2 The average proportion of five exuded carboxylates and their relationships with leaf manganese concentration in monoculture.

Fig. S3 Correlation between rhizosheath acid phosphatase activity and leaf manganese concentration in monoculture.

Fig. S4 Funnel plot to evaluate the possibility of publication bias in the meta-analysis.

Fig. S5 Correlations between relative complementarity effect and the relative change of leaf phosphorus concentration.

Fig. S6 Correlations between relative change of specific root length, root hair length, and root hair density of species that was inefficient at P mobilization and that of leaf manganese concentration.

Methods S1 Fourier-transform infrared spectroscopy analysis.

Methods S2 A detailed description of the data compilation and statistical analysis of the meta-analysis.

Table S1 Information of the experiment and the tested species.

Table S2 Information of the species combinations.

Table S3 The calibration curve range in two species of 34 calibration samples with known species composition used.

Table S4 Statistical parameters of the Fourier transform infrared models in terms of calibration.

Table S5 Aboveground phosphorus concentration and content in monoculture and mixture.

Table S6 PERMANOVA tests based on root phosphorus-mobilization traits between each species pair in monoculture.

Table S7 Classification of efficient phosphorus (P)-mobilizing species and species that are inefficient at P mobilization based on the literature as cited.