

# Carbon and phosphorus exchange rates in arbuscular mycorrhizas depend on environmental context and differ among co-occurring plants

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## Summary

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**Key words:** arbuscular mycorrhiza, P-for-C exchange ratio, resource allocation, soil extractable P, symbiosis.

- Phosphorus (P) for carbon (C) exchange is the pivotal function of arbuscular mycorrhiza (AM), but how this exchange varies with soil P availability and among co-occurring plants in complex communities is still largely unknown.
- We collected intact plant communities in two regions differing c. 10-fold in labile inorganic P. After a 2-month glasshouse incubation, we measured <sup>32</sup>P transfer from AM fungi (AMF) to shoots and <sup>13</sup>C transfer from shoots to AMF using an AMF-specific fatty acid. AMF communities were assessed using molecular methods.
- AMF delivered a larger proportion of total shoot P in communities from high-P soils despite similar <sup>13</sup>C allocation to AMF in roots and soil. Within communities, <sup>13</sup>C concentration in AMF was consistently higher in grass than in blanketflower (*Gaillardia aristata* Pursh) roots, that is P appeared more costly for grasses. This coincided with differences in AMF taxa composition and a trend of more vesicles (storage structures) but fewer arbuscules (exchange structures) in grass roots. Additionally, <sup>32</sup>P-for-<sup>13</sup>C exchange ratios increased with soil P for blanketflower but not grasses.
- Contrary to predictions, AMF transferred proportionally more P to plants in communities from high-P soils. However, the <sup>32</sup>P-for-<sup>13</sup>C exchange differed among co-occurring plants, suggesting differential regulation of the AM symbiosis.

## Introduction

At least 70% of plant species form symbioses with arbuscular mycorrhizal fungi (AMF; Brundrett, 2009). In some cases, AMF may provide most of the phosphorus (P) needed by plants (Smith *et al.*, 2003) in exchange for an average 6% of plant assimilated carbon (C; Hawkins *et al.*, 2023), although estimates of resource exchange vary widely. Also, our knowledge of AM function stems almost exclusively from controlled glasshouse or *in vitro* experiments involving few plant and fungal species. Thus, it is uncertain how representative such findings are of resource exchange in natural communities with patchy resource availability, variation among species in mycorrhizal dependency, multiple stressors, and local adaptation of symbionts.

Previous work has shown that host plants benefit most from AM when plants are P – not nitrogen (N) – limited (Johnson, 2010), and that proportionally more P is delivered by

AMF in low-P conditions (van't Padjé *et al.*, 2021). However, P delivered by AMF in low-P soils may 'cost' the plant more C (Andrino *et al.*, 2021), due to greater energy cost for the fungus to acquire P when P is more limiting. If C transfer from plants is insufficient, AMF can store P (Hammer *et al.*, 2011) and/or relocate P within the mycelium to optimize C transfer from plants (Whiteside *et al.*, 2019). Thus, despite being obligately dependent on plants for their C, AMF can control the allocation of P. Plants also possess some control, because C allocation to AMF and AM colonization are often reduced in high-P soils (Treseder, 2004; Konvalinková *et al.*, 2017). Reduced C allocation to AMF may also occur in extremely low-P soils (Teste *et al.*, 2016), where AMF may themselves be P-limited (Treseder & Allen, 2002). Under such circumstances, plants may rely on other strategies to acquire P (Lambers *et al.*, 2008). Soil P availability is likely a strong driver of symbiotic function in the field.

Resource exchange also varies greatly among plant and fungal species. For example, co-occurring plants differ in the amount of C they allocate to AMF (Walder *et al.*, 2012; Grman & Robinson, 2013; Faghihinia & Jansa, 2022), which in turn may affect how much P they obtain from AMF (Lekberg *et al.*, 2010; Kiers *et al.*, 2011; Fellbaum *et al.*, 2014). This could help explain some of the documented differences in AM dependency, that is the growth and fitness responses by plants to AMF, which sometimes differ broadly among functional groups (Wilson & Hartnett, 1998). For example, forbs tend to be more highly colonized and benefit more from AMF than grasses (especially cool-season grasses) in many ecosystems (Bunn *et al.*, 2015), possibly because grasses tend to be more fine-rooted (Hetrick, 1991). High mycorrhizal dependency may limit a plant's ability to regulate C flow to AMF, however, leaving more AM-dependent plants susceptible to AMF parasitism when P is nonlimiting (Grman, 2012). Just as plants differ as hosts, AMF taxa vary greatly in their ability to deliver P to plants (Pearson & Jakobsen, 1993; Ravnskov & Jakobsen, 1995; Smith *et al.*, 2003; Lendenmann *et al.*, 2011). Fungal taxa with high extraradical growth may be more effective in acquiring P (Hart & Reader, 2002; Maherali & Klironomos, 2007), although differences in function of the interfacial membranes may also play a role (Smith *et al.*, 1994). P fertilization may favor AMF that preferentially develop inside the root, leading to suggestions of reduced mutualism (Johnson, 1993). However, it is possible that AMF provide other functions, such as pathogen protection, in more fertile soils (Lekberg & Waller, 2021). Whether and how AM function differs among co-occurring plants and fungi in soils of varying P availability is not well known.

In contrast to most glasshouse and *in vitro* experiments, plants in nature are often co-limited by several nutrients and may simultaneously be exposed to both pathogens and drought, all of which AMF have the potential to mitigate (Delavaux *et al.*, 2017). Consequently, trade relationships may not be optimized solely for P-for-C exchange, resulting in plants with a bet-hedging strategy that supports multiple fungal taxa and functions (Lekberg & Koide, 2014). Many experiments also do not consider the effects of local adaptation (but see Johnson *et al.*, 2010; Revillini *et al.*, 2016; Williams *et al.*, 2017), whereby symbiotic function has been shaped by long-term exposure to particular environmental conditions. For example, AMF from low-P soils form more arbuscules (indicative of greater P delivery) than AMF from high-P soils in roots of P-limited plants (Johnson *et al.*, 2010), and long-term P fertilization may reduce P transfer from AMF to plants (Williams *et al.*, 2017). Whether these changes are due to shifts in function within AMF taxa or result from changes in composition is often unknown. Ultimately, we know very little about resource exchange in AM except under highly controlled conditions in which individual plants are colonized by few fungal species – possibly from disparate environments – and are exposed to single or few stressors.

To determine whether P-for-C exchange differs among co-occurring forbs and grasses and depends on long-term exposure to different soil P availabilities, we removed intact grassland communities from the field and labelled them with C and P isotopes. We selected grasslands in two regions that varied 10-fold in

extractable soil inorganic P and that harbored the common forb blanketflower (*Gaillardia aristata* Pursh) and native bunchgrasses. We predicted that (1) AMF produce greater extraradical biomass and deliver a greater proportion of total plant P in communities where soil P availability is lower, (2) P-for-C exchange depends on soil P availability such that plants obtain more P per C from AMF where P availability is higher, (3) due to its expected greater AM dependency as a forb, blanketflower obtains a greater proportion of its total P from AMF than co-occurring grasses, especially in low-P soils, and, consequently (4) blanketflower allocates more C to AMF than co-occurring grasses.

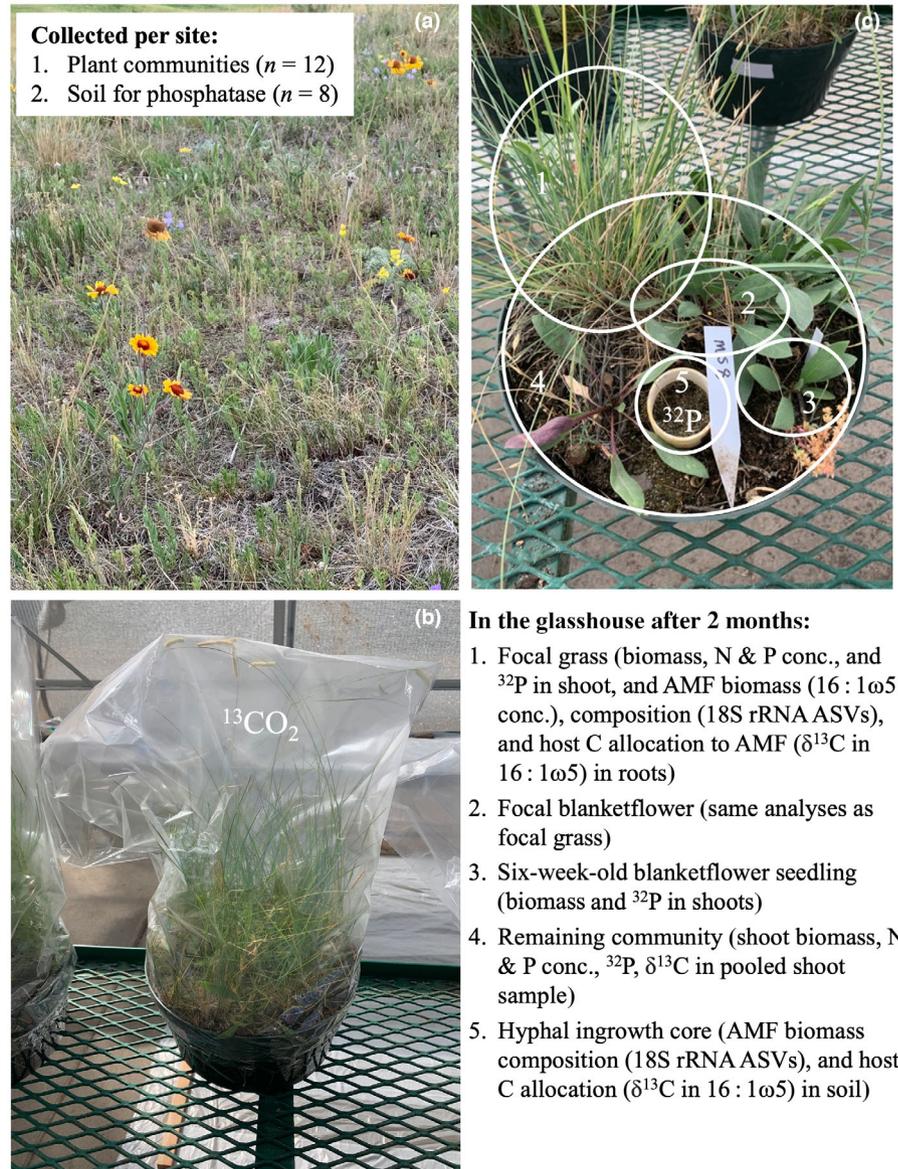
## Materials and Methods

### Site characteristics, collection of plant communities, and experimental setup

We selected three grassland sites in Montana (Drummond (46.654, -113.223), MPG Ranch (46.697, -114.023), Mount Sentinel (46.837, -113.97)), and three in North Dakota (Central Grasslands (46.727, -99.429), Chase Lake WMA (47.037, -99.414) Davis Ranch (47.355, -100.267)), as these two regions within the USA share many plant species yet differ in soil P availability (Smith *et al.*, 2019). Mean annual temperature and precipitation vary from 4.4°C and 400 mm (North Dakota) to 7.7°C and 350 mm (Montana). Soils in all sites are classified as fine to gravelly, loamy Mollisols, and all six sites harbor blanketflower and diverse native bunchgrasses (Fig. 1). In mid-June, 2020, when plants approached peak biomass, we targeted *c.* 1-ha area within each site and carefully extracted 12 intact communities (*c.* 25 cm Ø × 12 cm deep) that each harbored at least one flowering blanketflower and a perennial grass that was flowering or had flowered the preceding year, to ensure similar phenology and maturity. Communities were transferred in individual free-draining pots to the University of Montana glasshouses in Missoula, MT, USA (Fig. 1).

To quantify AMF P uptake and delivery to plants, we removed *c.* 200 ml of soil from each community in order to insert a root-free 5 cm × 12 cm tall cylindrical hyphal ingrowth core. Ingrowth cores were constructed following Johnson *et al.* (2001) with some modifications using PVC pipes, with holes (1 cm Ø) drilled to replace *c.* 30% of the surface area with 35 µm nylon mesh (Component Supply, Fort Meade, FL, USA), which was glued to the sides and bottom (Gorilla glue, Cincinnati, OH, USA) to ensure no openings larger than those of the mesh. Soil removed from the core was sieved through a 2 mm sieve and split into two pools, one mixed with sand 1 : 1 (v/v) and used to fill the ingrowth core, and the second placed in a cooler for chemical analyses described below. The soil corer and sieve were brushed clean of soil and drenched in 70% EtOH between samples to minimize cross-contamination of AMF among communities.

To directly compare AMF P delivery to plants without potential biases due to differences in plant age and local adaptation, we planted 2-wk-old blanketflower seedlings (Wind River Seed Co., Manderson, WY, USA) into all communities 2 wk after the installation of the ingrowth cores (Fig. 1). To minimize above



**Fig. 1** Twelve communities of flowering blanketflower (*Gaillardia aristata*, yellow flowers in photograph) and grasses were removed from each of six sites (a); three located in a region of high soil P availability (western Montana) and three located in a region of low soil P availability (eastern North Dakota). Communities were transported to a glasshouse, where a hyphal ingrowth core was inserted and a blanketflower seedling was planted into each community. After a 2-month acclimation period, all shoots were labelled with  $^{13}\text{CO}_2$  (b),  $^{32}\text{P}$  was added to the hyphal ingrowth core, and after an 8-d chase period, plants were destructively harvested (c). Four of the 12 communities per site were randomly selected as controls to measure non-AMF movement of P; hyphal ingrowth cores were rotated before  $^{32}\text{P}$  labelling to sever any arbuscular mycorrhiza fungi (AMF) connections between the  $^{32}\text{P}$  source and plants and rotated daily thereafter to ensure their absence.

and belowground competition, we transplanted seedlings into a 5 cm  $\times$  12 cm deep volume containing soil extracted from communities, sieved and mixed with sand as above, and cut adjacent vegetation within  $c.$  1 cm at the soil surface. We also randomly identified one blanketflower and one grass in each community to target for individual measurements, measured their distance to the ingrowth core and, when possible, identified the grass to species (Supporting Information Dataset S1). All communities then acclimated for 6 wk under ambient light and were watered as needed with tap water.

To assess whether selected soil edaphic properties differed between communities from high- and low-P sites, we quantified available N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) in fresh soil collected when installing the hyphal ingrowth core (Hart *et al.*, 1994) and available P (Bray-1) in air-dried soil (Bray & Kurtz, 1945; Table 1). Extracts were analyzed colorimetrically using a Synergy 2 Microplate Reader (BioTek, Winooski, VT, USA) after Weatherburn (1967),

Doane & Horwath (2003), and D'Angelo *et al.* (2001). The lowest values of soil  $\text{NO}_3^-$  availability were at the detection limit,  $0.1 \text{ mg kg}^{-1}$  (Dataset S1). To explore whether high- and low-P sites also differed in labile and moderately labile organic P pools using the first three stages of the Hedley fractionation procedure, resin P (pH 8.5), 0.5 M  $\text{NaHCO}_3$ -extractable P, and 1 M  $\text{NaOH}$ -extractable P (Hedley *et al.*, 1982), on one pooled sample per site (collected when installing the hyphal ingrowth core). Total P in the  $\text{NaHCO}_3$  and  $\text{NaOH}$  extracts was determined using acid persulfate digests (Rowland & Haygarth, 1997). Inorganic P and total P pools were measured using molybdate colorimetry (Murphy & Riley, 1962; Kuo, 1996). Organic P pools were calculated by difference. These analyses confirmed the higher soil P availability in Montana (high-P) than in North Dakota (low-P) as documented previously (Smith *et al.*, 2019). N availability did not differ between high- and low-P sites, nor did soil pH (measured in water, 1 : 1 v/v, Table 1).

**Table 1** Soil, plant, and arbuscular mycorrhiza fungi (AMF) properties in communities collected from three high-P sites (Montana) and three low-P sites (North Dakota).

Region	High-P (Montana)	Low-P (North Dakota)	$P_{\text{Region}}$
Soil properties			
Soil pH in water	6.80 (0.38)	6.53 (0.38)	0.64
Total organic P (mg kg <sup>-1</sup> )	1178 (302)	684 (79)	0.24
Soil P availability (Bray1; mg kg <sup>-1</sup> )	32.4 (9.54)	2.97 (0.22)	<b>0.028</b>
Soil Pase (mg pNP g <sup>-1</sup> soil h <sup>-1</sup> )*	271 (18.6)	421 (19.4)	<b>&lt;0.001</b>
Soil NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	4.84 (0.41)	9.36 (0.81)	0.10
Soil NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	0.99 (0.30)	3.92 (0.67)	0.19
Community properties			
Shoot biomass (g)	10.5 (0.66)	15.2 (0.69)	0.08
Forb-to-grass ratio	2.60 (0.86)	0.44 (0.09)	<b>0.009</b>
Shoot N conc. (mg g <sup>-1</sup> )	14.6 (0.46)	13.4 (0.49)	0.35
Shoot P conc. (mg g <sup>-1</sup> )	3.29 (0.14)	1.56 (0.04)	<b>0.005</b>
Shoot <sup>32</sup> P conc. (kBq g <sup>-1</sup> )	4.92 (0.75)	0.63 (0.13)	<b>0.032</b>
<sup>32</sup> P per total P (kBq mg shoot P <sup>-1</sup> )	1.40 (0.19)	0.40 (0.08)	<b>0.008</b>
Blanketflower seedlings			
Shoot biomass (g)	0.12 (0.03)	0.09 (0.02)	0.35
Shoot <sup>32</sup> P conc. (kBq g <sup>-1</sup> )	16.5 (4.86)	2.76 (0.92)	<b>0.005</b>
AMF in hyphal ingrowth core			
AMF biomass (mmol 16 : 1ω5 C g soil <sup>-1</sup> )	0.64 (0.12)	0.54 (0.07)	0.77
C allocation to AMF in soil (δ <sup>13</sup> C in 16 : 1ω5)	37.5 (4.20)	29.5 (4.42)	0.45
AMF richness in soil (no. of ASVs)	29.8 (1.44)	28.1 (2.04)	0.78
P-for-C exchange			
Shoot <sup>32</sup> P conc./δ <sup>13</sup> C in 16 : 1ω5 in core	0.19 (0.04)	0.03 (0.01)	0.06

Blanketflower seedlings were planted into each community at the beginning of the 2-month glasshouse incubation when soil properties were measured, and community and AMF properties were measured at harvest following <sup>13</sup>CO<sub>2</sub> and <sup>32</sup>P labelling and an 8 d chase period. The δ<sup>13</sup>C values were corrected for natural abundance and <sup>32</sup>P values were corrected for background, quench, and decay. Means (SE) were compared by ANOVA with site nested within region (except by *t*-test for soil pH and soil organic P). *P*-values < 0.05 are shown in bold, *n* = 8 per site, except for soil pH, and total soil organic P for which one pooled sample per site was analyzed.

\*Soil Pase activity was measured on separate soil samples, *n* = 8 per site.

To determine whether plants and soil biota rely on additional means to acquire P in low-P sites, we measured soil acid and alkaline phosphatase activities on eight samples per site (0–12 cm deep), which we collected 1 yr after the communities in June 2021. Soil was sieved (2 mm) and stored cool until analysis within 14 d. For the analyses, 1 g subsamples of field moist soil were incubated with the enzyme-specific substrate for 1 h at 37°C, then filtered and analyzed spectrophotometrically (Dick *et al.*, 1997; Parham & Deng, 2000; Dick, 2011). Controls received the substrate-specific *p*-nitrophenyl phosphate right before filtration.

### Dual-isotope labelling and harvest

Following 2 months in the glasshouse, in mid-August 2020, eight communities per site (total of 48) were randomly selected for <sup>13</sup>CO<sub>2</sub> labelling. The four remaining communities per site were left as unlabelled controls (described below). For the <sup>13</sup>CO<sub>2</sub> labelling, each community was enclosed in a 60 × 45 cm non-permeable oven bag (ClearBags, North Las Vegas, NV, USA) and incubated at ambient conditions with 10 ml 99% <sup>13</sup>CO<sub>2</sub> (Sigma-Aldrich) for 2.5 h at midday on two consecutive days (Fig. 1). We chose multiple, short labellings, which were effective at incorporating a strong <sup>13</sup>C signal in previous experiments (Lekberg *et al.*, 2013). Immediately after the second labelling,

two small leaf subsamples (< 5 mg dry weight) were collected from the focal blanketflower and grass in each community to estimate <sup>13</sup>C assimilated during labelling (Stable Isotope Facility, UC Davis, CA, USA). Samples were also collected from the four unlabelled communities from each grassland to determine background δ<sup>13</sup>C.

Following <sup>13</sup>CO<sub>2</sub>-labelling, all 12 communities per site were labelled with 1 ml of 1 MBq <sup>32</sup>P in the form of H<sub>3</sub><sup>32</sup>PO<sub>4</sub> in HCl-free water (314 TBq mmol P<sup>-1</sup>, Perkin Elmer, Boston, MA, USA). We used a syringe and disposable needle and the <sup>32</sup>P was added to the center bottom of the hyphal ingrowth core and released upward steadily as the needle was withdrawn to ensure an even vertical distribution within the core. Immediately before labelling, cores in the four control communities per site were rotated carefully to sever the AMF hyphal connections between the <sup>32</sup>P source and plants, allowing us to quantify non-AM mediated <sup>32</sup>P transfer. Cores in the control communities were rotated daily, and all watering was done outside the cores to minimize <sup>32</sup>P movement out of the core via water. Frequent rotation in a similarly textured soil did not affect important soil parameters in a previous study (Leifheit *et al.*, 2014).

Communities were destructively harvested 8 d after <sup>32</sup>P labelling. We chose 8 d because earlier work showed an exponential increase in radioactivity within plants during the first 10 d (Johansen *et al.*, 1993; Johnson *et al.*, 2001) and rapid loss of <sup>13</sup>C

following labelling (Drigo *et al.*, 2010). At harvest, roots and shoots of the focal blanketflower and grass were separated from the remaining plants in the community. To ensure  $^{32}\text{P}$  concentrations in shoots could be directly linked to  $^{13}\text{C}$  concentrations in roots and allow for estimates of P-for-C exchange, only roots that were still attached to the shoots were collected. One focal blanketflower, one grass plant, and one blanketflower seedling had died, each in a different community from one high-P site (Mt. Sentinel), resulting in 11 replicates from that site. For the planted blanketflower seedlings, only shoots were collected due to very low root biomass and many severed roots. The remaining live shoot biomass was collected from each community and separated into grasses and forbs.

All five shoot samples per community (blanketflower, grass, blanketflower seedling and remaining grass, and forb biomass) were oven-dried at 65°C to constant weight. After weighing, the remaining forb and grass biomass was pooled into one sample per community for subsequent analyses. Root samples (blanketflower and grass only) were placed in a cooler, frozen at -20°C and freeze-dried (Labconco, KS City, MO, USA). Roots and shoots were ground separately using a 1600 MiniG tissue homogenizer and cell lyser (Spex SamplePrep, Metuchen, NJ, USA). The ingrowth cores were examined at harvest to ensure no roots had breached the mesh. A soil sample (1 cm × 10 cm deep) was taken from the center of each ingrowth core and freeze-dried as above. Fig. 1 outlines all the sample pools and associated analyses.

### Sample processing for $^{32}\text{P}$ and $^{13}\text{C}$ analysis and shoot nutrients

Between 30 and 40 mg of ground shoot tissue was digested in nongummed cigarette paper in 2 ml 18 M  $\text{H}_2\text{SO}_4$  : 30%  $\text{H}_2\text{O}_2$  (1 : 1 v/v) at 360°C for 1.5 h and diluted to 10 ml with distilled water, separately for the focal blanketflower, grass, blanketflower seedling, and pooled remaining biomass from each community ( $n=12$  per site except for seedlings where  $n=8$ ; Dataset S1). Incorporated  $^{32}\text{P}$  was measured by Cerenkov counting on a Beckman LS 6500 liquid scintillation counter (Beckman Coulter Life Sciences, Indianapolis, IN, USA), and counts per minute (CPM) were corrected for quench, decay, and background and converted to kBq. Rotating the ingrowth core before  $^{32}\text{P}$  labelling – thus rupturing the AMF connection between the  $^{32}\text{P}$  source and shoots – effectively eliminated radioactivity in plants relative to communities with static cores (0.02 vs 3.36 kBq g biomass<sup>-1</sup>,  $P<0.001$ ). This indicates that the vast majority of the  $^{32}\text{P}$  in shoots in the static core communities was delivered by AMF. In one blanketflower sample from one low-P site (Davis Ranch), we set the value at zero because the enrichment value was slightly lower than the control values. Additional aliquots were analyzed for total N and P concentrations on a plate reader (Jensen, 1962; Watanabe & Olsen, 1965).

Ground shoot tissue of the focal blanketflower, the grass, and the pooled remaining biomass was analyzed for  $\delta^{13}\text{C}$  at harvest. Following Řezáčová *et al.* (2018), we used whole cell (phospholipid, glycolipid, and neutral lipid) fatty acid 16 : 1ω5 as a proxy

for AMF biomass, and estimated  $^{13}\text{C}$  allocation from shoots to AMF using the  $\delta^{13}\text{C}$  in 16 : 1ω5. Some Gram- bacteria also contain phospholipid fatty acid 16 : 1ω5, which can complicate assessments of AMF biomass. However, this should be less of an issue in this study because Gram- bacteria are typically in low abundance in roots (Lekberg *et al.*, 2022), and neutral lipid fatty acid 16 : 1ω5 dominates when AMF is present in soil (Vestberg *et al.*, 2012). Lipids were extracted separately from freeze-dried soil from the ingrowth core, blanketflower, and grass roots, converted to methyl esters and analyzed on a gas chromatograph coupled to an isotope-ratio mass spectrometer (Konvalinková *et al.*, 2017). Area and retention times were compared with the internal standard 19 : 0. One sample each of grass and blanketflower roots, as well as soil from one control (i.e. unlabelled) community from each grassland site were analyzed to determine background  $\delta^{13}\text{C}$ .

### Characterization of AMF communities in roots and soil

To assess whether AMF communities differed between high- and low-P sites in the hyphal ingrowth cores and blanketflower and grass roots, we used methods outlined in Bullington *et al.* (2021). DNA was extracted from 250 mg of ingrowth core soil ( $n=8$  per site) and 25 mg of roots using DNeasy PowerSoil Pro and DNeasy Plant Pro isolation kits, respectively, following the manufacturer's instructions (Qiagen, Germantown, MD, USA). We did not have sufficient root mass for all samples, so blanketflower and grass sample numbers ranged from 2 to 6 and 1 to 7 per low-P site, and 5 to 8 and 7 to 8 samples per high-P sites, respectively.

The small subunit rRNA gene region was amplified using the primer pair WANDA-AML2 (PCR1; Lee *et al.*, 2008; Dumbrell *et al.*, 2011). We chose this primer pair because it amplifies all major AMF families without apparent bias (Egan *et al.*, 2018) and sequences can be compared with an expert-curated database (Öpik *et al.*, 2010). Following index linker addition by PCR (PCR2), amplicons were purified using AMPure XP beads (Beckman Coulter Genomics, Brae, CA, USA), and pooled based on band strength to equalize concentrations before sequencing. Amplicon libraries were sequenced at the University of Montana Genomics Core facility using a MiSeq v2 kit (500 cycles) on an Illumina MiSeq sequencing platform (Illumina Inc., San Diego, CA, USA).

Processing of raw sequence data was performed using QIIME2 (2018.4, <https://qiime2.org/>; Bolyen *et al.*, 2019). Forward reads were trimmed to 200 bp, which covers the informative region (Lee *et al.*, 2008), and all sequences were quality filtered and de-replicated using the Q2-DADA2 plugin to produce sequence clusters with 100% similarity (Callahan *et al.*, 2016). Amplicon sequence variants (ASVs) were assigned taxonomy using the *MaarjAM* database (Öpik *et al.*, 2010) and the QIIME2 q2 feature classifier (<https://github.com/qiime2/q2-feature-classifier>) with a confidence threshold of 90%. We removed all ASVs that did not match with at least 85% similarity and 85% coverage to *MaarjAM* sequences. To verify the AMF identity of ASVs, we constructed a phylogenetic tree using the FASTTREE plugin (Price

*et al.*, 2010) in QIIME 2 with non-AMF outgroups and sequences extracted from the *MaarjAM* database. We rarefied to a resampling depth of 1400 and 250 sequences in soil and root samples, respectively, and all samples were retained at this depth. Sequences have been submitted to the Sequence read archive on NCBI under accession number PRJNA962124.

In cases where more roots were collected than were required for fatty acid and molecular analyses, we quantified overall AM colonization, as well as colonization by vesicles and arbuscules on trypan blue-stained roots (Phillips & Hayman, 1970) by scoring their presence within the field of vision at 200 $\times$  magnification and 50 intercepts.

### Calculations and statistical analyses

To estimate the proportion of total plant P delivered by AMF (required for our first and third predictions), we divided shoot  $^{32}\text{P}$  concentration by shoot total P concentration for either the whole community or for the blanketflower and grass individually. The P-for-C exchange in AM was estimated by calculating the ratio of shoot  $^{32}\text{P}$  concentrations and  $\delta^{13}\text{C}$  in 16:105 in blanketflower and grass roots and soil from the hyphal ingrowth core. In all cases, we subtracted the natural background  $\delta^{13}\text{C}$  in shoot tissue and 16:105 in roots and soil. Where natural abundance values differed significantly ( $P < 0.05$ ) between high- and low-P sites, possibly as a result of drought history or differences in photosynthetic pathway (Ehleringer *et al.*, 1986), we used regional-specific natural abundance values. Acid and alkaline phosphatase values were combined before analyses.

Differences in soil inorganic N and P, soil phosphatase activity, community biomass and shoot N and P concentrations, seedling and community  $^{32}\text{P}$  concentrations, and soil AMF biomass and richness between high- and low-P regions were determined by nested two-way ANOVA, with the region as a fixed effect and site nested within the region as a random effect. Differences between regions in soil pH and organic P were assessed by *t*-test. To examine differences between blanketflower and grasses in shoot biomass, shoot N and P concentrations, shoot  $^{13}\text{C}$  and  $^{32}\text{P}$  acquisition, root AMF biomass and richness, and C allocation to AMF, we used a mixed model ANOVA, with high-P and low-P region and plant species as fixed effects, high-P:low-P region  $\times$  plant species as interaction, and communities nested within site, and sites nested within either high-P or low-P regions as random effects. All ANOVAs were performed in JMP PRO v.17 (SAS Institute Inc., Cary, NC, USA).

The relationship between the proportion of total P delivered by AMF and soil P availability in each community was assessed by multiple linear regression (MLR; in JMP PRO v.17), including community biomass in the model. Because this model provided only a modest improvement (partial  $R^2 = 0.21$ ) over the predictive value of soil P availability alone ( $R^2 = 0.38$ ), we only present the simple correlation here. The effects of community biomass, distance to the  $^{32}\text{P}$  label in the in-growth core, and seedling biomass on seedling  $^{32}\text{P}$  concentration and content were also tested with MLR. For both blanketflower and grass, MLR was used to test for the effects of plant biomass, allocation to AMF in roots,

and difference in  $\delta^{13}\text{C}$  from labelling to harvest on  $^{32}\text{P}$  concentration and content. Lastly, we used MLR to test for the effects of soil P availability, distance of plants to the  $^{32}\text{P}$  label in the in-growth core, and shoot N:P on the P-for-C exchange ratio. We used bivariate Pearson correlation for linear relationships and Spearman rank correlations when relationships were nonlinear. A Wilcoxon rank sum test in JMP PRO was used to compare AMF hyphal, arbuscule, and vesicle frequencies between grasses and blanketflower. Variables were transformed when necessary to meet model assumptions before analysis.

Potential differences in fungal composition were examined in vegan in R (Oksanen *et al.*, 2019). To assess whether ASV relative abundance differed in ingrowth cores in high- and low-P sites, and between blanketflower and grass roots, we calculated Bray–Curtis distances using the *vegdist* function. We performed a PERMANOVA (Anderson, 2005) with algorithm ADONIS2 to test for compositional differences, which we visualized by conducting nonmetric multidimensional scaling using the *metaMDS* function.

## Results

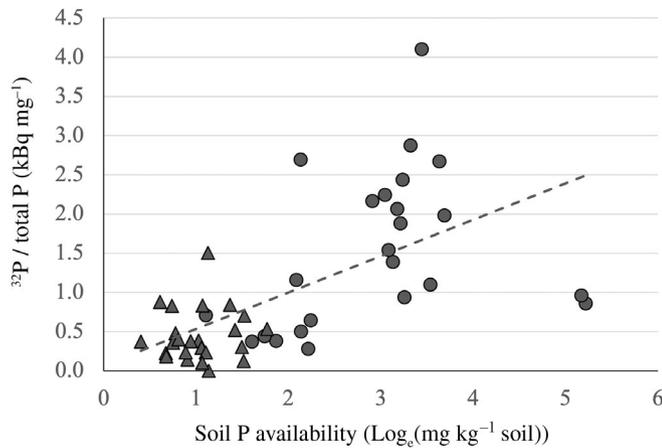
### Plant communities and AM function in high- and low-P sites

Communities collected from low-P sites were more grass-dominated, and shoot biomass trended higher than in communities from high-P sites (Table 1). Shoot N concentration did not differ in communities from high-P and low-P sites, but P concentration was more than twice as high in communities from high-P sites (Table 1). Differences in shoot  $^{32}\text{P}$  concentration were even greater, in both absolute and proportional terms (Table 1). Thus, contrary to our predictions, AMF delivered a greater proportion of total P to communities where soil P availability was higher (Fig. 2). Soil phosphatase activity, on the other hand, was higher in low-P sites (Table 1).

Biomass of blanketflower seedlings planted into each community did not vary between high-P and low-P sites (Table 1). However, seedling  $^{32}\text{P}$  concentrations mirrored patterns observed in established plants and were more than five times greater when grown in communities collected from high-P sites (Table 1). There were no effects of aboveground community biomass or proximity of seedlings to the  $^{32}\text{P}$  source on seedling  $^{32}\text{P}$  content or  $^{32}\text{P}$  concentration (data not shown).

Despite differences in soil P availability and  $^{32}\text{P}$  concentration in shoots of established plants and blanketflower seedlings, we found no difference in 16:105 fatty acid concentrations in the hyphal ingrowth cores between communities from high- and low-P sites, suggesting similar extraradical AMF abundance (Table 1). Supporting this,  $^{13}\text{C}$  enrichment in 16:105 (indicative of allocation of recent assimilate to AMF and an active symbiosis) also did not differ between communities from high-P and low-P sites (Table 1). Thus, plants tended to obtain more P per C in high-P soils (Table 1).

We detected a total of 603 ASVs in the hyphal ingrowth cores across all communities, with a clear dominance of Glomeraceae.



**Fig. 2** Relationship between soil P availability and amount of  $^{32}\text{P}$  delivered from arbuscular mycorrhiza fungi (AMF) to plant shoots relative to the total P measured in all aboveground biomass in communities collected in three low-P (triangles) and high-P (circles) sites. As soil P availability increased, AMF delivered proportionally more P to plants (Pearson's  $r = 0.62$ ,  $P < 0.001$ ,  $n = 8$  per site).

The composition of AMF taxa within families differed between communities collected from high- and low-P sites ( $P < 0.001$ ; Fig. S1), but AMF richness in the cores did not (Table 1).

### Comparisons of AM function and AMF community composition between blanketflower and grasses

Reflecting differences in forb:grass biomass ratios in communities, blanketflower biomass was higher in communities from high-P sites, whereas grass biomass trended higher in communities from low-P sites (Table 2). Shoot P concentration did not differ between blanketflower and grasses, but reflected soil P

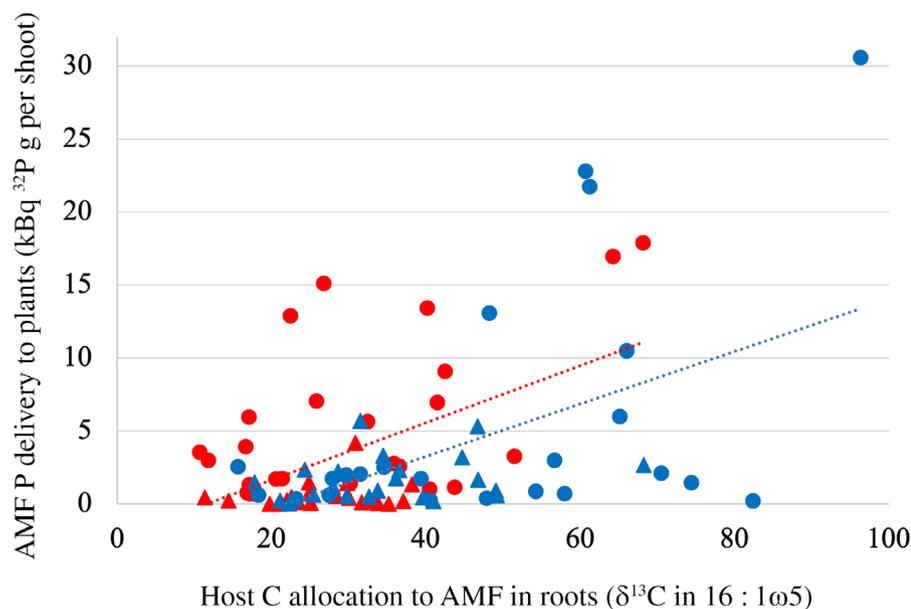
availability and was higher in communities from high-P sites. Shoot N concentration, on the other hand, was higher in blanketflower than in grasses but did not differ between high- and low-P sites (Table 2).

Similar to shoot biomass, we found significant interactions between region and plant species in many variables associated with AM resource exchange (shoot  $^{32}\text{P}$  concentration,  $^{13}\text{CO}_2$  assimilation, and AMF biomass; Table 2). For example,  $^{32}\text{P}$  concentration – and thus P delivery from AMF – was higher in blanketflower than in grass shoots in communities from the high-P sites, but grasses had higher  $^{32}\text{P}$  concentration than blanketflower in communities from low-P sites (Table 2). When AMF delivery was relativized based on total shoot P, it did not differ between blanketflower and grasses in communities from high-P sites but was higher in grasses than in blanketflower for communities from low-P sites (Table 2). Differences in  $^{32}\text{P}$  delivery may have been related to a plant's ability to assimilate and allocate more C to AMF, because shoot  $^{32}\text{P}$  concentration was positively related to differences in shoot  $\delta^{13}\text{C}$  between labelling and harvest (indicative of C allocation to AMF and/or respiratory losses) for both blanketflower ( $F_{1,40} = 3.42$ ,  $P < 0.01$ ) and grasses ( $F_{1,42} = 3.73$ ,  $P < 0.001$ ). Further indicating the role of AMF, we found a positive relationship between  $\delta^{13}\text{C}$  in 16:1ω5 in roots and  $^{32}\text{P}$  concentration in shoots for both plant types (Fig. 3). However, even though relationships for both plant types were positive,  $\delta^{13}\text{C}$  in 16:1ω5 was consistently higher in grass roots (Table 2), and for every unit  $^{13}\text{C}$  allocated to AMF in roots, blanketflower obtained more  $^{32}\text{P}$  (Fig. 3). Another difference between plant types was that  $^{32}\text{P}$ -for- $^{13}\text{C}$  exchange ratios varied with soil P availability and shoot N:P for blanketflower ( $F_{1,39} = 6.52$ ,  $P = 0.02$ ;  $F_{1,39} = 5.32$ ,  $P = 0.03$ , respectively); as P became more available and as shoot P indicated greater N limitation, blanketflower obtained more P per C (Figs 4, S2). No such relationships were

**Table 2** Comparisons of shoot biomass, nutrient concentrations,  $^{32}\text{P}$  delivery from arbuscular mycorrhiza fungi (AMF) to plants (both concentration and uptake per unit P in plant biomass), plant  $^{13}\text{CO}_2$  assimilation,  $^{13}\text{C}$  allocation from plants to AMF ( $\delta^{13}\text{C}$  in 16:1ω5 in roots), and AMF biomass and richness in roots in co-occurring blanketflower and grasses in communities collected from three high-P sites (Montana) and three low-P sites (North Dakota).

Variable	High-P (Montana)		Low-P (North Dakota)		$P_{\text{Region}}$	$P_{\text{Species}}$	$P_{\text{R} \times \text{S}}$
	Blanketflower	Grass	Blanketflower	Grass			
Shoot biomass (g)	1.32 (0.29) <sup>a</sup>	0.76 (0.14) <sup>b</sup>	0.68 (0.10) <sup>ab</sup>	0.85 (0.11) <sup>ab</sup>	0.86	0.64	<b>0.014</b>
Shoot N ( $\text{mg g}^{-1}$ )	19.1 (0.54)	15.3 (0.93)	19.7 (0.83)	13.8 (0.58)	0.89	<b>&lt;0.001</b>	0.19
Shoot P ( $\text{mg g}^{-1}$ )	3.39 (0.17)	3.07 (0.23)	1.42 (0.07)	1.43 (0.09)	<b>&lt;0.001</b>	0.21	0.26
Shoot $^{32}\text{P}$ conc. ( $\text{kBq g}^{-1}$ )	6.04 (1.14) <sup>a</sup>	5.55 (1.77) <sup>b</sup>	0.65 (0.18) <sup>c</sup>	1.69 (0.31) <sup>ab</sup>	0.06	0.27	<b>&lt;0.001</b>
$^{32}\text{P}$ per total P ( $\text{kBq mg}^{-1}$ shoot P)	1.83 (0.37) <sup>a</sup>	1.68 (0.46) <sup>ab</sup>	0.49 (0.15) <sup>b</sup>	1.33 (0.25) <sup>a</sup>	0.27	0.17	<b>&lt;0.001</b>
$\delta^{13}\text{C}$ shoots after labelling	114.3 (9.86) <sup>a</sup>	79.5 (7.41) <sup>b</sup>	59.4 (3.02) <sup>b</sup>	57.8 (5.50) <sup>b</sup>	<b>0.029</b>	<b>0.004</b>	<b>0.049</b>
$\delta^{13}\text{C}$ shoots at harvest	38.2 (3.12) <sup>a</sup>	24.5 (3.13) <sup>b</sup>	23.4 (2.09) <sup>b</sup>	27.9 (3.25) <sup>b</sup>	0.27	<b>0.022</b>	<b>&lt;0.001</b>
$\delta^{13}\text{C}$ loss in shoots ( $\delta^{13}\text{C}$ labelling – $\delta^{13}\text{C}$ harvest)	76.1 (9.63)	54.6 (6.27)	34.3 (2.67)	29.9 (4.02)	<b>0.026</b>	0.096	0.31
C allocation to AMF in roots ( $\delta^{13}\text{C}$ in 16:1ω5)	31.9 (3.27)	49.1 (4.48)	26.0 (1.56)	34.9 (2.38)	0.49	<b>&lt;0.001</b>	0.08
AMF biomass ( $\text{mmol 16:1}\omega\text{5 C g}^{-1}$ root)	27.0 (2.84) <sup>b</sup>	70.1 (7.95) <sup>a</sup>	47.1 (7.18) <sup>ab</sup>	46.9 (6.99) <sup>ab</sup>	0.99	<b>0.003</b>	<b>&lt;0.001</b>
AMF richness in roots (no. of ASVs)	22.4 (1.76)	20.2 (2.00)	29.0 (4.11)	27.2 (4.91)	<b>0.020</b>	0.87	0.92

Values are means (se),  $n = 6-8$  communities per site within each region at harvest following a 2-month incubation in the glasshouse, labelling with  $^{32}\text{P}$  and  $^{13}\text{CO}_2$ , and a chase period of 8 d. The  $\delta^{13}\text{C}$  values were corrected for natural abundance and  $^{32}\text{P}$  values were corrected for background, quench, and decay. Means were compared by two-way ANOVA with region and plant species as fixed effects and site nested in region and community nested in site as random effects. Significant differences ( $P < 0.05$ ) are highlighted in bold. For significant interactions, means that share a letter did not differ statistically by Student's  $t$ -test.



**Fig. 3** Relationship between C allocation to arbuscular mycorrhiza fungi (AMF) in roots (measured as  $\delta^{13}\text{C}$  in 16 : 105) and P delivery from AMF to shoots (measured as  $^{32}\text{P}$  in shoot tissue) in blanketflower (red; Pearson's  $r = 0.51$ ,  $P < 0.001$ ) and co-occurring grasses (blue; Pearson's  $r = 0.53$ ,  $P < 0.001$ ) grown in plant communities collected from three high-P sites (circles) and three low-P sites (triangles). Grasses consistently obtained less  $^{32}\text{P}$  per  $^{13}\text{C}$  allocated to AMF in roots compared with blanketflower. All  $^{13}\text{C}$  values were corrected for natural abundance and  $^{32}\text{P}$  values were corrected for background, quench, and decay;  $n = 6\text{--}8$  per site.

observed for the grasses. Proximity to the  $^{32}\text{P}$  source (3–10 cm) did not affect  $^{32}\text{P}$  concentrations in either blanketflower or grass shoots, suggesting factors other than distance to source dictated resource delivery.

Even though grasses always allocated more  $^{13}\text{C}$  to AMF in roots than blanketflower (based on  $\delta^{13}\text{C}$  in 16 : 105), AMF biomass was only higher in grass roots in communities from high-P sites (Table 2). Despite this higher AMF biomass, grasses obtained less  $^{32}\text{P}$  than blanketflower in high-P sites. This may be related to the particular AM structures within roots. On the subset of samples where we had enough roots to assess percent AM colonization (mostly from high-P sites, Dataset S1), the proportion of arbuscules (exchange structures) was higher in blanketflower ( $Z = 2.22$ ,  $P = 0.03$ ,  $n = 12$ ) whereas vesicular (storage structures) abundance trended higher in grasses ( $Z = -1.83$ ,  $P = 0.07$ ,  $n = 12$ ; Fig. S3).

Similar to patterns in AMF biomass, we observed clear differences in AMF composition in roots of blanketflower and grasses in communities from high-P, but less so in the low-P sites ( $P_{\text{Region} \times \text{plant type}} < 0.001$ , Fig. S1). This interaction appeared to be driven by a greater abundance of Claroideoglomeraceae in grass roots in communities from high-P sites, whereas Glomeraceae dominated in all other roots (Fig. S1). In all, we observed 401 ASVs in root samples across both regions, with a higher overall AMF richness in plants from low-P sites (Table 2).

## Discussion

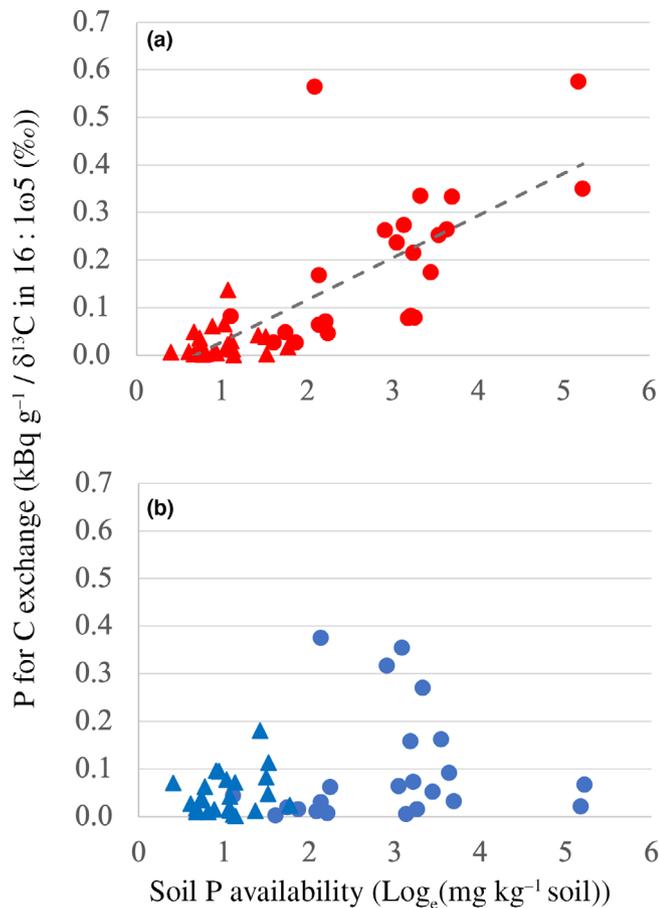
Most of what we know about AM function stems from experiments in controlled environments involving few plant and fungal taxa, but whether such studies accurately reflect processes in natural, complex communities where resource availabilities and environmental stressors vary greatly is uncertain. Extracting intact communities from sites that differed greatly in soil available P, we show that – for this range of soil P availability – AMF

delivered a greater proportion of total plant P in communities from high rather than low-P sites. We also show that co-occurring plant species varied in the amount of C they delivered to AMF, as well as their ability to regulate P-for-C exchange based on soil P availability, suggesting C trade is not solely driven by P exchange. These findings challenge some of our current understanding of resource exchange in AM.

### AMF delivered a greater proportion of P in communities from high-P, not low-P soils

Previous work under simplified conditions has shown that AMF deliver a greater proportion of total plant P when P is less available (Williams *et al.*, 2017; van't Padje *et al.*, 2021) and that AMF may become redundant and even parasitic when plants are N- rather than P-limited (Johnson *et al.*, 2015; Bennett & Groten, 2022). Yet, in our study, AMF delivered more P (both in absolute and proportional terms) where soil P availability was higher (Fig. 2) and where shoot N : P ratios in communities suggested clear N limitation (Fig. S4; Koerselman & Meuleman, 1996). Blanketflower seedlings used as phytometers showed the same pattern.

Part of this may be due to the differences in AMF communities we observed in high- and low-P sites (Fig. S1), because AMF taxa differ in their ability to deliver P to plants (Smith *et al.*, 2003). Regardless, this prompts the question why plants continue to allocate C to AMF in high-P soils where AMF may exacerbate N limitation (Riley *et al.*, 2019) and become parasitic (Johnson *et al.*, 2015)? We can think of at least five possible explanations. First, AMF may reduce the ability of some plant species to acquire their own P (Smith *et al.*, 2003; Grace *et al.*, 2009), rendering them dependent on AMF for P acquisition even when P availability is high. Indeed, suppression of plant genes involved in the plant's P-starvation response and direct P uptake seems to be greater the more P is delivered by AMF in



**Fig. 4** Changes in P-for-C exchange between arbuscular mycorrhiza fungi (AMF) and blanketflower (a) and grasses (b) with increasing soil available P. Phosphorus delivery from AMF to plants was measured as <sup>32</sup>P in shoot tissue, and C delivery from plants to AMF was measured as <sup>13</sup>C in 16 : 1 ω<sub>5</sub> in roots. As soil available P increased, blanketflower obtained more P per C delivered to AMF (Pearson's  $r = 0.76$ ,  $P < 0.001$ ), whereas no directional change was observed in grasses. Triangles show values from the three low-P sites in North Dakota, and circles show values from the three high-P sites in Montana. All <sup>13</sup>C values were corrected for natural abundance and all <sup>32</sup>P values corrected for background, quench, and decay.

some plant species (Burleigh, 2002). Second, modelling suggests that it may be energetically more favorable for plants to assimilate and trade C with the fungus than to uptake P directly in high-P soils (Grman *et al.*, 2012), and that AMF benefits to plants may increase linearly with nutrient availability (Bachelot & Lee, 2018). This, however, clearly contradicts much empirical work, especially when N is limiting (Johnson *et al.*, 2015), suggesting more work is needed to understand this paradox. Third, plants often upregulate photosynthesis in response to AM symbiosis establishment (Kaschuk *et al.*, 2009) and C may therefore not be limiting (Corrêa *et al.*, 2012). Fourth, plants may 'pay' AMF for other services, including N acquisition, drought tolerance, pathogen protection (Augé, 2001; Delavaux *et al.*, 2017), and defense against herbivores (Jung *et al.*, 2012), which were not assessed here. Finally, P availability may change with time, and maintaining AM symbionts may be regarded as a bet-hedging strategy (Veresoglou *et al.*, 2022).

Regional differences in AMF P delivery could also stem from an inability of AMF to deliver P in low-P grasslands, because most of the P is unavailable and the AMF themselves may be P-limited. Indeed, AMF deliver more P in very low-P soil when P is added (Dickson *et al.*, 1999), possibly because AMF P limitations are overcome. The higher phosphatase activity in the low-P sites suggests a reliance on additional means to acquire P by plants, other soil biota, and AMF (Joner & Johansen, 2000; Koide & Kabir, 2000; Fall *et al.*, 2022) and support previous findings in very low-P soils (Lambers *et al.*, 2017). This can coincide with low extraradical hyphal density (Teste *et al.*, 2016), but this was not observed here despite a 10-fold variation in labile soil inorganic P. It is possible that the lack of differences is due to a hump-shaped relationship between soil P availability and fungal biomass where fungi are P-limited at low-P availabilities, and C-limited at high-P availabilities (Bolan *et al.*, 1984; Treseder & Allen, 2002). Alternatively, AMF biomass may be less responsive to natural variation in soil P availability in the field (as P may only be one of many variables that affect AM associations) than indicated by fertilization experiments (Treseder, 2004). Indeed, sampling along natural gradients of soil P availability has reported similar internal and external AM colonization in several studies (Treseder & Allen, 2002; Powers *et al.*, 2005; Johnson *et al.*, 2010). Overall, our study builds on previous work and illustrates that relationships between P delivery, soil P availability, and AMF biomass in more complex systems may differ from those expected based on shorter-term controlled experiments.

Grasses allocated more C to AMF in roots and appeared less able to regulate resource exchange than blanketflower

Previous work under axenic conditions shows that roots that allocate more C to AMF receive more P via the mycorrhizal pathway (Lekberg *et al.*, 2010; Hammer *et al.*, 2011). Our results suggest this may also happen in more complex settings with widely varying resource availabilities and AMF communities, because we found positive relationships between C allocation to AMF and P delivery to plants for both blanketflower and grasses (Fig. 3). Yet for every unit of C allocated to AMF in roots, grasses obtained less P. Similar disparities among plant species have been observed before, prompting suggestions that some plant species subsidize AM associations for others (Grime *et al.*, 1987; Walder *et al.*, 2012). Care should be taken when interpreting current and previous findings, however, as they require assessments of all pools during the same time interval. We observed P-for-C exchange during the same period, but we were unable to assess C contribution to extraradical hyphae by individual plants. It is possible, therefore, that blanketflower allocated more C to AMF outside than inside roots, which may have led us to underestimate C costs. Also, we used total fatty acids to quantify C allocation to AMF and fungal biomass, which may be biased toward storage rather than membrane lipids (Lekberg *et al.*, 2022). The greater AMF biomass in grass roots – especially in communities from high-P grasslands – could therefore primarily reflect storage structures, which agrees with the observed trend of more vesicles in grass roots. Regardless of these limitations, the fact that

blanketflower roots contained more arbuscules, and the possibility that a greater proportion of AMF biomass was in the soil than in the roots with this species, are consistent with expectations of greater P acquisition and transfer from AMF.

Blanketflower and grasses also varied in their apparent ability to regulate exchange rates. As P became more available and shoot N : P ratios indicated greater N limitation, P was obtained for less C in blanketflower but not in grasses (Figs 4, S2). Blanketflower thus responded in ways that are consistent with biological market theory (Werner *et al.*, 2014) but grasses did not. Our results may indicate a reduced dependency on AMF for P acquisition among the grasses, consistent with a 'do-it-yourself strategy' (Bergmann *et al.*, 2020; Han *et al.*, 2022). However, grasses always allocated more C to AMF in roots than blanketflower, which is inconsistent with the idea of lesser dependency. Grasses harbored different AMF taxa than blanketflower (Fig. S1) and fungal taxa can differ drastically in their P-for-C exchange ratio (Pearson & Jakobsen, 1993; Ravnskov & Jakobsen, 1995; Smith *et al.*, 2003), as well as other services they may provide (Sikes *et al.*, 2009; Delavaux *et al.*, 2017). Follow-up experiments involving mycorrhizal and nonmycorrhizal plants in which root vs hyphal P uptake is compared (Smith *et al.*, 2003), and where other services are also assessed, are required to reveal potential differences in AM dependency between blanketflower and grasses.

### Limitations and future directions

Our approach, despite comprising complex communities, nevertheless had some limitations. The well-watered conditions in the glasshouse prompted substantial growth during the 2-month incubation and likely differed from field conditions in ways that could have affected AM physiology. Also, we refer to grasses as a homogeneous group throughout this paper, but in reality, they comprised multiple species with different photosynthetic pathways and possible differences in AM dependency (Hetrick *et al.*, 1988). This could have obscured relationships between P-for-C exchanges and soil P as well as shoot N : P. Likewise, while P uptake tracked P availability, other factors could have driven observed patterns as we did not manipulate P directly.

Differences in enrichment and natural abundance could also complicate interpretations of resource transfer, although in our study they likely resulted in conservative estimates. For example, the greater  $^{13}\text{C}$  assimilation in blanketflower from high-P sites could result in higher  $\delta^{13}\text{C}$  in 16 : 105 despite similar C allocation to AMF. If so, the favorable P-for-C exchange we observed in blanketflower would have been even more favorable. Similarly, the higher P availability in high-P grasslands might have diluted the  $^{32}\text{P}$  relative to  $^{31}\text{P}$ , but this would have resulted in a conservative estimate of P transfer in high-P soils. It is also possible that more  $^{32}\text{P}$  was adsorbed to soil surfaces in the low-P grasslands, but differences in P sorption are biologically relevant and can contribute to the disparities in AMF P acquisition and transfer we observed. Finally, because AMF can allocate resources within a common mycorrhizal network to optimize fitness (Lekberg *et al.*, 2010), the  $^{13}\text{C}$  measured in roots of individual plants was

not necessarily assimilated by that plant. However, because  $^{13}\text{C}$  assimilation,  $\delta^{13}\text{C}$  in 16 : 105, and shoot  $^{32}\text{P}$  concentrations correlated positively with each other, we likely did capture dynamics within individual plants.

In conclusion, our approach of combining dual isotopic labeling in similarly composed plant communities under contrasting soil conditions provides new insight into the factors controlling resource exchange in mycorrhizal symbioses.

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### Competing interests

None declared.

### Author contributions

YL and LA-W designed the study, sampled communities, and ran statistical analyses. YL maintained communities, labelled, harvested, and processed samples, and wrote the first draft. JJ quantified  $^{13}\text{C}$  in AMF-specific lipids. MED characterized and analyzed AMF communities. AS and MM analyzed soils for N and P concentrations. CZ ran soil phosphatase analyses. and DJ and WEH helped YL with  $^{32}\text{P}$  analyses. RTK, along with all the co-authors, edited the manuscript draft and provided critical input to interpret the data.

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## Data availability

Sequencing files for AMF communities are available at NCBI under the accession no. (PRJNA962124). Raw data required to repeat all analyses are submitted as [Supporting Information](#).

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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** Raw data required to repeat most analyses.

**Fig. S1** NMDS of arbuscular mycorrhiza fungi (AMF) communities in ingrowth cores and plant roots from communities collected in three high-P and three low-P sites harboring blanketflower and grasses, and the relative abundance of AMF families within each sample type across replicates.

**Fig. S2** Changes in P-for-C exchange between arbuscular mycorrhiza fungi (AMF) and blanketflower and AMF and grasses in communities collected in three low-P sites in North Dakota and three high-P sites in Montana with increasing shoot N : P.

**Fig. S3** Hyphal, vesicular, and arbuscular colonization in a subset of blanketflower and grass roots for which sufficient root material was left after fatty acid and molecular analyses.

**Fig. S4** Shoot N : P in blanketflower and grasses in communities collected in three high-P sites and three low-P sites, means  $\pm$  SE,  $n = 8$  per site.

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