Title: Agnostic fungi: plant traits and tissue stoichiometry explain nutrient transfer in common arbuscular mycorrhizal networks of temperate grasslands

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Abstract:

Plants and mycorrhizal fungi form close mutualistic relationships that affect the structure and function of ecosystems. Recent studies show how certain plant communities can form associations with the same mycorrhizal fungus leading to preferential transfer of carbon and limiting nutrients, such as nitrogen, between different species through common mycorrhizal networks. This phenomenon has been documented in a wide range of ecosystems but its mechanisms remain poorly understood. In an experimental prairie-pasture plant system, replicated at three sites across a 520 km latitudinal gradient in the Pacific Northwest, USA, we identified general processes that reconcile competing hypotheses related to resource transfer in common mycorrhizal networks. We used stable isotope tracers, paired with analyses of fungal DNA and plant and fungal community structure and traits, to quantify above- and belowground allocation and transfer of carbon and nitrogen between 18 different plant species. We applied isotopically enriched carbon (^{13}C) and nitrogen (^{15}N) as a pulse of carbon dioxide (CO_2) and ammonia (NH_3) to the leaves of target "donor" species common across experimental sites. At each site, we performed >1360 measurements of foliar and root isotopic enrichment, which revealed morphological traits and tissue stoichiometry as the most important predictors of interspecific resource transfer. Assimilation of isotopic tracers was similar between labeled

“donor” plants with no significant difference between sites for labeled “donor” plants with no significant difference in transfer between species among experimental treatments, including rainfall exclusion or species diversity. However, allocation of carbon and nitrogen to roots and transfer to “receiver” species varied significantly between functional groups (annual/perennial or grass/forb) due to differences in tissue stoichiometry. Our findings points to a simple mechanistic answer for long-standing questions regarding mutualism and transfer of resources between plants via mycorrhizal networks, an explanation that can lead to general predictions of preferential flow of limiting resources in other ecosystems.

Introduction

Plant-mycorrhizal associations are thought to have emerged as rudimentary root systems over 400 million years ago, facilitating the expansion of terrestrial life that followed (Kenrick & Strullu-Derrien, 2014). The transformative power of early fungal symbioses is still evident today in all major plant lineages, from bryophytes to angiosperms, all of which form mycorrhizal connections (van der Heijden et al. 2015). Over 85% of all contemporary flowering plant species form symbioses with fungi, with arbuscular mycorrhizal (AM) associations being the most common (Brundrett 2009). Today, the relationships between plants and AM fungi dominates managed and unmanaged landscapes and are estimated to be responsible for up to 80% of global primary productivity (van der Heijden et al. 2015). Fungi are gregarious and form partnerships with more than one individual plant (Selosse et al. 2006). By extension, it has been widely hypothesized that the multi-plant-fungal relationships form “common mycorrhizal networks” (CMNs) which facilitate carbon and nutrient transfer between organisms, beyond the immediate plant-fungus mutualism formed by individuals. This relationship is often simplified to the mutual exchange of carbon-based compounds created by plant photosynthesis for limiting soil nutrients mined by fungi (Smith and Read 2008). However, a synthesis of empirical research at macro- and micro-levels of ecological organization reveals a gap in understanding of structural and functional properties that could predict how carbon and nutrient are transferred in CMNs (Silva and Lambers 2021).

The literature holds myriad (often complimentary, but sometimes contradictory) hypotheses that could explain the CMN mutualism as a key structural and functional component of ecosystems. For example, the “Wood Wide Web” hypothesis emerged from the analysis of isotopically labeled carbon transferred between plants, presumably through fungal mycorrhizae (Simard et al. 1997). This observation set the foundation for economic analogies based on the idea that certain plants which allocate carbon to sustain common fungal symbionts can also benefit from shared nutrients, while plants associating with different mycorrhizal fungi cannot. The “economics” hypothesis (Kiers et al. 2011) proposes that plants and fungi engage in “trades” of nutrients mined by fungi in exchange for carbon-based photosynthates from plants (Fellbaum et al. 2014, Werner and Dubbert 2016, Averill et al. 2019). In this hypothesis, the terms of trade between plant and fungi are mediated by supply and demand for limiting resources, which could create a dynamic market emerging from interactions between environmental, biochemical, and

biophysical variables. Complementing the economic analogy, the “kinship” hypothesis proposes that phylogenetic distance within or across species promotes functional gradients and preferential flow of resources in CMNs (Tedersoo et al. 2020), in which transfer of carbon and nutrients are predicted to be more frequent and abundant between related individuals (e.g., seedlings and trees of the same species) than between unrelated individuals (Pickles et al. 2017).

The past two decades have seen extensive but inconclusive research on these hypotheses and how they relate to empirical measurements of CMN structure and function. There is a conspicuous ambiguity separating theory and empirical observations. On the one hand, economic analogies suggest that the reciprocally regulated exchange of resources between plants and fungi in CMNs should favor the most beneficial cooperative partnerships (Kiers et al. 2011, Fellbaum et al. 2014). On the other hand, reciprocal transfer is only found in a subset of symbionts under specific conditions, while amplified competition in CMNs is a more common observation (Walder and van der Heijden 2015, Weremijewicz et al. 2016). At the core of this controversy is the question of whether CMNs actively support fungal growth at the expense of plant resource demands (i.e., a fungi-centric view) or function as passive channels through which plants regulate resource fluxes (i.e., a plant-centric view). Data exist to support both of these opposing views (see Silva and Lambers 2021, Figueiredo et al. 2021); therefore we posit that CMNs are neither plant- nor fungi-centric. Perhaps more than gregarious, AM fungi might be “agnostic” with respect to plant species partnerships, which would imply that that the rates and direction of resource transfer in AM CMNs are ultimately a reflection of sink-source strength gradients.

To test the “agnostic fungi” hypothesis, we investigated how interactions between general biophysical and biogeochemical processes operating at the soil-plant-atmosphere interface could explain resource transfer in CMNs better than previous plant- or fungi-centric analogies. We studied how soil resource-use efficiency and plant traits that regulate physiological performance affect the transfer of carbon and nitrogen in paired experiments where we also measured fungal DNA, plant community structure, and plant traits to test the effects of relatedness in resource transfer. Our experimental setting includes high- and low-diversity prairie and pasture experimental sites under ambient conditions and rain exclusion shelters, designed to affect soil water and nutrient mass flow, replicated at three different sites across a 520 km latitudinal gradient. Due to the latitudinal and natural climatic difference in communities across sites, not all species were present at all sites (Table S1); however, all “donor” species and all “receiver”

functional groups were present at all sites. This allowed us to draw general inferences about the structure and function of AM CMNs in temperate grasslands under a broad range on ecological and environmental conditions.

Methods

We conducted our experiment at three sites situated on a 520 km latitudinal transect that spans cool, moist (northern site; Tenino, WA) to warm, moist (central site; Eugene, OR) to warm, dry (southern site; Selma, OR) climates. Half of our plots were manipulated to restore a high-diversity prairie community (n = 10 per site) while the other half of the plots were the existing introduced low-diversity pasture grass community (n = 10 per site). Manipulated high-diversity plots were mowed, raked, received herbicide, and seeded in 2014-2015, followed by seeding in fall 2015, 2016, and 2017 (Reed et al. 2019). We erected rainout shelters that excluded 40% of the rainfall on half the plots at each site (n = 10 rain exclusion, 10 control per site, except the central site where there were only 8 rain exclusion plots; Fig. 1).

Plots were either planted high diversity prairie communities or previously existing pasture communities, and all plants in our system have the potential to form associations with AM fungi. Half the plots were under rainout shelters that had little to no effect on aboveground community structure or function, where significant differences exist between plant functional groups with respect to ecophysiological performance and tissue stoichiometry (Dawson et al. *In Press*). We labeled perennial plants central to each plot (hereafter, 'donors') and monitored leaf ^{15}N and ^{13}C for the surrounding plants ('receivers') for up to 21 days. In addition, we sampled roots and soils at 21 days post-labelling. We analyzed fungal DNA collected from the root samples, as well as stable isotopes in all samples. Our experimental design was nested in a multi-year experiment where in situ data loggers were used to continuously measure variables in all the manipulated plots. We calculated soil matric potentials to account for soil differences between sites (Saxton and Rawls 2006). Rain exclusion only changed soil temperature at the northern site (Fig. 1c) and soil matric potential at the central site (Fig. 1d).

This network of sites has been established since 2010 and has been extensively studied since then (Reed et al. 2019, 2021a, 2021b, 2021c, Peterson et al. 2020) including work on mycorrhizal fungi (Wilson et al. 2016). Previous results show that the rain exclusion treatment significantly changed soil water potential, which should also affect nutrient uptake through mass

flow. At each site, pasture plots that have not been seeded by additional species adjoin the high diversity plots. These are dominated by one to a few species of introduced perennial grasses which we targeted as donors in pasture plots. For high diversity plots, we targeted native perennial species that are common to ambient and drought treatments at all sites, and that have shown divergent changes in productivity in response to a warming-induced decline in mycorrhizal colonization attributed to that treatment's drying effect (Wilson et al. 2016). Despite those differences, we did not find significant changes in the average plant community composition or productivity under rain exclusion, which also did not affect morphological and functional traits (e.g. specific leaf area, iWUE, and C:N ratios) of the functional groups we selected for this experiment (Dawson in press) (Reed et al. 2021c). Therefore, this experimental setting is ideal to test general hypotheses related to how plant-fungal relationships mediate carbon and nutrient dynamics in CMNs under different ecological and environmental conditions.

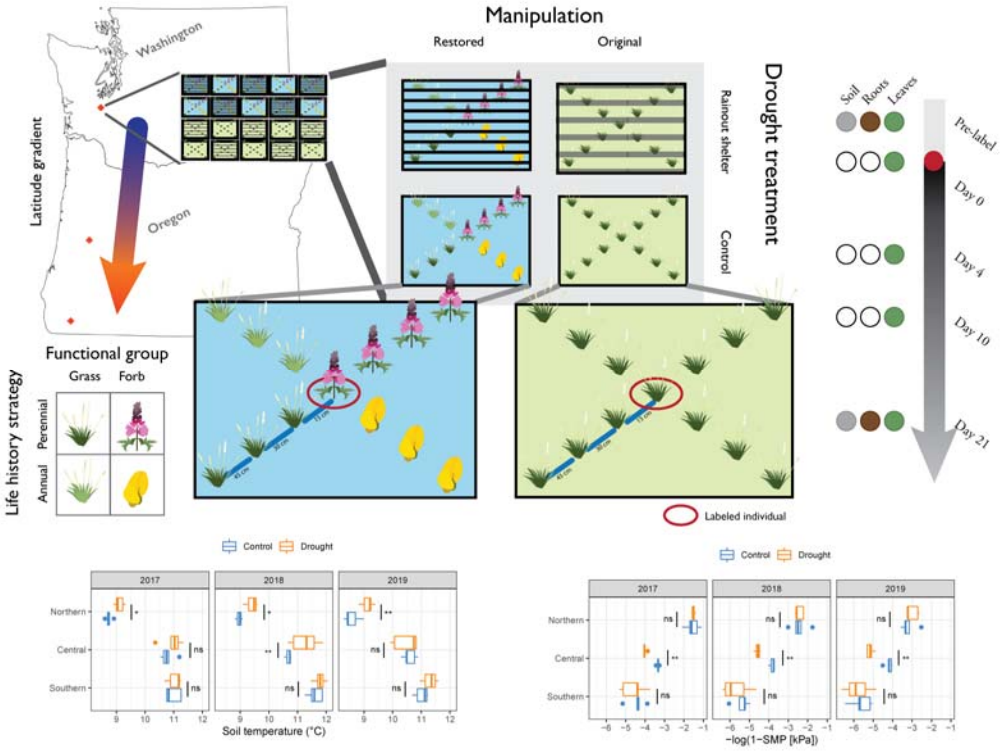

Figure 1. Schema of experimental set up, sampling, and effects of rainout shelters. Effects of rain exclusion treatment are shown on soil matric potential and soil temperature at each site for each growing season. Growing season is defined as 1 Oct to 30 Jun and each season is labeled by the spring months of the growing year (e.g. 2018 is 1 Oct 2017 to 30 Jun 2018).

Isotopic labelling

On a sunny day between 11AM and 3PM, we applied isotopically enriched carbon (^{13}C) and nitrogen (^{15}N) as a pulse of carbon dioxide (CO_2) and ammonia (NH_3) to the leaves of target "donor" species common across experimental sites. At each site, we selected a healthy perennial forb (*Sidalcea malviflora* in diverse prairie plots [except in one plot where we used *Eriophyllum lanatum* due to a lack of *S. malviflora*], *Alopecurus pratensis, Schedonorus arundinaceus,* or *Agrostis capillaris* in low diversity pasture plots) at the center of each plot to receive the isotopic labels. We performed the labeling experiment using custom-made field-deployable clear chambers with internal fans built to allow gas mixing and fast in situ assimilation of isotopic labels, following established protocols developed in previous isotopically labeling studies (Silva et al. 2015, e.g., Earles et al. 2016, Sperling et al. 2017). We covered the donor plant with a clear plastic cylinder and injected gas in sequence at 20 minute intervals. For $^{13}CO_2$, we made three injections of 2 mL pure CO_2 (enriched at 98 atm % ^{13}C) to double the amount of CO2 in the chamber each time. For NH3, we made two injections of 10 mL pure NH3 (98 atm% 15N). The dates of application were based on peak NDVI at each site (Reed et al. 2019). We sampled leaves from each donor plant immediately after labeling (time point 0) as well as from all plants approximately 4 days (time point 1), 10 days (time point 2), and 21 days (time point 3) post-labelling as logistics permitted (Fig 1, Table S2). By applying enriched gases only to the aerial portions of established perennial donor plants we introduced the isotopic tracer to both the plant and mycorrhizal fungal tissues without exposing the soil to the tracers. At the end of the experiment we harvested entire plants and surrounding rhizospheres at time point 3 and kept them in cool conditions until processing. We separated the roots and rhizospheres, and collected roots from each plant, selecting approximately ten ~3cm fine root fragments per sample (i.e. third order or finer, where available) for DNA extraction. All roots and rhizosphere soils were stored at -80º C until processing.

Baseline and Resource Transfer Calculations

Before isotopic labeling, we collected soil, leaf, and roots from each site. We collected soils in late spring and early summer 2019 to a 20 cm depth in each plot. From these soil samples, we removed root fragments that represented the typical roots seen in each plot. We

collected leaves for each species in each plot; however, these leaves were contaminated with ^{15}N during transport. To replace these, we separately sampled leaves from biomass samplings performed at the same plots, ensuring that annual and perennial grasses and forbs were represented at each site. The biomass samples were collected in late spring and early summer 2019. Baseline ^{13}C values were calculated from the original samples because the contamination only occurred with ^{15}N.

We oven-dried all samples at 65° C to constant mass and encapsulated them for stable isotope analysis. All stable isotope analysis was done at UC Davis Stable Isotope Facilities using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). We calculated the amount of carbon and nitrogen in each plant compartment (leaves and roots) using standard label recovery equations (Silva et al 2015), using baseline values measured before application of the labelled gases to capture background variations in isotopic composition of unenriched leaves, roots, and soil samples

At each relevant point in space and time, we calculated % nitrogen and % carbon derived from label (%NDFL and %CDFL, respectively) as follows (He et al. 2006, Kramer et al. 2002, Silva et al. 2015 Nature).

Equation 1

$$\frac{atm\%_{post-label} - atm\%_{baseline}}{atm\%_{labelling\ gas}}$$

Baseline values were calculated from the site- and perenniality-specific values for each plant material. Site-specific baseline soil and root isotope ratios represent the whole community because of interconnected rhizospheres where is it is not possible to identify specific species. In all cases, baseline values fell within the expected range for our region (Fig 2).

We used the baseline ^{13}C values to calculate intrinsic water-use efficiency using the method of Dawson et al. (*in press*).

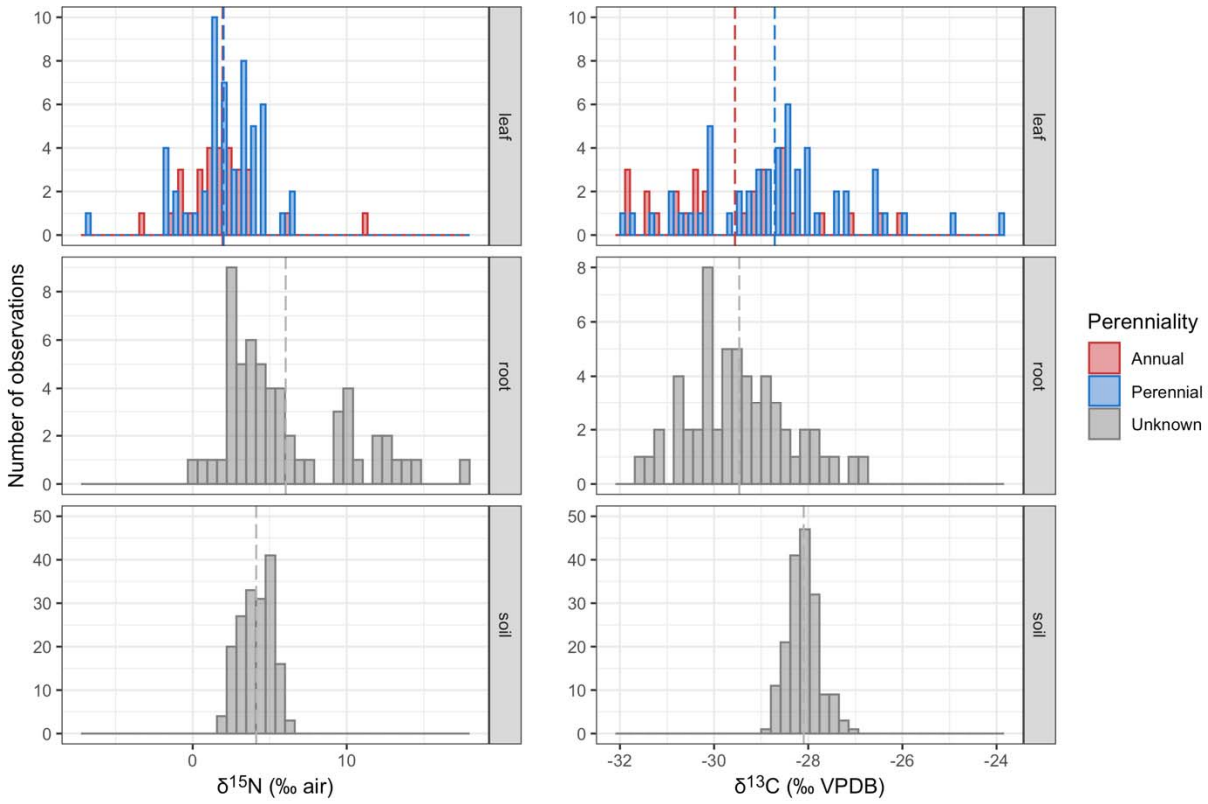

Figure 2. Natural abundance of stable isotopes in leaves, roots, and soil before labelling. Dashed lines indicate mean value.

To better understand post-labelling soil enrichment, we selected a subset of rhizosphere soils that represented six donor plants at each site divided equally between high and low diversity plots, and selected the three most highly ^{15}N enriched interspecific receivers in each plot. In addition, we sampled three most highly enriched interspecific receivers at each site and diversity combination (if they had not been sampled previously). Because pasture plots were sometimes monodominant grasses, we sampled the top three enriched intraspecific receivers at each site and treatment. In total, this came to 48 post-labelling soil samples in 29 plots.

Fungal analysis

We extracted DNA from roots of 450 plants harvested at time point 3 using Qiagen DNeasy Powersoil HTP kits (Qiagen, Hilden, Germany). We only analyzed DNA from roots, not from the soils collected from each plant's rhizosphere. We characterized each sample's AM fungal composition with a two-step PCR protocol that amplified a ~550bp fragment of the SSU

rRNA gene (the most well-supported region for AM fungal taxonomic resolution (Dumbrell et al. 2011)). We used WANDA (5'- CAGCCGCGGTAATTCCAGCT- 3') and AML2 (5'-GAACCCAAACACTTTGGTTTCC-3') primers (Lee et al. 2008, Langmead and Salzberg 2012). We used primers with unique indices so we could multiplex several projects on a single run. We quantified successful PCR amplicons with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Waltham, MA, USA) on a SpectraMax M5E Microplate Reader (Molecular Devices, San Jose, CA, USA) before purifying with QIAquick PCR Purification kits (Qiagen). We sequenced the purified pools on the Illumina MiSeq platform (paired-end 300bp, Illumina Inc., San Diego, CA, USA) at the University of Oregon Genomics and Cell Characterization Core Facility (Eugene, OR, USA). We used a custom, in-house bioinformatics pipeline to demultiplex reads and filter out duplicate reads generated by PCR duplication using unique molecular identifiers (UMIs) inserted during PCR processing.

We assigned amplicon sequence variants (ASVs) using the dada2 pipeline with standard quality filtering and denoising parameters (Callahan et al. 2016). The dada2 pipeline maintains strain-level diversity at the scale of individual sequence variants rather than clustering sequences into OTUs. This fine-scale measure of fungal sequence diversity was particularly important for our analyses to maintain the greatest chance of detecting a single AM fungal 'individual' in multiple plant root samples. Taxonomy was assigned to ASVs using the MaarjAM database (Öpik et al. 2010). We used a Bayesian mixture model to scale ASV counts within and across samples to avoid artificial taxon abundance biases (Anders and Huber 2010).

Data analysis

We performed all analyses in R ver. 4.0.4 (R Core team 2015). We tested the relationship between NDFL and plant traits and site conditions with a mixed-effect ANOVA. We constructed a phyloseq object (McMurdie and Holmes 2013) and used iGraph, metagMisc, DESeq2, and RCy3 (Nepusz and Csardi 2006, Love et al. 2014, Mikryukov 2017, Gustavsen et al. 2019) to create networks for each plot. Nodes represented individual plants and edges between nodes showed where plants shared at least one fungal partner. We weighted edges based on how many fungal ASVs were shared between plants. We used tidygraph (Petersen 2022) to calculate degrees of connectivity, or how many plants each individual plant was connected to in each plot (Fig. 3). We also calculated whether each receiver shared fungal ASVs with the central donor

plant. We visualized individual plot networks in CytoScape. We created a non-metric multi-dimensional scaling (NMDS) plot using the vegan package demonstrating the mycorrhizal fungal community similarity between plants (Oksanen et al. 2022).

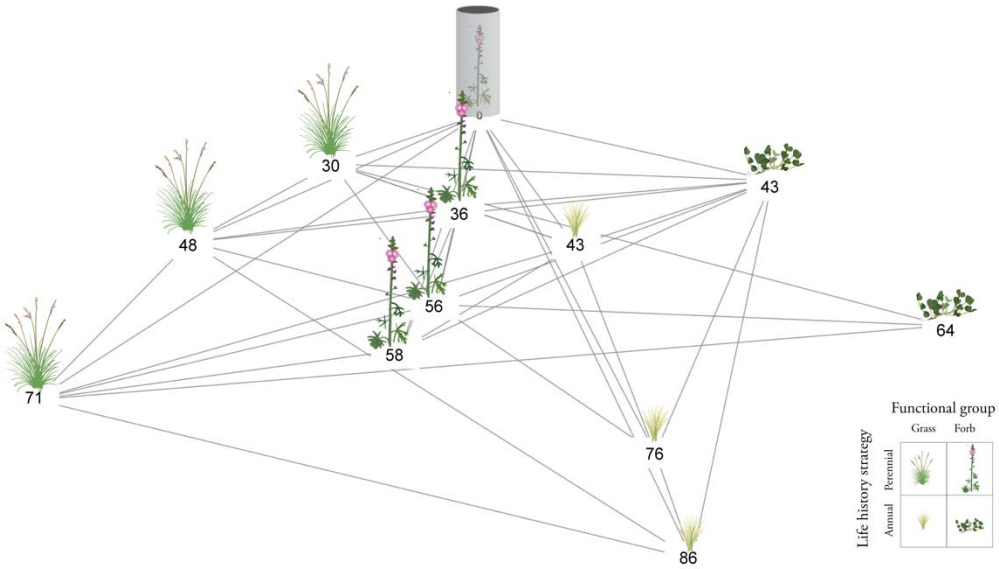

Figure 3. Example of how networks were constructed for each plot. The grey cylinder indicates the donor plant for the plot. Numbers beneath the receivers are the distance (in centimeters) from the donor. Degrees were calculated as how many plants each individual plant was connected to; for example, the perennial grass at 71 cm has 7 degrees.

Results

Assimilation of isotopic tracers was similar between labeled "donor" plants with no significant differences on average between sites or experimental treatments within sites, including rainfall exclusion or species diversity (Fig S1). At all sites, foliar assimilation of ^{15}N and ^{13}C by donor plants led to enrichment levels ranging from approximately 5-fold to one order of magnitude higher than baselines, with only a few that were less than 2-fold from baseline (two donor individuals *of S. malviflora* at the northern site and two donor individuals of *S. arundinaceus* at the southern site). Foliar enrichment levels decreased consistently at all sites and treatments over the twenty-one day sampling period, suggesting translocation within plants and interspecific transfer between species. We found significant spatial and temporal differences in foliar and root isotope ratios in donors and receivers resulting from interspecific transfer of

carbon and nitrogen (Fig 4; Table 1). Receiver foliar enrichment levels did not correlate with donor foliar enrichment levels within the same plot (Fig S2).

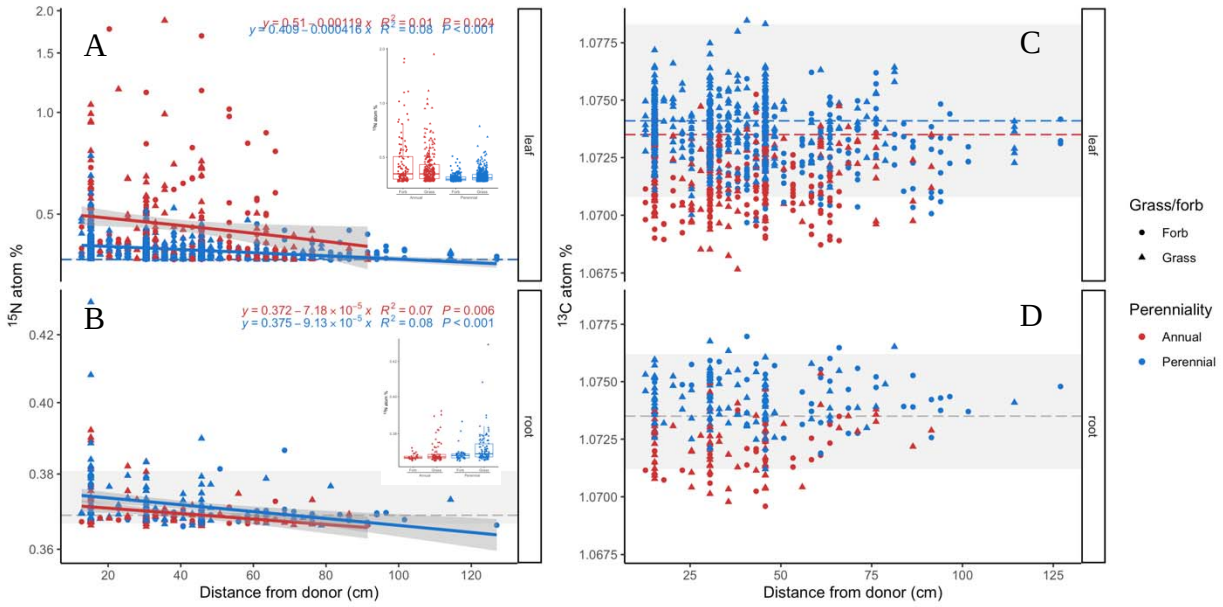

Figure 4. Decreased receiver enrichment with distance from donor in leaves and roots. Dashed lines indicate natural abundance means; grey boxes indicate range of natural abundance variation shown in figure 2. We interpret this as no systematic enrichment of 13C; however, there is high 15N enrichment in leaves. Y-axes are log10 scale. Note that the y-axis scales are different between 15N leaves and 15N roots. Inset box shows distribution of 15N atm% for enriched (greater than natural abundance) annual and perennial leaves and roots.

Table 1. ANOVA results effects on nitrogen derived from label (NDFL)

	χ^2	**P-value**
Fixed effects		
Perenniality	32.095	**<0.001**
Grass/forb	18.673	**<0.001**
iWUE	0.010	0.921
Degree of connectivity	1.149	0.284
C:N	46.115	**<0.001**
Site	0.360	0.548
Drought treatment	0.534	0.465
Diversity treatment	2.802	0.094
Functional diversity	1.713	0.191
Distance from donor	20.965	**<0.001**
Time from labelling	82.693	**<0.001**
Random effect		
Plot		**<0.001**

Allocation of ^{13}C and ^{15}N tracers to roots and subsequent transfer to "receiver" species did not follow expected patterns (e.g., despite donors being perennial, annual receivers were more enriched, indicating that relatedness did not drive the transfer, Fig. 4, Table 1) but varied significantly between functional groups due to their intrinsic differences in physiological traits and tissue stoichiometry (Table 1). We selected 18 representative annual/perennial and grass/forb species of receiver plants, which revealed significant differences between functional groups for NDFL (Fig 5) but no detectable CDFL relative to baseline (Fig. 4).

Rain exclusion treatment, plot diversity treatment, and site did not affect interspecific transfer of carbon or nitrogen (Fig S; ANOVA, $P > 0.05$, table 1). We observed significant

differences in nitrogen transfer by functional group, mirroring intrinsic differences in tissue stoichiometry and iWUE, despite no significant enrichment in soils collected from the rhizosphere of those same plants (fig S4). We did, however, detect a low level of soil enrichment in 4 out of 23 donor soil samples (ranging from 0.382 to 0.479 atm% 15N). Annuals had greater ^{15}N foliar enrichment compared to perennials (ANOVA, $P < 0.001$, table 1, fig 4 insets). Foliar enrichment decreased over both time and space (ANOVA $P < 0.001$). On average, annuals had a lower nitrogen content and higher C:N than perennials (table 1). Annuals had an average of 0.16% ±0.23 SD NDFL at the first sampling; they ranged from 0 to 1.53%. Perennials had an average of 0.04% ±0.03 NDFL at the first sampling; they ranged from 0.01 to 0.15%. C:N affected NDFL (table 1), but NDFL did not correlate with either one (Fig. 5).

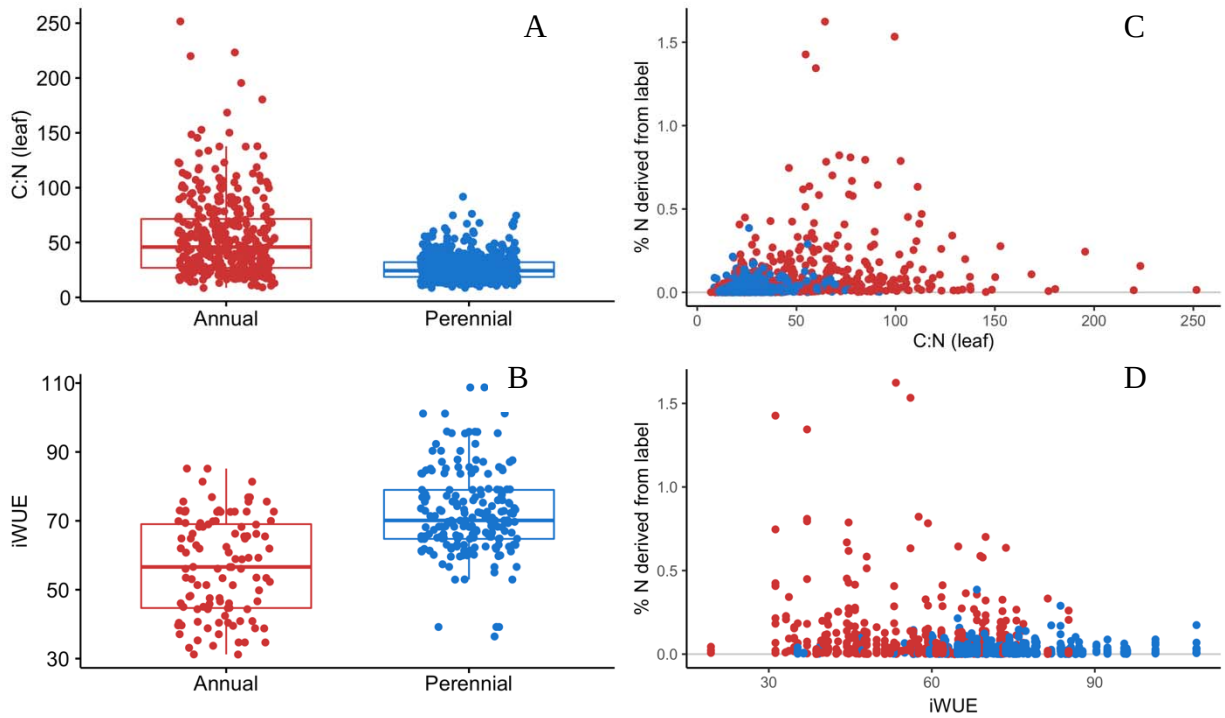

Figure 5. Nitrogen content by perenniality and intrinsic water-use efficiency. A) Leaf C:N by perenniality. B) iWUE by perenniality as measured before labelling. C) Percent ^{15}N derived from label (DFL) compared to C:N in leaves. D) Percent ^{15}N DFL compared to iWUE in leaves at all time points. Grey line indicates 0%.

Our analysis of fungal community composition shows a high degree of connectivity between plants of different species but no obvious pattern of connectedness that could explain preferential nutrient transfer by functional groups. Overall, plants in all experimental plots and sites were highly connected. We infer this from fungal communities within the roots of each donor and receiver plant where we found that 97.25 % ± 8.01 (SD) of all plants within each experimental plot shared at least one fungal DNA sequence variant (ASV) with another plant of the same plot. All plants shared at least one ASV with the plot donor plant, with fungal communities being apparently agnostic with respect to functional groups (Fig 6). Annual plants shared fungi with more plants in the same plot (4.74 plants ± 2.62 SD) compared to perennials (4.04 plants ± 2.49 SD ; t-test, P = 0.019; table S3), but degrees of connectivity did not predict transfer (table 1).

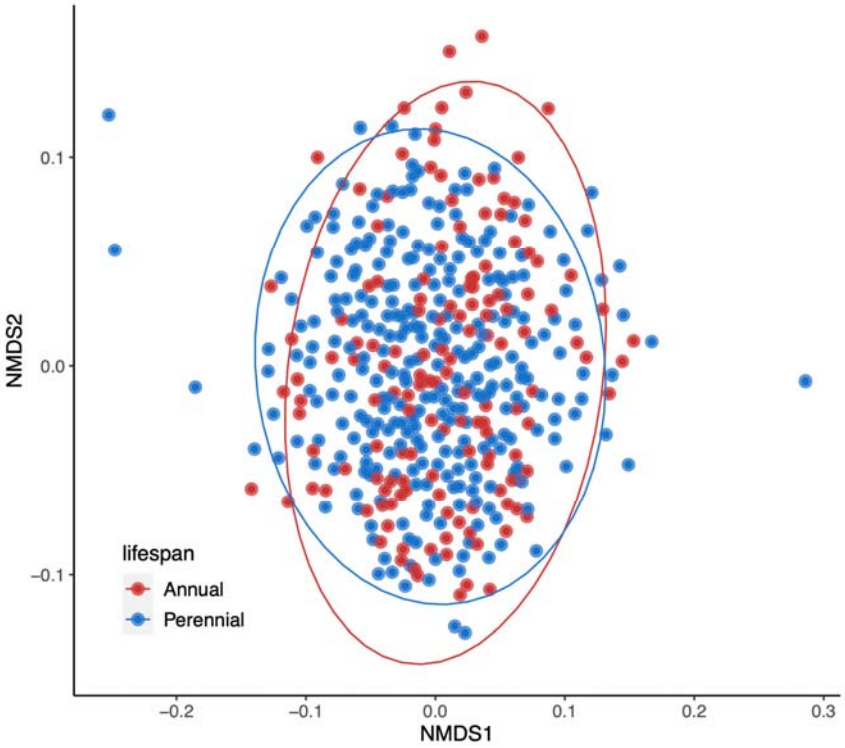

Figure 6. NMDS of fungal communities. Each point is an individual plant ordinated by its fungal community.

Discussion

We designed this experiment to be informed by previous hypotheses on how carbon and nitrogen transfer occurs in highly connected CMNs of the Pacific Northwest. We conducted

repeated spatiotemporal sampling of isotopic enrichment levels at increasing distances from donor species, days to weeks after labelling, and in well-established communities exposed to multiple years of experimental treatments, expecting to find evidence of kinship (i.e., greatest resource transfer in related plants), driven by CMN economics (i.e., ^{15}N transfer rates coinciding with ^{13}C investment in root and fungal mass). Our data do not support either hypothesis and instead suggest AM fungi are "agnostic" with respect to plant species partnerships, forming CMNs where the rates and direction of resource transfer ultimately reflects a sink-source strength in ecophysiological performance and stoichiometric gradients. We found a ^{15}N signature in leaves that was especially strong in annual receivers, indicating preferential transfer of limiting resources to that functional group. However, we did not find ^{13}C enrichment in most samples, above- or belowground, and both ^{15}N and ^{13}C signatures of receiver plants were independent of donor or soil enrichment levels.

Our dual-isotope (^{13}C and ^{15}N) labelling approach was imposed upon an existing climate experiment to investigate in the terms of trade between carbon and nitrogen from assimilation to translocation among plant compartments and fungal partners. Our results are consistent with earlier experiments performed in greenhouse experiments under low stress (e.g., temperature controlled and irrigated twice daily) (Silva et al. 2015). However, post-assimilation translocation did not support previous data. Plants acquire CO_2 and NH_3 from the atmosphere and release those same gases into their surroundings, exhibiting a foliar compensation point at which the evolution of gases is equal to assimilation (Farquhar et al. 1980). This compensation point depends on the partial pressure of CO_2 or NH_3 in the mesophyll, and therefore on its partial pressure in the atmosphere, with an increase in leaf uptake expected for both gases as their concentration rises. Our pulse experiment worked for both gases, causing lasting enrichment levels in the leaves of doner species and, over time, in the leaves of receiver individuals of other species, suggesting translocation and transfer (Fig 4 and S1). This result represents an integrated measure of the total foliar assimilation of NH_3 and CO_2 after a single pulse of isotopically enriched gases.

Nitrogen enrichment levels remained high in leaves and many roots at the end of the experiment, allowing us to measure NDFL across the community and infer the main drivers of N transfer. However, carbon enrichment levels faded before plants were harvested approximately 21 days post-labelling, either due to losses via respiration or redistribution in CMNs where it

became undetectable in all but a few cases. After controlling for variation in assimilation rates, we found that annual plants received greater ^{15}N enrichment than perennial plants. Plants closest to the donor were most enriched, and ^{15}N enrichment decreased over time (fig 4). We did not detect ^{13}C enrichment in roots, shoots, or soils. Annuals had lower total nitrogen content than perennials, mirroring intrinsic differences in stoichiometry and water-use efficiency (although with no significant correlation found; Fig 5), indicating that kinship did not drive this transfer as all donor plants were perennials. Although this experiment was conducted along a latitudinal climate gradient with rainout shelters, which affected soil water content (fig 1), neither treatment nor site affected our results.

The major drivers of differences in allocation of ^{13}C and ^{15}N to roots and subsequent transfer to "receiver" species were the intrinsic difference in physiological traits and tissue stoichiometry between functional groups (annual/perennial or grass/forb; table 1). This result points to a simple mechanistic explanation to reconcile long-standing questions regarding mutualism and hierarchical transfer of resources between plants via mycorrhizal networks, an explanation that can lead to general predictions of preferential flow of limiting resources in other ecosystems. For example, previous studies in northern California under environmental conditions similar to those found in our southernmost experimental site showed rapid (days to weeks) transfer of ^{15}N applied to the leaves of EM pines to surrounding annual AM plant receivers (He et al. 2006). Those results were interpreted as evidence for agnostic CMNs because "direct fungal connections are not necessary for N transfer among plants" and "leaves of the annual plants had greater ^{15}N derived from source (NDFS) and were more enriched (^{15}N at % excess and $\delta^{15}N$ values) than perennial receivers, irrespective of the mycorrhizal type". Similarly, as proposed by He et al. (2006), our observation of agnostic ^{15}N transfer from donor to receivers suggests that annual plants, with their extensive root systems, were a strong sink for N which could be explained by stoichiometric gradients that affect root exudation and recapture of N-containing materials from rhizodeposition. Our data corroborate rapid agnostic transfer among AM plants, with no detectable enrichment in root or soil ^{13}C near roots 21 days post-labelling, but do not allow us to determine which mechanism is responsible for the ^{15}N transfers.

We found a significant degree of connectivity between plants within each given treatment and site, with a near complete overlap in their shared CMN communities (Fig 6). This overlap could explain the lack of support for the kinship hypothesis in our dataset and offers further

support for stoichiometric gradients as the fundamental control of terms of trade in CMNs. The marketplace hypothesis proposes that host plants provide the mycorrhizal fungus with carbon in the form of simple sugars. The rate which fungi take up these sugars is controlled by their affinity to the host. Different C sources from a host trigger changes in fungal monosaccharide transporter gene expression, causing fungi to uptake N from the soil (Fellbaum et al. 2012). However, host and fungus-specific gene expression differences would be weak in our study sites because our DNA results demonstrate that our plants were highly connected by shared arbuscular mycorrhizal (AM) fungal ASVs. In our case, the ^{15}N we applied to leaves could have mineralized, been recycled at the soil/fungal interface, and converted into basic organic compounds such as amino acids (e.g. via glutamine synthetase or argininosuccinate synthase) which act as charge balance in the catabolic arm of the urea cycle. The fact that we supplied a carbon source (acetate) independent from the C supply of the host could have reduced N transport in the AM symbiosis. Apoplastic transport would then explain how some of the labeled C ended up in plants even though most of it is expected to go to the fungal symbionts. This mechanism also explains how N and mineral elements (e.g. P) are transferred through CMNs from plant to plant even though they are not expected to limit fungal growth (Ward et al. 2022).

Prior work suggests that plant-to-plant transfer of nitrogen via fungi is unlikely. More likely the nitrogen label “leaked” into the soil where fungi harvested it and transferred it to another, connected, plant (Ingraffia et al. 2021). Regardless, greater fungal connectivity increases nitrogen transfer (He et al. 2009, Ingraffia et al. 2021). This suggests that the nitrogen transfer we observed in our system was mediated by fungi. However, because we had few plants that were disconnected from the network, we cannot say with certainty that connectivity is necessary for this transfer.

In conclusion, we found that plant-soil stoichiometric gradients and functional traits were the strongest drivers of resource sharing in grassland CMNs. We interpret this finding as evidence of biophysical and biophysical sinks, in which nutrients are allocated to plants with the greatest need for those nutrients, either through a ‘passive’ mycorrhizal network or direct uptake from soil, or that nutrients should be allocated through water flow. Expanding on previous studies, we propose that agnostic fungi facilitate spatiotemporal dynamics of carbon and nitrogen through CMNs in was that are neither plant- nor fungi-centric. That is, plants and fungi that are

located closer together in space and with stronger demand for resources over time are more likely to receive larger amounts of those limiting resources.

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