

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

**Authors:** Hilary Rose Dawson, Katherine L. Shek, Toby M. Maxwell, Paul B. Reed, Barbara Bomfim, Scott D. Bridgham, Brendan J. R. Bohannon, Lucas C.R. Silva

## Supplemental Information

**Table S1. List of species at each site with number of leaf samples per species.** Donor species are indicated in bold.

Species	Annual/ perennial	Grass/forb	WC	TW	DC	Fungal symbiont
<i>Agrostis capillaris</i> L.	Perennial	Grass	121	--	--	AM <sup>1</sup>
<i>Alopecurus pratensis</i> L.	Perennial	Grass	--	210	--	AM <sup>1</sup>
<i>Aphanes occidentalis</i> L.	Annual	Forb	3	--	--	AM* <sup>2</sup>
<i>Bromus diandrus</i> Roth	Annual	Grass	49	--	--	AM* <sup>1</sup>
<i>Bromus hordeaceus</i> L.	Annual	Grass	35	42	96	AM* <sup>1</sup>
<i>Elymus repens</i> (L.) Gould	Perennial	Grass	--	--	63	AM* <sup>1</sup>
<i>Eriophyllum lanatum</i> (Pursh) Forbes	Perennial	Forb	8	--	--	AM <sup>2</sup>
<i>Festuca idahoensis</i> Elmer ssp. <i>roemeri</i> (Pavlick) S. Aiken	Perennial	Grass	18	--	--	AM* <sup>1</sup>
<i>Geranium dissectum</i> L.	Annual	Forb	15	81	--	AM <sup>2</sup>
<i>Holcus lanatus</i> L.	Perennial	Grass	--	6	--	AM <sup>1</sup>
<i>Koeleria macrantha</i> (Ledeb.) Schult.	Perennial	Grass	18	--	--	AM <sup>2</sup>
<i>Lotus corniculatus</i> L.	Perennial	Forb	--	9	--	AM <sup>1</sup>
<b><i>Schedonorus arundinaceus</i> (Schreb.) Dumort.</b>	Perennial	Grass	--	--	130	AM <sup>1</sup>
<b><i>Sidalcea malviflora</i> ssp. <i>virgata</i> (DC.) A. Gray ex Benth.</b>	Perennial	Forb	66	129	119	AM <sup>3</sup>
<i>Trifolium subterraneum</i> L.	Annual	Forb	48	--	--	AM <sup>1</sup>
<i>Veronica arvensis</i> L.	Annual	Forb	3	--	--	AM <sup>1</sup>
<i>Vicia sativa</i> L.	Annual	Forb	--	14	--	AM* <sup>1</sup>
<i>Vulpia</i> spp.	Annual	Grass	54	8	21	AM <sup>1</sup>
		<b>Total samples</b>	438	499	429	

Notes: Fungal symbiont data derived from <sup>1</sup>Chaudhary et al. (2016), <sup>2</sup>Soudzilovskaia et al. (2020), and <sup>3</sup>Dickie et al. (2013). \* indicates “to genus,” that strategy was extrapolated from a sister species given a lack of data on this species. Nutrient-use strategies include arbuscular mycorrhizal (AM) and nitrogen fixation (N-fixer).

**Table S2. Collection dates for each time point at each site.** Days since labeling given in parentheses after date.

Site	Label application (time point 0)	Time point 1	Time point 2	Time point 3
<i>Ideal days since labeling</i>	0	4	10	21
<b>Northern</b>	June 10	June 14 (4)	June 20 (10)	July 2 (22)
<b>Middle</b>	May 29	June 3 (5)	June 11 (13)	June 18 (20)
<b>Southern</b>	May 9	May 13 (4)	May 22 (9)	May 30 (21)

**Table S3. Baseline values used to calculate amount derived from label (DFL).**

Site	Drought treatment	Roots				Leaves					
		<i>N</i> atm%	±SD	<i>C</i> atm%	±SD	Annual/perennial	Grass/forb	<i>N</i> atm%	±SD	<i>C</i> atm%	±SD
North	Control	0.370	0.001	1.073	0.001	Annual	Forb	0.367	0.001	1.07	0.001
							Grass	0.367	0.000	1.071	0.003
						Perennial	Forb	0.367	0.000	1.072	0.001
							Grass	0.368	0.000	1.075	0.001
	Rain exclusion treatment	0.370	0.001	1.073	0.001	Annual	Forb	0.367	0.001	1.072	0.001
							Grass	0.367	0.000	1.073	0.000
						Perennial	Forb	0.367	0.000	1.072	0.002
							Grass	0.368	0.000	1.075	0.001
Central	Control	0.368	0.001	1.073	0.001	Annual	Forb	0.367	0.001	1.071	0.001
							Grass	0.366	0.001	1.073	0.001
						Perennial	Forb	0.368	0.003	1.071	0.001
							Grass	0.367	0.003	1.074	0.002
	Rain exclusion treatment	0.368	0.001	1.073	0.001	Annual	Forb	0.367	0.001	1.071	0.001
							Grass	0.367	0.001	1.072	0.001
						Perennial	Forb	0.366	0.000	1.071	0.001
							Grass	0.366	0.000	1.075	0.002
South	Control	0.368	0.000	1.074	0.001	Annual	Forb	0.366	0.000	1.071	0.000
							Grass	0.368	0.001	1.074	0.001
						Perennial	Forb	0.367	0.000	1.074	0.001
							Grass	0.367	0.000	1.074	0.000
	Rain exclusion treatment	0.368	0.001	1.074	0.001	Annual	Forb	0.366	0.000	1.071	0.000
							Grass	0.367	0.001	1.074	0.002
						Perennial	Forb	0.367	0.001	1.074	0.001
							Grass	0.367	0.000	1.074	0.001

**Table S4. ANOVA results effects on leaf carbon derived from label (%CDFL).** Only receiver leaves enriched with <sup>13</sup>C with associated DNA data were included in the analysis (n = 92). Leaf %NDFL results available in Table 1.

	DF	$\chi^2$	P-value
<i>Fixed effects</i>			
<b>Annual/perennial</b>	<b>1</b>	22.91	<b>&lt;0.001</b>
<b>Grass/forb</b>	1	12.59	<b>&lt;0.001</b>
<b>Same species as donor</b>	1	11.76	<b>0.001</b>
Degree of connectivity	1	1.77	0.183
iWUE	1	0.18	0.674
C:N	1	2.30	0.130
<b>Site</b>	<b>2</b>	6.32	<b>0.043</b>
Drought treatment	1	1.27	0.260
Restoration treatment	1	2.80	0.094
Distance from donor	1	0.00	0.958
Time from labelling	1	1.38	0.241
<b>Annual/perennial: Grass/forb interaction</b>	NA	NA	NA
<i>Random effect</i>			
<b>Plot</b>			<b>&lt;0.001</b>

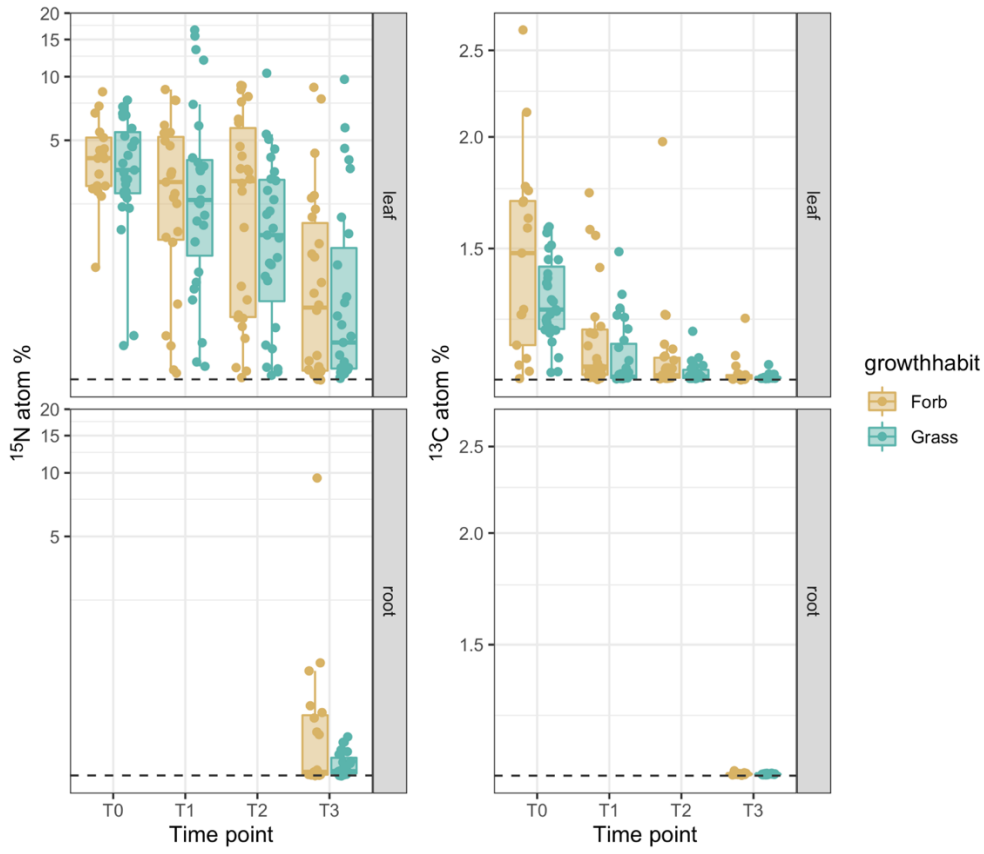
**Table S5. List of fungal ASVs by annuals and perennials.**

<b>Fungal taxon</b>	<b>No. of perennial plants assoc. with</b>	<b>No. of annual plants assoc. with</b>
<i>Acaulospora sp877</i>	13	0
<i>Claroideoglossum sp744</i>	20	7
<i>Claroideoglossum sp745</i>	18	5
<i>Claroideoglossum sp746</i>	18	7
<i>Claroideoglossum sp749</i>	14	4
<i>Claroideoglossum sp751</i>	25	10
<i>Claroideoglossum sp757</i>	11	1
<i>Claroideoglossum sp758</i>	11	1
<i>Claroideoglossum sp781</i>	18	13
<i>Claroideoglossum sp782</i>	59	36
<i>Claroideoglossum sp783</i>	38	20
<i>Claroideoglossum sp784</i>	47	21
<i>Claroideoglossum sp811</i>	19	9
<i>Claroideoglossum sp812</i>	12	4
<i>Claroideoglossum sp813</i>	15	10
<i>Claroideoglossum sp815</i>	21	11
<i>Claroideoglossum sp816</i>	19	7
<i>Claroideoglossum sp817</i>	30	8
<i>Glomus sp1024</i>	11	1
<i>Glomus sp1025</i>	12	4
<i>Glomus sp1029</i>	23	4
<i>Glomus sp1031</i>	20	7
<i>Glomus sp104</i>	12	3
<i>Glomus sp113</i>	23	10
<i>Glomus sp114</i>	6	16
<i>Glomus sp115</i>	21	15
<i>Glomus sp1349</i>	13	8
<i>Glomus sp1351</i>	18	8
<i>Glomus sp1352</i>	15	5
<i>Glomus sp148</i>	22	11

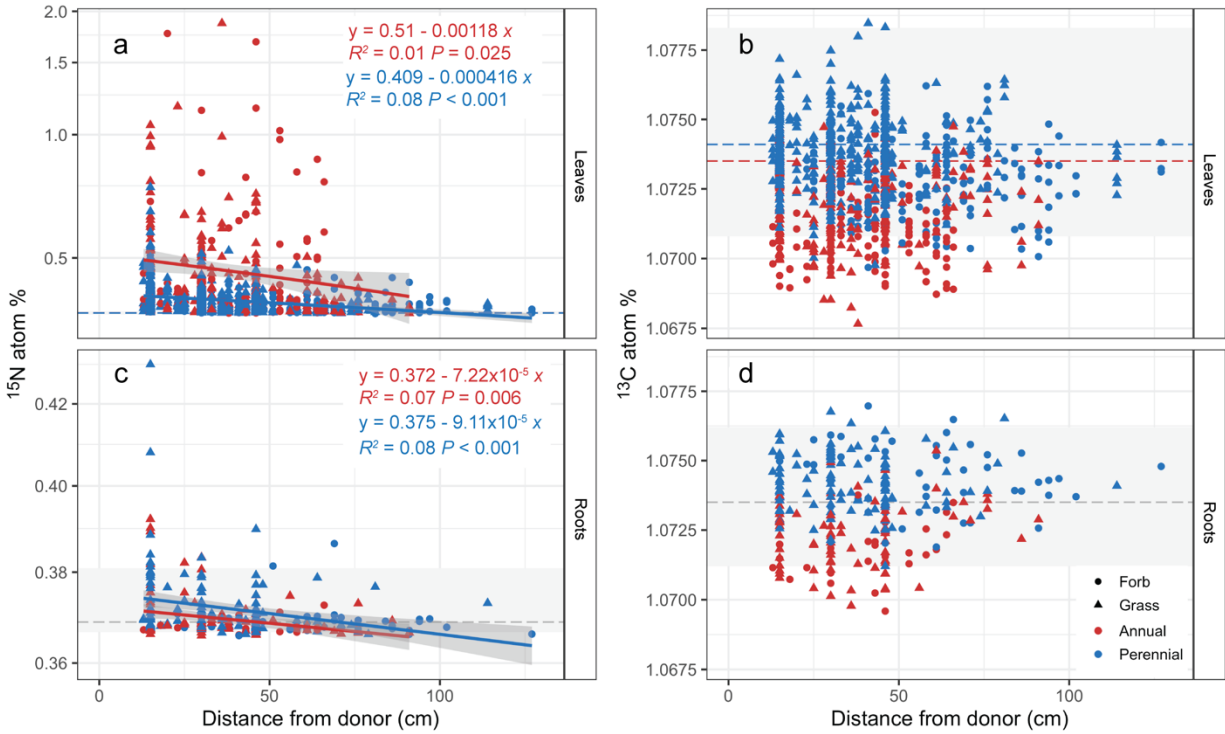
<b>Fungal taxon</b>	<b>No. of perennial plants assoc. with</b>	<b>No. of annual plants assoc. with</b>
<i>Glomus sp149</i>	12	6
<i>Glomus sp150</i>	27	14
<i>Glomus sp168</i>	21	12
<i>Glomus sp169</i>	13	6
<i>Glomus sp170</i>	15	6
<i>Glomus sp177</i>	11	1
<i>Glomus sp191</i>	14	10
<i>Glomus sp470</i>	15	6
<i>Glomus sp472</i>	14	4
<i>Glomus sp488</i>	13	2
<i>Glomus sp539</i>	13	3
<i>Glomus sp541</i>	19	3
<i>Glomus sp565</i>	15	7
<i>Glomus sp566</i>	12	1
<i>Glomus sp612</i>	13	3
<i>Glomus sp614</i>	11	6
<i>Glomus sp648</i>	12	2
<i>Glomus sp651</i>	25	10
<i>Glomus sp661</i>	11	4
<i>Glomus sp662</i>	14	3
<i>Glomus sp663</i>	12	8
<i>Glomus sp667</i>	13	1
<i>Glomus sp668</i>	14	3
<i>Glomus sp669</i>	14	5
<i>Glomus sp670</i>	20	2
<i>Glomus sp692</i>	14	1
<i>Glomus sp700</i>	11	2
<i>Glomus sp701</i>	12	12
<i>Glomus sp703</i>	19	13
<i>Glomus sp83</i>	11	0
<i>Glomus sp85</i>	12	3

<b>Fungal taxon</b>	<b>No. of perennial plants assoc. with</b>	<b>No. of annual plants assoc. with</b>
<i>Glomus sp904</i>	18	3
<i>Glomus sp905</i>	11	2
<i>Glomus sp906</i>	17	6
<i>Glomus sp917</i>	29	12
<i>Glomus sp918</i>	85	48
<i>Glomus sp919</i>	45	32
<i>Glomus sp92</i>	11	0
<i>Glomus sp920</i>	53	33
<i>Glomus sp921</i>	17	3
<i>Glomus sp938</i>	14	8
<i>Glomus sp986</i>	12	1
<i>Paraglomus sp301</i>	19	4
<i>Paraglomus sp302</i>	26	5
<i>Paraglomus sp303</i>	15	4
<i>Paraglomus sp304</i>	25	5
<i>Scutellospora sp1080</i>	12	6
<i>Unknown sp21</i>	12	4

Table only includes fungal species associated with at least 10 plants.



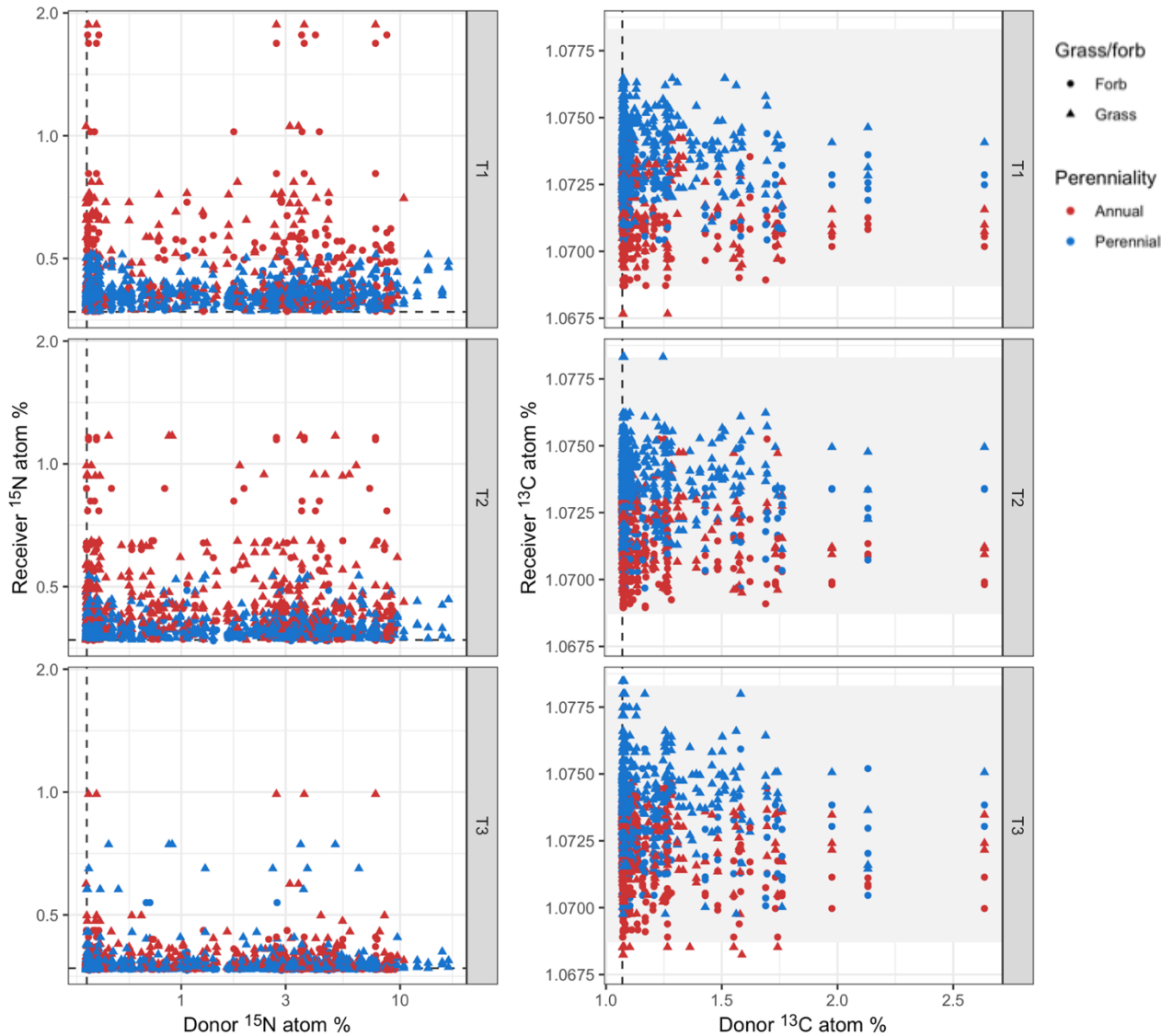
**Figure S1. Enrichment decreases over time in labelled plants.** Time points roughly correspond with T0 = time of labelling, T1 = 4 days later, T2 = 10 days later, T3 = 21 days later. Y-axis is in log<sub>10</sub> scale.



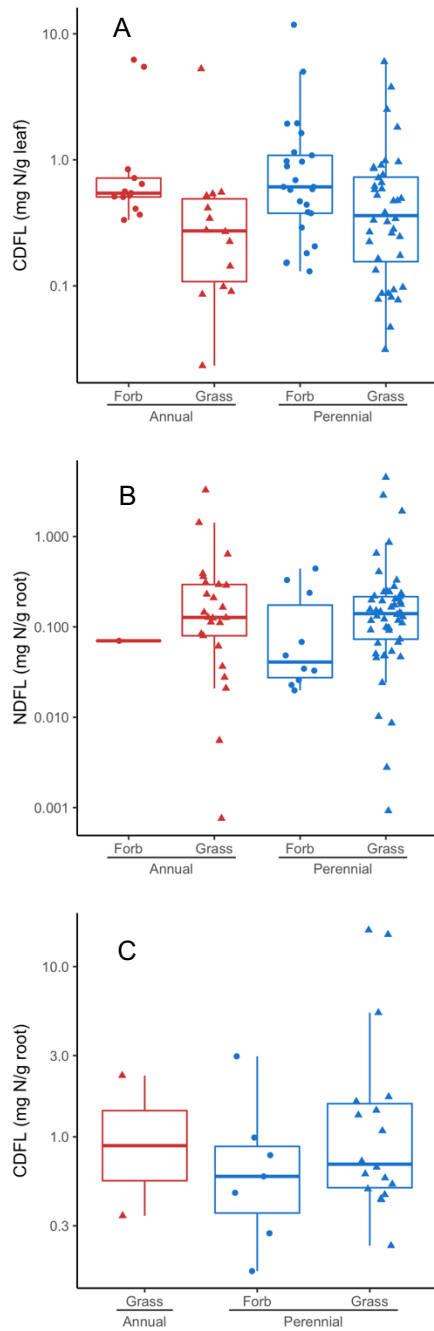
**Figure S2. Decreased receiver enrichment with distance from donor in leaves and roots.**

Dashed lines indicate natural abundance means; grey boxes indicate range of natural abundance variation shown in Fig. 2. There is no systematic enrichment of  $^{13}\text{C}$ ; however, there is high  $^{15}\text{N}$  enrichment in leaves. Y-axes are log<sub>10</sub> scale. Note that the y-axis scales are different between  $^{15}\text{N}$  leaves and  $^{15}\text{N}$  roots. See Fig. 4 and Fig. S\*\* for boxplots of enrichment data by grass/forb and annual/perennial.

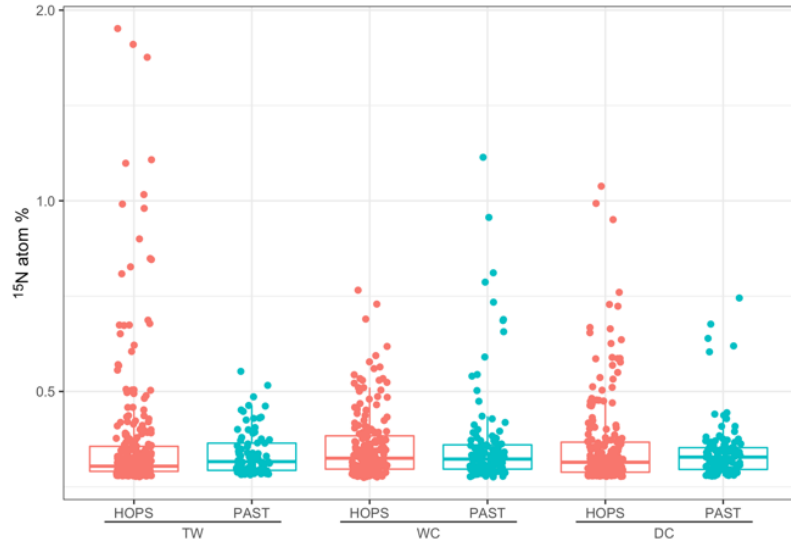




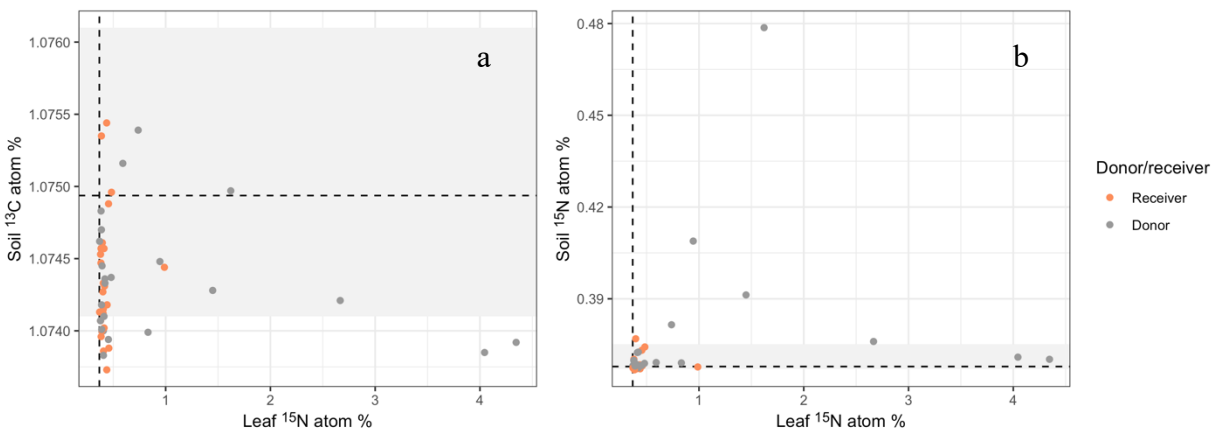
**Figure S3. Receiver enrichment did not correlate with donor enrichment.** Dashed lines indicate natural abundance means; grey boxes indicate range of natural abundance variation shown in Figure 2. (Grey boxes are too small to be visible for  $^{15}\text{N}$ .)  $^{15}\text{N}$  axes are log10 scaled. Note there is a sizeable difference between X- and Y-axes of both elements. Time point facets are time point 1 (~4 days post label), time point 2 (~10 days post label), and time point 3 (~21 days post label). Donors are all sampled at time point 0 (immediately after labelling). Note that five plots are missing from this graph (one each from northern/high diversity/control, northern/low diversity/rain exclusion, central/high diversity/rain exclusion, central/low diversity/rain exclusion, southern/high diversity/control) due to a lack of donor DNA data.



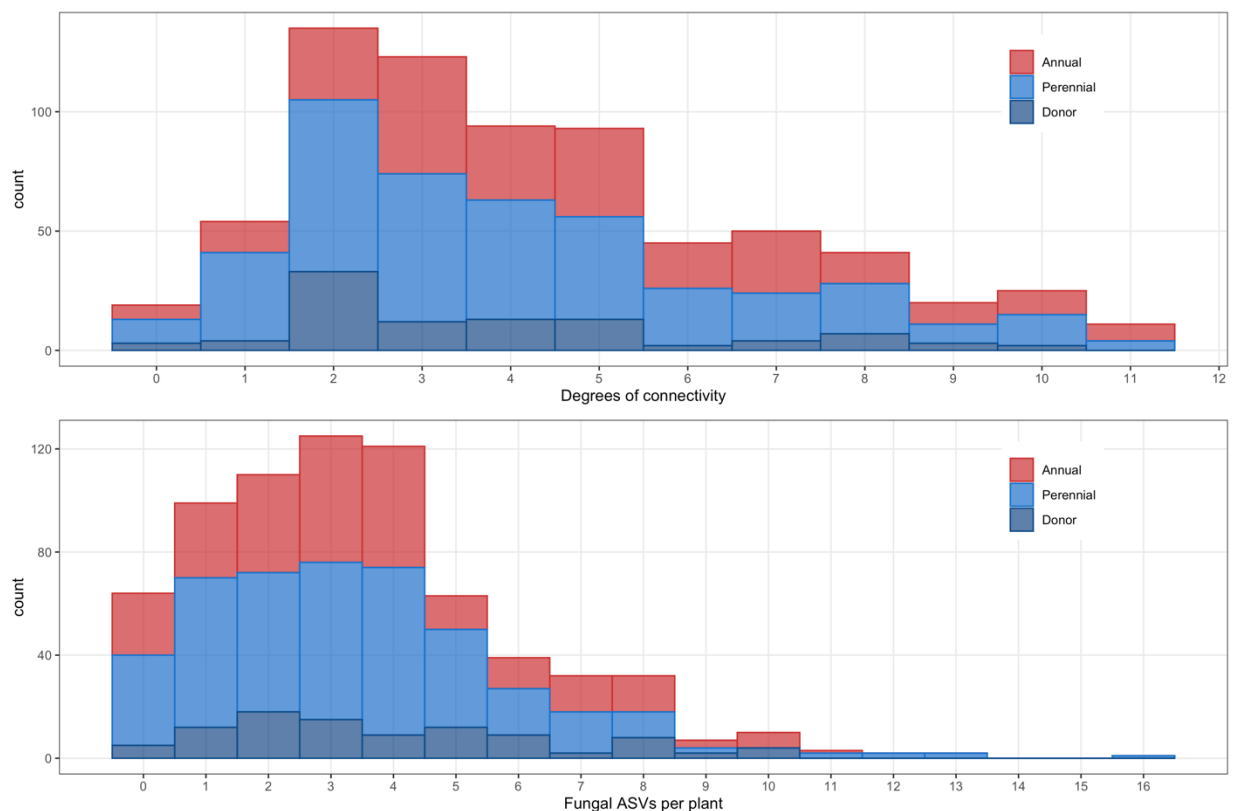
**Figure S3: Enrichment derived from label for A) CDFL in leaves, B) NDFL in roots, and C) CDFL in roots.** Points represent enriched individual receiver plants with associated DNA data at one time point of sampling. In A all three post-enrichment time points are shown; in B and C only time point 3 is shown. Y-axis is log<sub>10</sub> scale. See Fig. S\*\* for values over distance and Fig. 4 for NDFL in leaves.



**Figure S5. No trend in  $^{15}\text{N}$  enrichment across site and rain exclusion treatment.** Y-axis is in log<sub>10</sub> scale. HOPS indicates restored prairie sites, PAST indicates pasture sites. Sites are ordered from north (TW) to central (WC) to south (DC).



**Figure S6. Limited soil enrichment 21 days after labelling.** Dashed lines indicate average baseline enrichment. Grey boxes indicate range of baseline enrichment. Samples are subset to individuals most highly enriched with foliar  $^{15}\text{N}$  at each site by restored prairie/introduced pasture as well as their associated donors.



**Figure S7. Histogram of connectivity metrics showing left skew.** Degrees of connectivity indicate how many plants an individual shared at least one fungal ASV with in the same plot. Data are from time point 1 (approximately four days after labeling). Donors are the plants that we applied the isotopic label to.

## SI References

- Chaudhary, V. B., Rúa, M. A., Antoninka, A., Bever, J. D., Cannon, J., Craig, A., Duchicela, J., Frame, A., Gardes, M., Gehring, C., Ha, M., Hart, M., Hopkins, J., Ji, B., Johnson, N. C., Kaonongbua, W., Karst, J., Koide, R. T., Lamit, L. J., ... Hoeksema, J. D. (2016). Data Descriptor: MycoDB, a global database of plant response to mycorrhizal fungi. *Nature*, 3:160028. <https://doi.org/10.1038/sdata.2016.28>
- Dickie, I. A., Martínez-García, L. B., Koele, N., Grelet, G. A., Tylianakis, J. M., Peltzer, D. A., & Richardson, S. J. (2013). Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. *Plant and Soil*, 367(1–2), 11–39. <https://doi.org/10.1007/s11104-013-1609-0>
- Soudzilovskaia, N. A., Vaessen, S., Barcelo, M., He, J., Rahimlou, S., Abarenkov, K., Brundrett, M. C., Gomes, S. I. F., Merckx, V., & Tedersoo, L. (2020). FungalRoot: global online database of plant mycorrhizal associations. *New Phytologist*, 227(3), 955–966. <https://doi.org/10.1111/nph.16569>