Supporting Information

Article title: Routes to Roots: Direct Evidence of Water Transport by Arbuscular Mycorrhizal Fungi to Host Plants

Authors: Anne Kakouridis, John A. Hagen, Megan P. Kan, Stefania Mambelli, Lewis J. Feldman, Donald J. Herman, Peter K. Weber, Jennifer Pett-Ridge, and Mary K. Firestone

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Methods S1: **Detailed ^{18}O Calculations and assumptions.** The ^{18}O values used in the calculations below can be found in bold in Table **S1**, both in ∂ notation in ‰ and in atom%. ^{18}O values are reported in ∂ notation in ‰ throughout the main text, figures, and supplement for ease of reading, but the calculations were performed in atom%.

Assumption #1: **The same volume of water crossed the air gap via liquid or vapor diffusion (i.e., not via AMF hyphae) from the no-plant compartment to the plant compartment and was taken up by roots in +AMF, -AMF, and ^{16}O microcosms**. The pore size of mesh used in +AMF and ^{16}O vs. -AMF microcosms was 18 µm and 0.45 µm, respectively; this difference was required to allow versus restrict AMF hyphae from crossing the air gap. These standard mesh sizes are routinely used by AMF researchers for studies of hyphal transport of water and solution-based N and P nutrients (e.g., Hodge *et al.*, 2001; Querejeta *et al.*, 2003; Egerton-Warburton *et al.*, 2007; Storer *et al.*, 2018).

Assumption #2: **For both +AMF and -AMF microcosms, the ^{18}O content of the water in the no-plant compartment soil mix was the same at t=0 and at harvest (t=3.5), 300.75‰ $\partial^{18}O$.** We did not directly measure the ^{18}O content of water in the no-plant compartment soil mix at t=0 (i.e., when the labeled water was injected) since obtaining this value would have required destructive sampling. We acknowledge that the ^{18}O content of the water in the soil mix may have slightly changed between t=0 and harvest 3.5 days later, due to natural abundance fractionation caused by evaporation and AMF water transport. If this were the case, both evaporated water and AMF-transported water would have been slightly ^{18}O depleted relative to the water remaining in the soil mix (Sharp, 2007; Poca *et al.*, 2019). However, the change in $\partial^{18}O$ due to evaporation or AMF transport would be quite small compared to the tracer-level $\partial^{18}O$ we added to the soil mix (3000‰), approximately 7‰ $\partial^{18}O$ for evaporation (Yong *et al.*, 2020) and approximately 3‰ for AMF-transport (Poca *et al.*, 2019). Therefore, we assume that natural abundance fractionation effects did not significantly influence our calculations.

Assumption #3: Any water crossing the air gap and reaching the roots via AMF hyphae, liquid diffusion, or vapor diffusion had the same ^{18}O content as water in the no-plant compartment soil mix, 300.75‰ $\partial^{18}O$. Fractionation attributable to evaporation/water vapor is ~7‰ (Yong *et al.*, 2020) and ~3‰ for AMF-transport (Poca *et al.*, 2019). These effects are small enough that we assume they did not significantly affect our calculations (see assumption #2).

Assumption #4: **The $\delta^{18}O$ of the water in the plant compartment sand mix at t=0 was the same for all microcosms. On average, between t=0 and harvest (t=3.5), the water in the sand mix had a $\delta^{18}O$ value of -4.89‰.** We did not directly measure the $\delta^{18}O$ value of the water in the sand mix at t=0 (i.e., the time when the labeled water was injected into the no-plant compartment) since obtaining this value would have required destructive sampling. However, all microcosms received the same water in the plant compartment throughout the experiment, and plant

compartments received their last watering 24 hours prior to the ^{18}O-labeled water injection into the no-plant compartments (t=-1). All plants were watered with natural abundance water that had a $\delta^{18}O$ value of -8.24‰, and in the ^{16}O microcosms, the $\delta^{18}O$ of the water in the plant compartment sand mix at harvest was -1.53‰. The difference between these values was likely due to fractionation within the plant compartment, caused by water evaporation and root and hyphal uptake of water (Poca *et al.*, 2019; Yong *et al.*, 2020). On average, if there had been no input of water from the no-plant compartment, the water in the sand mix of all microcosms would have had a $\delta^{18}O$ value of -4.89‰ between t=0 and harvest (the average between -8.24‰ and -1.53‰.).

Mixing model: To calculate the volume of soil mix water transported by AMF to host plants, we used a standard isotope mixing model, following the approach described in Hayes (2004), but substituting V_T - V_K for V_I and solving for V_K:

$$V_K = \frac{V_T \cdot (F_T - F_I)}{F_K - F_I} \quad \textbf{(1)}$$

Where:
V_K = volume of water from the no-plant compartment transpired by plants (mL)
V_I = volume of water from the plant compartment transpired by plants (mL)
V_T = total volume of water transpired by plants (mL)
F_T = ^{18}O value of transpired water (atom% ^{18}O)
F_I = ^{18}O value of water in the sand mix in plant compartment (atom% ^{18}O)
F_K = ^{18}O value of water in the soil mix in no-plant compartment (atom% ^{18}O)

In -AMF microcosms:
V_{K1} = volume of water that crossed the air gap as liquid or vapor diffusion (mL)
V_{T1} = volume of water transpired by -AMF plants (mL)
F_{T1} = ^{18}O value of transpired water of -AMF plants (atom% ^{18}O)
F_I = ^{18}O value of water in the sand mix in plant compartment = 0.1991 atom% ^{18}O
F_K = ^{18}O value of water in the soil mix in no-plant compartment = 0.2601 atom% ^{18}O

In +AMF microcosms:
V_{K2} = volume of water that crossed the air gap via AMF, plus via liquid or vapor diffusion (mL)
V_{T2} = volume of water transpired by +AMF plants (mL)
F_{T2} = ^{18}O value in transpired water of +AMF plants (atom% ^{18}O)
F_I = ^{18}O value of water in the sand mix in plant compartment = 0.1991 atom% ^{18}O
F_K = ^{18}O value of water in the soil mix in no-plant compartment = 0.2601 atom% ^{18}O

Solving the mixing model equation (1) for each +AMF and -AMF microcosm on days 1, 2 and 3, we obtained V_{K1} and V_{K2} values. We averaged V_{K1} and V_{K2} across all microcosms and days (and named the averages $\overline{V}_{K1}$ and $\overline{V}_{K2}$). We used the difference $\overline{V}_{K2}$ - $\overline{V}_{K1}$ to calculate V_{K3}, the volume

of water transpired that came from the no-plant compartment via AMF. We calculated the standard deviation of V_{K3}, $\sigma_{3,}$ from the standard deviation of $\overline{V}_{K1}$, σ_1, and of $\overline{V}_{K2}$, σ_2. We then calculated the 95% CI from σ_3.

V_{K3} = water transported by AMF and taken up by roots = $\overline{V}_{K2}$ - $\overline{V}_{K1}$ = 0.885 mL = 34.6% of $\overline{V}_{T2}$
95% CI of V_{K3} = $V_{K3} \pm 1.96 \cdot \frac{\sigma_3}{\sqrt{n}}$ = 0.885 ± 0.268
Where:
$\overline{V}_{T2}$ = average volume of water transpired by +AMF plants over three days = 2.56 mL
n =18 (6 microcosms per treatment · 3 individual days of transpired water collection)
$\sigma_3 = \sqrt{(\sigma_1{}^2 + \sigma_2{}^2)} = 0.581$
$1.96 \cdot \frac{\sigma_3}{\sqrt{n}}$ = 0.268 = 10.5% of V_{T2}

On average, **water travelling via AMF across the air gap and taken up by roots accounted for 0.885 ± 0.268 mL (95% CI) of the transpired water per day; this is equivalent to 34.6 ±10.5% (95% CI) of the total transpired water by the +AMF plants.**

We also calculated the values described above using a Monte Carlo analysis with associated error propagation. In this approach, we used data from our three independent treatments (+AMF, -AMF, and ^{16}O control) and randomly grouped individual replicates from the treatments. To do so, results from the six replicates for each treatment were separated by day (Day 1, 2 and 3), and randomly paired with the other treatments from the same day to calculate transpiration due to AMF (3,744 pairings per day, 11,232 total). Means, standard deviations, and 95% confidence intervals were calculated by day and for the whole experiment using the number of replicates per treatment (6) for n and $\alpha = 0.05$ (Zar, 1984). Our Monte Carlo analysis yielded the result 34% ± 15% (95% CI), which is not statistically different from the value we calculated above.

Table S1: **Data used in statistical analyses and ^{18}O calculations.** Values that appear in the main text are in **bold**. Means ± standard errors were obtained by one-way ANOVA & Fisher LSD test. Means ± standard errors are averages of six microcosms per treatment, except for transpired water volumes and ^{18}O contents that are averages of eighteen samples per treatment (six microcosms · three individual days of transpired water collection).

		'+AMF'	'-AMF'	'^{16}O'
transpired water	volume of transpired water at time = day 1 (mL)	2.46 ±0.41	1.29 ±0.22	2.20 ±0.32
	volume of transpired water at time = day 2 (mL)	2.52 ±0.51	1.19 ±0.17	2.65 ±0.21
	volume of transpired water at time = day 3 (mL)	2.69 ±0.28	1.54 ±0.30	2.66 ±0.51
	sum of volume of transpired water over three days (mL)	**7.67 ± 1.10**	**4.03 ±0.52**	7.51 ±0.81
	average volume of water transpired per day over three days (mL)	**2.56 ±0.23**	**1.34 ±0.13**	2.51 ±0.21
	^{18}O content of transpired water at time = day 1 (‰; atom% ^{18}O)	115.62 ±24.50; 0.2232	19.20 ±2.61; 0.2040	4.78 ±1.47; 0.2011
	^{18}O content of transpired water at time = day 2 (‰; atom% ^{18}O)	128.81 ±17.86; 0.2258	40.27 ±3.95; 0.2082	4.90 ±1.28; 0.2011
	^{18}O content of transpired water at time = day 3 (‰; atom% ^{18}O)	136.85 ±14.47; 0.2274	54.38 ±1.93; 0.2110	5.18 ±1.48; 0.2012
	average ^{18}O content of transpired water over three days (‰; atom% ^{18}O)	**127.09 ±10.73; 0.2255**	**37.95 ± 3.86; 0.2077**	**4.95 ±0.77; 0.2011**
plant compartment	mass of dry sand-clay mixture (g)	745.1	745.1	745.1
	gravimetric water content of sand-clay mixture at harvest (%)	16.5 ±0.6	15.9 ±1.0	16.1 ±0.5
	volume of plant compartment filled with sand-clay mixture (cm^3)	612.5	612.5	612.5
	volume of water added at time = -1 day (mL) (last plant watering)	10.0	10.0	10.0
	^{18}O content of water used for watering (‰; atom% ^{18}O)	-8.24 ±0.03; 0.1985	-8.24 ±0.03; 0.1985	-8.24 ±0.03; 0.1985
	^{18}O content of water in sand-clay mixture at harvest (‰; atom% ^{18}O)	162.19 ±10.80; 0.2325	80.70 ±12.51; 0.2162	-1.53 ±1.97; 0.1998
	average ^{18}O content of water in sand-clay mixture over three days (‰; atom% ^{18}O)	76.98 ±6.23; 0.2155	36.23 ±7.22; 0.2074	**-4.89 ±1.04; 0.1991**
	above ground biomass at harvest (mg)	559.2 ±64.5	535 ±49.7	769.2 ±76.6
	below ground biomass at harvest (mg)	805.3 ±80.5	801.9 ±75.2	814.5 ±88.8
	above ground biomass %C	37.73 ±0.42	36.04 ±0.59	37.03 ±0.60
	above ground biomass %N	0.89 ±0.02	0.77 ±0.12	0.81 ±0.02
	above ground biomass C:N	42.63 ±1.37	47.41 ±2.22	48.00 ±6.79
	above ground biomass %P	**0.29 ±0.05**	**0.12 ±0.012**	0.25 ±0.012
	total P added as Rorison's solution (µmol)	10.11	20.22	10.11
	total P added as bone meal (µmol)	76.93	76.93	76.93
	total N added as Rorison's solution (µmol)	403.30	806.60	403.30
	total N added as bone meal (µmol)	111.38	111.38	111.38
	total Fe added as Rorison's solution (µmol)	6.82	13.62	6.82
no-plant compartment	mass of dry soil-sand mixture (g)	230.0	230.0	230.0
	gravimetric water content of soil-sand mixture at harvest (%)	11.6 ±0.3	11.1 ±1.1	9.9 ±0.4
	volume of no-plant compartment filled with soil-sand mixture (cm^3)	183.8	183.8	183.8
	^{18}O content of water used for watering (‰; atom% ^{18}O)	-8.24 ±0.03; 0.1985	-8.24 ±0.03; 0.1985	-8.24 ±0.03; 0.1985
	^{18}O content of water added at t=0 (‰; atom% ^{18}O) (input water)	3000; 0.7957	3000; 0.7957	-8; 0.1985
	volume of water added at t=0 (mL) (input water)	20.0	20.0	20.0
	^{18}O content of water in soil-sand mixture at harvest (‰; atom% ^{18}O)	**300.75 ±50.88; 0.2601**	N/A	N/A
	total P added as Rorison's solution (µmol)	101.1	101.1	101.1
	total P added as bone meal (µmol)	76.93	76.93	76.93
	total N added as Rorison's solution (µmol)	403.20	403.40	403.20
	total N added as bone meal (µmol)	111.38	111.38	111.38
	total Fe added as Rorison's solution (µmol)	6.82	6.82	6.82

Figure S1: **Assembly of a microcosm. 1**, Laser cut acrylic panels; **2**, Nylon mesh; **3**, Acrylic washers; **4**, Metal screws.

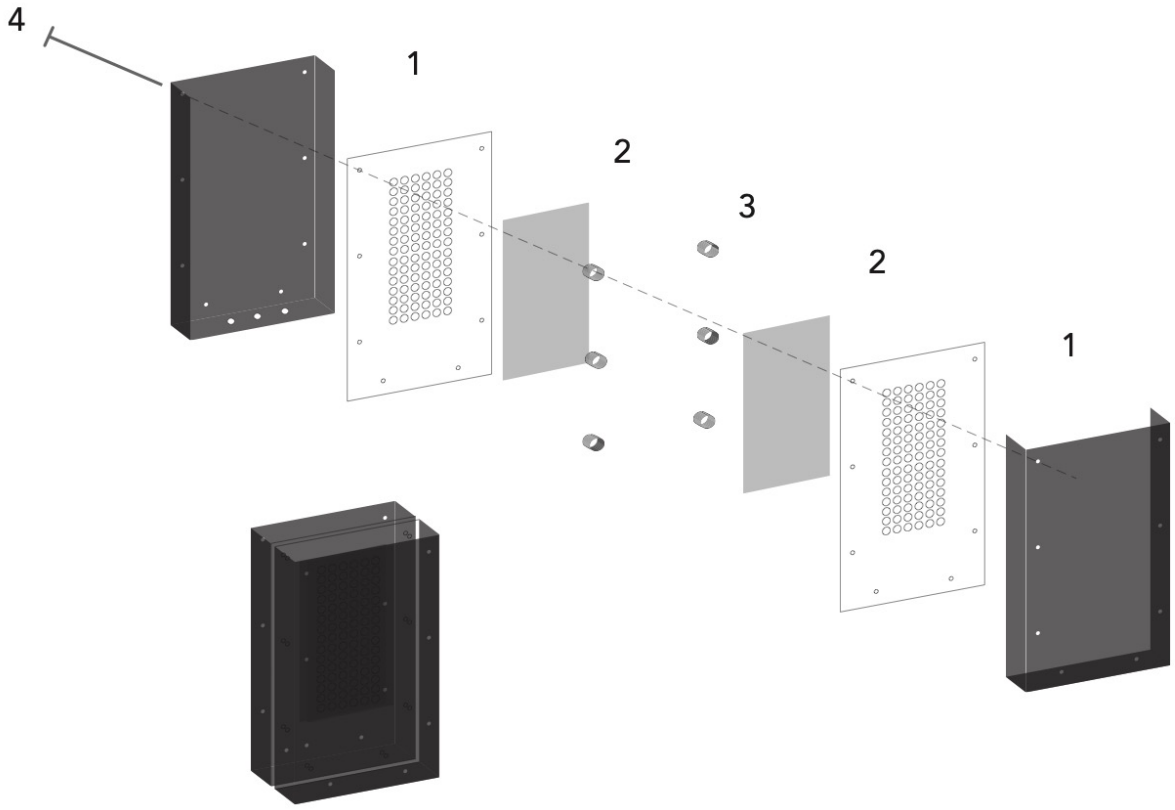
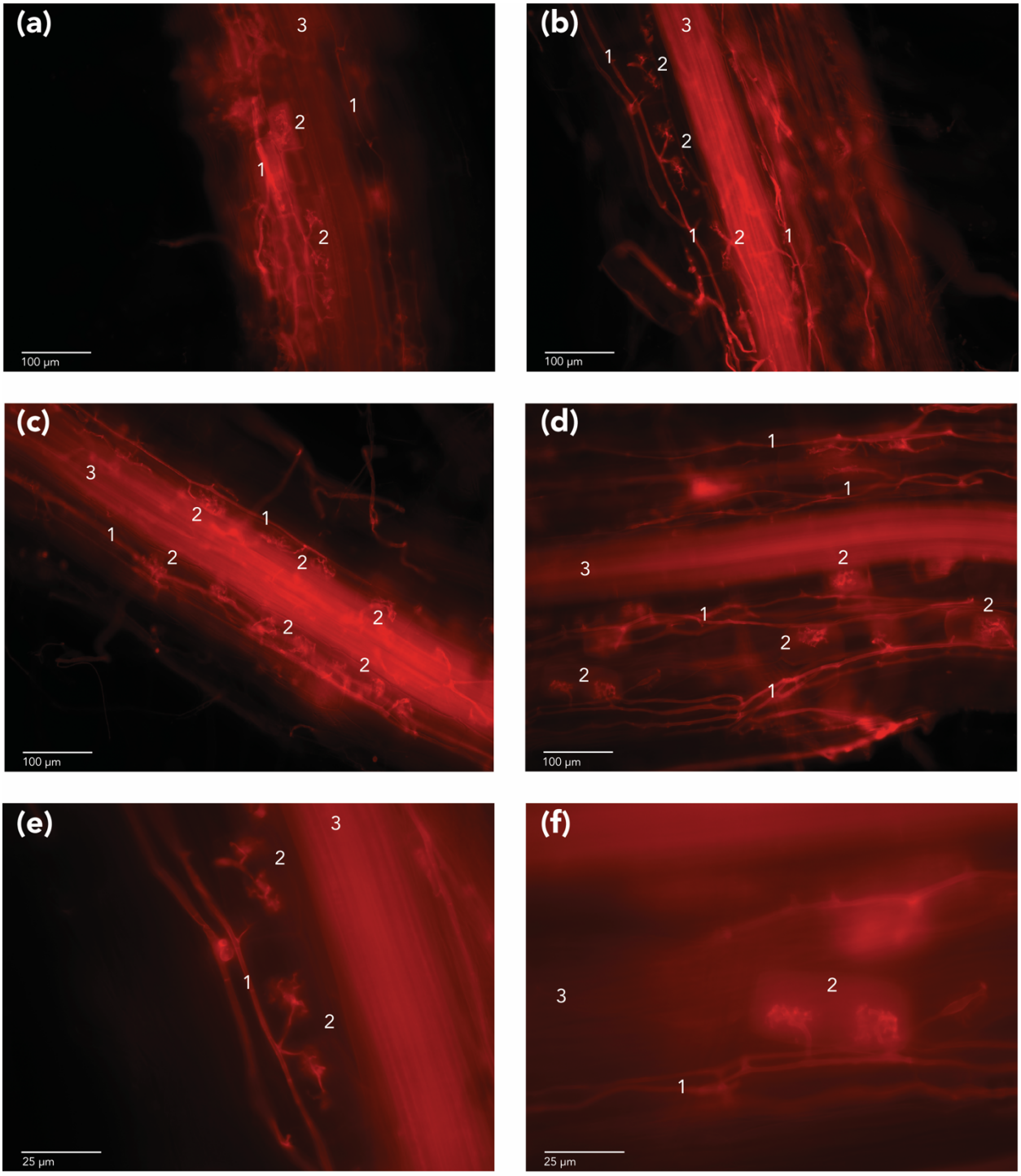


Figure S2: **Fluorescence images** of *Avena barbata* roots dyed with acid fuchsin showing AMF structures. In **(a-f)**: **1**, Hypha; **2**, Arbuscule; **3**, Root.



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