

Addendum

Efflux of hydraulically lifted water from mycorrhizal fungal hyphae during imposed drought

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Apart from improving plant and soil water status during drought, it has been suggested that hydraulic lift (HL) could enhance plant nutrient capture through the flow of mineral nutrients directly from the soil to plant roots, or by maintaining the functioning of mycorrhizal fungi. We evaluated the extent to which the diel cycle of water availability created by HL covaries with the efflux of HL water from the tips of extramatrical (external) mycorrhizal hyphae, and the possible effects on biogeochemical processes. Phenotypic mycorrhizal fungal variables, such as total and live hyphal lengths, were positively correlated with HL efflux from hyphae, soil water potential (dawn), and plant response variables (foliar ¹⁵N). The efflux of HL water from hyphae was also correlated with bacterial abundance and soil enzyme activity (P), and the moistening of soil organic matter. Such findings indicate that the efflux of HL water from the external mycorrhizal mycelia may be a complementary explanation for plant nutrient acquisition and survival during drought.

In environments that experience seasonal or extended drought, plant productivity, resource partitioning, and competition are limited by the availability of water and mineral nutrients. One mechanism that is important to whole plant water balance in these environments is hydraulic lift (HL), a passive process driven by gradients in water potential among soils layers. Soil water is transported upwards from deep moist soils and released into the nutrient-rich upper soil layers by root systems accessing both deep and shallow soil layers.¹ HL water may improve the lifespan and activity of fine roots in a wide variety of plant life forms.²

Hydraulic lift may also have a second ecological function in facilitating plant nutrient acquisition.² It been hypothesized that HL water could enhance the supply of nutrients to roots through mass flow or diffusion,³ or trigger episodes of soil biotic activity such as microbe-mediated nutrient transformations^{4,5} that are analogous to the increased inflow of nitrogen (N) into roots and flushes of carbon (C) and N mineralization respectively that follow precipitation events.^{4,6} However, few data currently exist with which to test these possibilities.

Hydraulically lifted water also sustains mycorrhizal fungi,^{7,8} a mutualism that enhances the acquisition of water and mineral nutrients in many terrestrial plant species. Mycorrhizal fungal hyphae provide comprehensive exploration and rapid access to small-scale or temporary nutrient flushes that may not be available to plant roots.⁹ This resource flow has often been assumed to be a unidirectional flux whereby resources are moved from source (soil) into the sink (plant) by the fungal hyphae. However, there is now evidence to suggest that the physiological plasticity of the peripheral extramatrical hyphae, and in particular the hyphal tips, permits the exudation, and subsequent reabsorption, of water and solutes.^{10,11} Laboratory experiments using pure cultures have demonstrated that water may be exuded from the hyphal tips, especially in fungal species with hydrophobic hyphae, along with a variety of organic molecules, such as free amino acids.^{10–13} At the same time, water, mobile minerals, amino acids and other low-molecular weight metabolites may be selectively and actively reabsorbed by mycorrhizal fungal hyphae.¹¹ However, quantitative data on the environmental impact of hyphal exudation and reabsorption is still largely lacking.

We ask: could the diel cycle of water availability created by HL produce a water efflux from hyphal tips and if so, would this be sufficient to impact biogeochemical processes? Is there also an opposite rhythm driven by plant transpiration so that any resultant soil solution is pulled towards hyphal tips and consequently, the host plant? By imposing drought on seedlings of *Quercus agrifolia* Nee (coast live oak; Fagaceae) grown in mesocosms (Fig. 1), we identified a composite of feedbacks that could influence nutrient capture with HL (Fig. 2). Our analyses provide support for the key predictions of the HL-nutrient cycling scenario including the efflux of HL water from the extramatrical hyphae (Fig. 3), moistening of soil organic

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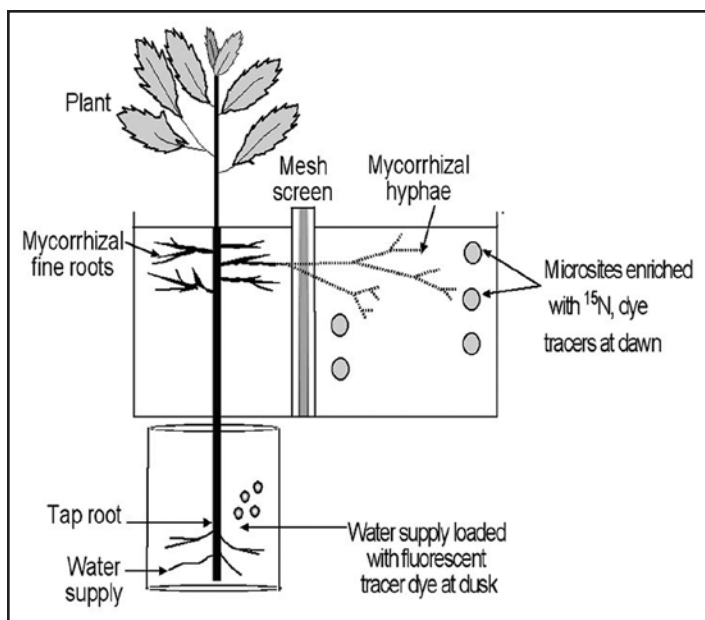


Figure 1. *Quercus* mesocosms demonstrating the plant, root, and hyphal compartments. Details of soil conditions, plant inoculation protocol, mycorrhizal fungi and dye injection methods are detailed in previous work (ref. 7) Point 1 (tap root compartment) denotes the region in which fluorescent tracer dyes were injected into the mesocosm at dusk to track the path of HL water. Point 2 (hyphal chamber) denotes spots adjacent to or distant from the mesh screen into which a small volume (200 μ l) of fluorescent and ^{15}N tracers (99% as $^{15}\text{NH}_4^{15}\text{NO}_3$) were injected at dawn to measure water and nutrient uptake by the external hyphae.

matter (Figs. 3 and 4), and the maintenance of soil microbial activity and nutrient capture (N, P; Table 1).

What Influence Does the Hyphal Efflux of HL Water have on the Distribution of Soil Moisture?

For both efflux and influx (or uptake) of HL-derived water, the available evidence suggests that efflux from the extramatrical hyphae was generally greater than influx, especially with increasing distance from the host plant (Fig. 4). As a result, soils that are well supplied with functional mycorrhizal hyphae are likely to remain moister (Table 1), particularly at depth (Fig. 4), than soils with low hyphal abundance. Because the major proportion of the extramatrical mycelia (~90%) is hydrophobic,¹⁰ this spatial redistribution of HL water can only occur via efflux from locally effective hyphal tips. Indeed, the morphological data provide clear evidence that HL water (as tracked by fluorescent dyes) can be leaked from the hyphal tips (Fig. 3) and subsequently adsorbed into soil particles (Fig. 3) or absorbed by soil organic matter (Fig. 3). The measured efflux rate in

Figure 3. Fluorescently-labeled structures recovered from the hyphal chamber of *Quercus* mesocosms following 80 days of soil drying and with nocturnal hydraulic lift. Yellow-green fluorescence indicates samples labeled with Lucifer yellow CH (LYCH), blue fluorescence denotes samples labeled with Cascade blue (CB) hydrazide. (A) CB-labeled leaf litter from the soil and (B) soil particle; (C) LYCH-labeled root fragment in the soil mixture with adherent extramatrical hyphae; (D) LYCH tracer dye fluorescence in labeled extramatrical hyphae and in efflux (arrow) from the hyphal tip onto organic matter; (E and F) external hyphae filled with LYCH (influx; arrow) and (G) background fluorescence in non-labeled extramatrical hyphae.

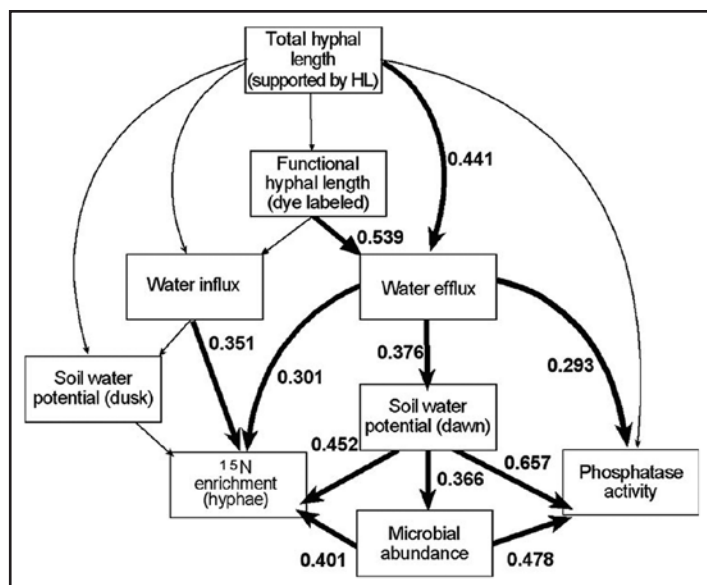
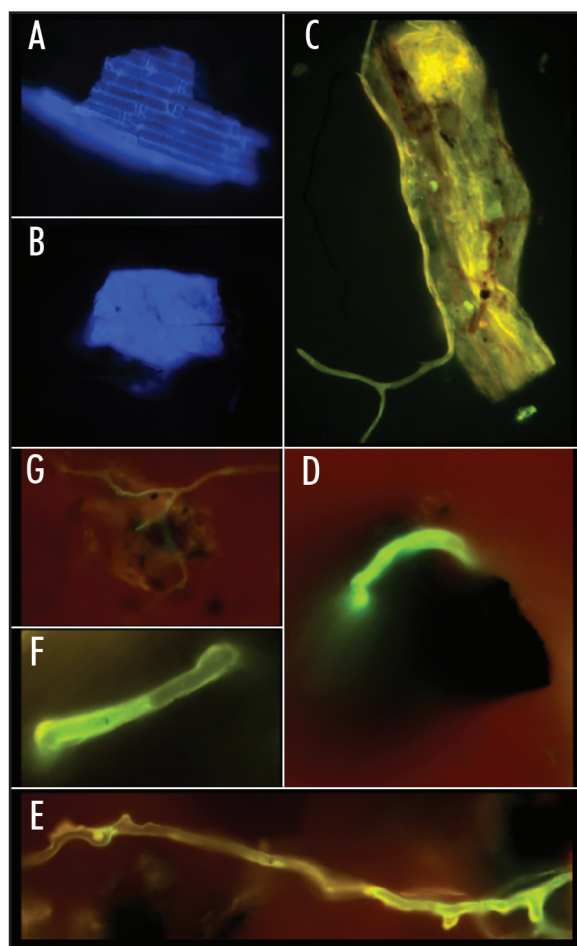


Figure 2. Path analysis of the influence of different soil and mycorrhizal factors on nutrient capture with HL, and resultant model showing the significant path coefficients among variables in the *Q. agrifolia* mesocosms. Lines with a single arrow denote possible cause-effect relationships. The partial correlation coefficients adjacent to each line indicate the strength of the association between the individual factors. Thick lines are statistically significant ($p < 0.05$) whereas thin lines indicate no significant relationship between parameters ($p > 0.05$) and only significant coefficients are given ($p < 0.05$).



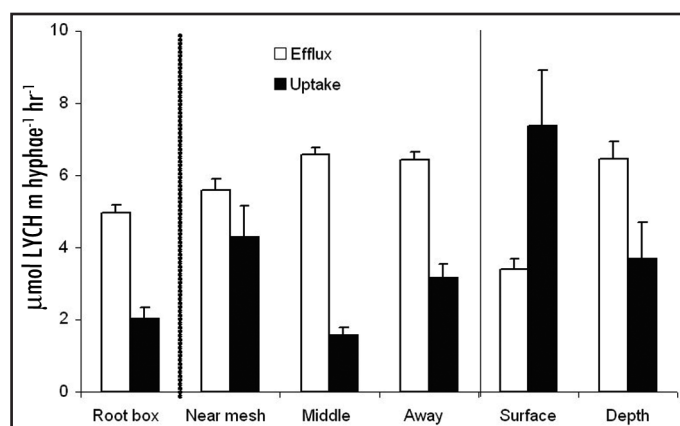


Figure 4. Measurements of hyphal efflux and influx based on the quantitative analysis of LYCH fluorescence intensity in soil solution. Fluorescent intensity values were converted to LYCH concentration using a standard curve generated for the dye since fluorescent intensity correlates with the number of fluorescent molecules in solution. Influx is the uptake of LYCH by hyphae as driven by plant transpiration demands (day), and measured efflux is the passive loss of LYCH from hyphae into the surrounding soil during HL (night). Vertical bars indicate the standard error of the means.

our experiments was, on average, $\sim 280 \mu\text{l}$ per kg soil. Thus, the scale of HL efflux roughly corresponds to the local (microsite) scale at which small differences in soil moisture could result in large increases in litter decomposition and the release of nutrients from organic materials that are then available for uptake and reimmobilization. At the surface, however, the net effect of water exudation appeared to be very small on account of the rapid sorption of water,¹³ or evaporation or capillary action.¹¹

What are the Potential Mechanisms by which Mycorrhizal Hyphae and HL Water Might Influence Nutrient Capture?

Although there are a number of physiological mechanisms by which mycorrhizal fungi supported by HL may enhance plant

nutrient acquisition, our findings (Fig. 2) indicate that factors may be allocated into two classes: indirect and direct. For our purposes, we consider indirect to be the increases in nutrient availability or capture that are the result of water efflux from mycorrhizal hyphae and thus are extrinsic to the fungus. Direct effects are defined as functions that could be directly related to the mycorrhizal fungal association.

Indirect effects. Nutrient capture or acquisition events appeared to be primarily linked to the indirect effects of HL efflux (Fig. 2). For N, this result might be due to microbial mechanisms (Fig. 2 and Table 1) since denitrifier (*nirS*), aerobic ammonia-oxidizing nitrifier (*amoA*) and other members of the domain Bacteria (16S rRNA genes) were found to inhabit the entire mesocosm soil environment. Alternatively, N may be directly acquired by EM hyphae sustained by HL and delivered to the plant root and shoot. The observed increases in plant and fungal ¹⁵N (4–6%) following the application of ¹⁵N-enriched patches in the hyphal compartment support this mechanism (Table 1). A further possibility is that EM hyphae capture N from the small ephemeral pulses created by the efflux of HL water from hyphal tips since N acquisition was correlated with both the efflux and influx of HL water from hyphae (Fig. 2). Because N uptake tends to become diffusion limited in drier soils, these data indicate that improved plant performance with HL is likely related to enhanced N, as well as water, availability and facilitated by mycorrhizal functioning.

In searching for mechanisms, one must also consider the enzymatic capabilities of EM fungi, especially those involved in the mobilization of P (Table 1). The capacity of EM fungi to produce enzymes involved in the mobilization of P (and organic forms of N) is well documented.¹⁵ Indeed, phosphatase activity was four-fold higher in the hyphal than plant compartment indicating that enzymes were actively mobilizing organic P materials. This notwithstanding, we found a disconnect between hyphal abundance and functioning (phosphatase activity). Pooled soil sampling may have masked any metabolic differentiation within the mycelia, or alternatively, the EM taxa utilized

Table 1 Summary of soil, microbial, mycorrhizal and plant parameters in plant or hyphal compartments

Trait	Compartment and Location		
	Plant	Hyphal (Near Mesh)	Hyphal (Away from Mesh)
ψ_s Dawn (MPa)	-4.19 (0.31) ^b	-2.04 (0.66) ^a	-2.09 (0.31) ^a
ψ_s Dusk (MPa)	-20.3 (2.10) ^b	-2.55 (0.49) ^a	-2.09 (0.30) ^a
Phosphatase activity ($\mu\text{g pNP g}^{-1} \text{ hr}^{-1}$)	346 (41) ^b	1289 (38) ^a	1128 (33) ^a
Microbial abundance (colonies $\text{g}^{-1} \text{ soil} \times 10^6$)	2.55 (0.28) ^b	4.72 (1.21) ^a	3.54 (0.37) ^a
Total hyphal length (AMF + EM; m $\text{g}^{-1} \text{ soil}$)	29 (13) ^b	235 (45) ^a	208 (52) ^a
Live hyphal length (dye-labeled AMF + EM hyphae; m $\text{g}^{-1} \text{ soil}$)	29 (3.5) ^b	75 (0.3) ^a	69 (2.1) ^a
*Abundance of microbial genes:			
16s rRNA	++	++	++
<i>nirK</i>	+	+	+
<i>nirS</i>	nd	nd	nd
<i>amoA</i>	++	++	++
§Percentage of ¹⁵ N incorporated into plant or fungal biomass	Old leaves 0.10 New leaves 5.74 Fine roots 1.42	Hyphae 4.34	Hyphae 5.70

Within each row, mean values with the same letter do not differ significantly at $p < 0.05$. *Microbial genes: + detected in soil; ++ abundant in soil; nd, not detected in sample. §Percentage of ¹⁵N uptake based on two-source mixing-model of $\delta^{15}\text{N}$ (‰) in plant and hyphal material following the spot application of ¹⁵NH₄¹⁵NO₃ to the hyphal compartment.

in the study (*Cortinarius*, *Cenococcum*) may make limited use of recalcitrant P sources. On the other hand, microbial abundance was positively correlated with both phosphatase activity and hyphal efflux (Fig. 2 and Table 1), suggesting that soil microbes were the major contributors to the observed phosphatase activity. If so, soil microbes in the hyphal compartment were neither limited by substrate supply (C) nor exposed to the adverse physiologic effects of soil drying. Under these circumstances, HL efflux contributes to microbial growth and biosynthesis, both of which may be especially important to the plant under drought conditions when soil hydraulic conductivity declines. Ectomycorrhizal hyphal and microbial communities are thus inseparably linked by this functional equilibrium. Further, this covariance introduces the possibility of multi-trophic interactions between oaks, EM fungi, and myco-rhizosphere bacteria¹⁶ supported by HL water.

Direct effects. Since mycorrhizal hyphae supported by HL tend to remain functional in dry soils,⁷ the direct role of the extramatrical hyphae appears to be to maintain nutrient acquisition at a number of spatial scales (Fig. 4). The increase in ¹⁵N in hyphal and plant tissues (Table 1), and the daytime uptake of the fluorescent tracer dyes by the extramatrical hyphae (Fig. 4) supports this proposition. The uptake of tracer dyes during the day also indicates that solute influx may be driven, at least in part, by plant transpiration.

What are the Implications for the Plant Community?

Although much of the observed effects of HL and water efflux on nutrient capture appeared to be the outcome of mycorrhizal-mediated events, the relative importance of different potential mechanisms underlying the HL efflux—indirect versus direct—could determine the importance of mycorrhizae in the local environment (soil, plant). If we consider that all the available soil habitat is occupied by hyphae, an assumption implicit in many mycorrhizal studies, and that the effects are largely indirect, then nutrient mobilization from natural organic substrates may be the key function of the vegetative mycelium with HL.¹⁷

On the other hand, should certain EM fungal taxa selectively promote the efflux of HL water or nutrient uptake at the local scale, then the identity, diversity and distribution of EM fungi in the rhizosphere and soil becomes very important in understanding the effects of HL. In the simplest conception of HL-nutrient cycling, the extramatrical mycelia of EM taxa can differ markedly in density, organization and extension, as well as biomass and functioning.²⁰ In addition, different EM fungi also forage at different spatial scales¹⁹ (see also Figs. 3 and 4). Further, EM functional diversity may include, for example, species that are tolerant of drier soils or more extreme diel fluctuations in soil water potential, can differentially use organic or inorganic substrates,¹⁵ differ in their C requirements,¹⁸ or produce long-range hydrophobic mycelia¹⁰ or dense mycelial mats that strongly affect nutrient availability.^{18,19} Such traits could further modify the relationship between hyphal abundance and performance in drier soils. Thus, a rhizosphere containing a diversity of EM species provides a complex environment that changes and evolves over the lifespan of the host plant and in response to edaphic factors. If such functional diversity is widespread, then the more important question now is elucidating the degree to which HL and mycorrhizal fungi influence plant nutrient uptake via the functional properties of individual EM fungi or their combined effects within an EM community.

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