**The arbuscular mycorrhizal fungus *Glomus hoi* can capture and transfer nitrogen from organic patches to its associated host plant at low temperature**

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**Running head:** AM growth and function at low temperature

**Key words:** arbuscular mycorrhizal fungi (AMF), temperature, extra-radical mycelium (ERM), organic material, nitrogen capture, mycorrhizal functioning.

**Abstract**

Arbuscular mycorrhizal fungi have been suggested to be of potential benefit in achieving sustainable agriculture systems. However, there is conflicting information on the degree to which arbuscular mycorrhizal fungi (AMF) can grow and function at soil temperatures typical of temperate regions. To resolve this conflict we grew *Plantago lanceolata* L. inoculated with *Glomus hoi* (UY 110) in microcosm units maintained at 12/10oC (day/night). The microcosms had two compartments, one planted and one not. The root-free compartment contained either an organic (15N:13C labelled milled shoot material) or a sand patch. When permitted access, *G. hoi* proliferated hyphae extensively in the organic patch material. Plant 15N content was a simple function of length density of extra-radical mycelium (ERM) in the patch and c. 6% of host plant N was derived from the patch. These results indicate that *G. hoi* not only grew at these realistic soil temperatures, but also conferred a nutritional benefit to its host.

**Introduction**

Arbuscular mycorrhizal fungi (AMF) play key roles in nutrient cycling and ecosystem functioning and are predicated to become increasingly important under more sustainable agricultural management systems (Jeffries et al., 2003; Gosling et al., 2006; Quilliam et al., 2010; Rooney et al., 2009; Verbruggen et al., 2010). However, there is a lack of knowledge about the responses of this key microbial group to environmental variability (Tibbett and Cairney, 2007; Hughes et al., 2008) and the extent to which they may grow and function at low temperature. AMF are affected by changes in external factors such as temperature both directly and also indirectly via effects on their host plant (Heinemeyer and Fitter, 2004). A reduction in internal root colonisation is commonly reported at temperatures below 15°C (Hetrick and Bloom, 1984; Zhang et al., 1995) and growth of mycorrhizal plants may be more reduced by low temperature than that of non-mycorrhizal plants (Baon et al., 1994; Liu et al., 2004). This finding has implications for symbiotic nutrient transfer with several studies reporting that AM colonisation only increases plant P capture at higher growth temperatures (Wang et al., 2002; Kytövitta and Ruotsalainen, 2007). The impact of temperature on the growth and development of AMF extra-radical mycelium (ERM) is, however, likely more important as this is the phase which is responsible for nutrient capture from the soil. Evidence from the field (Rillig et al., 2002), pot experiments (Gavito et al., 2003; Heinemeyer and Fitter, 2004) and monoxenic cultures (Gavito et al., 2005) suggests that ERM growth is sensitive to low temperature. Collectively, these data suggest AMF growth and functioning is reduced at low temperature, yet such reports seem counter-intuitive to the wide distribution and abundance of AMF in soils that commonly experience temperatures below 15oC for most of the year (Tibbett and Cairney, 2007). In the UK for instance, average soil temperatures in South East England at 10 cm depth are *c*. 10oC, with temperatures >15oC confined to three summer months (<http://www.ecn.ac.uk/aboutecn/database.htm>).

We studied AM development and nutrient scavenging by the ERM of *Glomus hoi* at 12/10°C day/night temperature, typical for natural soils in temperate regions and compared it to the findings from previous experiments carried out at considerably higher temperatures. *G. hoi* was selected as the AMF because we have previously demonstrated that plant N uptake from an organic patch by *G. hoi* was a simple function of hyphal length density under warmer conditions (*c*. 19oC; Hodge et al., 2001).

**2. Materials and methods**

*2.1. Microcosms*

We studied the nutrient foraging ability of this AMF directly by using a two compartment (each 14.5 x 14.5 x 15 cm) microcosm unit separated by a 20 µm nylon mesh which permitted only the fungus access to the second compartment. This compartment contained either a patch of the organic material (see section 2.2 below; treatment OM + AMF) or sand (control patch; treatment S + AMF). In addition, to follow the intrinsic rate of decomposition of the organic material in the absence of the AMF, compartmented units were established as above but in which the AMF was denied access to the second compartment by means of a 0.45 µm nylon mesh (treatment OM - AMF). Microcosms were filled with an autoclaved (121ºC; 30 min) sand:Terragreen® (OIL-DRI Ltd, Cambridgeshire, UK) mixture (1:1 v/v). The plant compartment also received 50 g FW of *G. hoi* inoculum (including root pieces and growth medium), 0.1 g superphosphate and 0.9 g rock phosphate. One pre-germinated *Plantago lanceolata* L. seedling was planted in the middle of the plant compartment. After 2 weeks plants were fed twice weekly with 50 ml of nutrient solution (as Leigh et al., 2009). Microcosm units were placed randomly in a growth cabinet (Conviron, Winnipeg, Canada) with 12/10 ºC (day/night) and 60-80 % relative humidity. Photosynthetically active radiation at plant height ranged between 360 - 370 µmol m-2 s-1. Moisture content of the medium was maintained at 0.081 – 0.104 m3 m-3.

*2.2. Organic patches*

Organic patches were created by mixing 9 g of autoclaved sand and 1 g of 15N:13C dual labelled milled shoot material of *Lolium perenne* L. (generated as described by Hodge et al., 1998). The organic patches contained 31.5 mg N (7.71 mg 15N) and 352.6 mg C (11.36 mg 13C). Control patches were 10 g of autoclaved sand only. Patches were added to the microcosms 116 d after planting. Four replicate microcosm units from each treatment were harvested at 29, 56 and 98 d after patch addition.

*2.3 Plant and mycorrhizal measurements*

Total root FW was recorded and a sub-sample of roots was taken to determine the colonization level by the mycorrhizal fungus (Hodge, 2003a). AMF hyphal length densities were recorded using a modified membrane technique (Hodge, 2003b). Root, shoot and patch material was oven dried (65 ºC, 72 h), DW recorded and milled in a ball mill to a fine powder for analysis of total C, N and 13C and 15N concentrations using CF-IRMS.

*2.4 Statistical analysis*

All data were checked for normality and homogeneity of variance using SPSS for Windows (v 16.0) and transformed where necessary. A Bonferroni post hoc test was applied to determine differences between means where main effects were significant. A paired t-test was used to compare the amount of 15N in the plant roots v shoots. When the data were non-parametrically distributed Kruskal-Wallis tests were performed with Bonferroni corrected Mann-Whitney U post hoc tests. Hyphae broke through the 0.45 µm mesh in one replicate from the OM - AMF treatment which was therefore excluded from the analysis. Differences referred to in the text were statistically significant with *P* < 0.05, unless otherwise stated and values reported are means ± standard errors.

**3. Results and discussion**

# Hyphal densities were low in the compartment where the AMF was excluded (< 0.07 m g-1 patch DW), but where *G. hoi* had access to the root-free compartment, ERM grew into both the sand and organic patches, although hyphal length densities were six times higher in the organic patches (Fig. 1). This difference in hyphal growth demonstrates that the tendency for AM hyphae to proliferate within organic material patches (St John et al., 1983; Hodge et al., 2001; Hodge and Fitter, 2010) also occurs at temperatures typical of temperate soils. Hyphal length densities reached 1.05 m g-1 patch DW 56 d after patch addition, comparable to other studies using similar organic patches but conducted under higher growth temperatures (see Leigh et al., 2009; Hodge et al., 2001). ERM growth is however, enhanced at elevated temperature. For example, after 158 d hyphal length densities in the organic patch were 3.82 m g-1 patch DW, which while comparable to the 3.79 m g-1 soil DW reported by Heinemeyer et al. (2006) at *c*. 25oC, was achieved only 75 d after planting. Although direct comparisons with other studies must be treated with caution due to differing experimental conditions, the results of this study suggest although markedly slower, development of the ERM at an ecologically relevant low temperature. This contrasts with the findings of Gavito et al. (2005) who observed that *G*. *intraradices* failed to develop an ERM at 12°C. Both %RLC and arbuscule frequency were low (6.6 ± 0.6 % RLC; 0.5 ± 0.1 % arbuscules), and were not significantly influenced by hyphal access to the root-free compartment, patch type or time. Reduced root colonisation levels are commonly reported at low temperatures. For example, 15 weeks after *Sorghum bicolor* plants were exposed to three different root zone temperatures, %RLC was 59% when the root zone temperature was 23oC but only 10% RLC for the roots exposed to 10oC (Liu et al., 2004). Similarly, %RLC for *Trifolium pratense* plants grown for 12.5 weeks was only 1.5% at 10oC compared to 19% at 20oC (Hetrick and Bloom, 1984).

Total plant biomass and N concentration were unaffected by the patch type or access treatments. Similarly, Leigh et al. (2009) did not find an effect on plant biomass when either *G. hoi* or *G. intraradices* had access to an organic patch of similar material despite the patch N contributing nearly one fifth of the total plant N. Moreover, while N concentrations were higher in plants colonized by *G. intraradices* that had access to the patch, colonization by *G. hoi* did not show the same effect (Leigh et al., 2009). In contrast, plant N content was higher (*F*2,26 = 4.166, *P* = 0.027) when the fungus had access to an organic patch compared with when it was excluded (29.66 ± 1.67 mg of N; OM + AMF vs. 25.83 ± 1.81 mg of N OM - AMF). Plant N content when the AMF had access to a sand patch was intermediate between these two (27.0 ± 1.70 mg of N; S + AMF). The second compartment contained no additional N other than that in the patch, thus AMF may have captured N from the nutrient solution which had diffused into this compartment. The interaction term between treatment and harvest however was not significant. Where AMF had access to an organic patch, plants captured 200 times more 15N than when AMF access to the patch was denied (0.38 ± 0.06 mg 15N with access v 0.002 ± 0.001 mg 15N no access), equivalent to 7.2 ± 1.8 % of the original patch 15N added by the final harvest. In contrast, when AMF had no access to the organic patch, plants captured < 0.05 % of the 15N originally added by the final harvest. In contrast, 13C levels in the plant tissue were never significantly different to natural background suggesting N was being transferred to the plant in inorganic form (see also Leigh et al., 2011). The form of N captured by the AMF is unknown and we cannot be certain that the 15N label in N captured by the fungus was in proportion to that in the organic material because of the possibility of differential labeling of soluble and structural materials. There also will have been some inorganic N present in the patch and its availability may have been enhanced by the drying and milling of the material. Nevertheless, there will also have been capture of 14N over the duration of the experiment; if we assume that patch 14N and 15N were taken up by the hyphae in proportion to their concentration in the patch, then 6.2 ± 1.2 % of the plant N came from the patch at 98 d. This value could have made an important contribution to plant nutrition and suggests that the transfer of N by AMF may provide a significant amount of ‘extra’ N to colonized plants in N-limited systems and help achieve sustainable farming practices.

We have recently demonstrated that such organic patches can be an important source of N for the AM fungus itself (Hodge and Fitter, 2010), thus patch N detected in the colonized roots may still be held in fungal structures. However, in the treatment where the AMF had access to the patch (OM + AMF) there was no difference (*P* = 0.603) in the amount of 15N in the roots versus the shoots indicating patch N was evenly distributed in the plant. Moreover, as 15N was also detected in the shoots true transfer of patch N from the fungus to the plant must have occurred. In addition, there was a positive relationship between hyphal length density in the organic patch and the 15N content of the host plant (Fig. 2) as reported by Hodge et al. (2001) but at a higher growth temperature (c. 19oC). That the relationship held within the last harvest implies the relationship was not merely a result of both parameters increasing through time (Fig. 2). In the study by Hodge et al. (2001) the slope of the regression was 0.226 whereas in this study the slope was less than half this value (i.e. 0.107) suggesting less N was captured per unit hyphal length at the lower temperature. This diminished N capture from the patch may have been due to lower N availability at 12oC as a consequence of a slower rate of decomposition at the lower temperature (Panikov, 1999; Liski et al., 2003). The relatively high C:N ratio of the organic patch material (i.e. 6.4:1 in Hodge et al., 2001; 11:1 in the present study) might also have slowed mineralization (Kaye and Hart, 1997; Hodge et al., 2000).

From the results of studies conducted under a wide range of conditions Gavito et al. (2005) concluded that temperatures > 18oC - < 30oC were required for good AM development and growth. Although, the exact optima within this range likely depends upon the AMF species in question and the habitat it originates from. Growth is of course still possible outside this range (see Tommerup, 1983). In contrast, optima ranges for soil bacteria and fungi have been found to be more narrow (i.e. 25-30oC see Pietikäinen et al., 2005) and generally higher than the temperatures experienced in most temperate soils. Outside this optimum, soil fungi are less adversely affected by lower, but more adversely affected by higher, temperatures than bacteria (Pietikäinen et al., 2005). The lower range reported by AMF than for other soil fungi is likely linked to the close association they form with the plant and in this study we found that the AMF was still able to proliferate in organic patches and transfer N to their host at 12oC albeit at a reduced level compared to studies conducted at higher temperatures (e.g. Leigh et al., 2009; Hodge and Fitter, 2010). However, we cannot tell from this study how much N the fungus retained for itself to support its own needs and how temperature influences the symbiotic nutrient exchange.

**4. Conclusion**

The results of this study demonstrate that *G. hoi* was able to proliferate in an organic patch and transfer N from that patch to its associated host plant at 12oC. Most studies upon the growth and functioning of AM symbiosis have been conducted at temperatures of 20 - 37°C (Tibbett and Cairney, 2007) which are not representative of average soil temperatures in temperate regions. Further research is needed at temperatures typical of those in the field to determine whether AM isolates have different temperature tolerances (Pringle and Bever, 2002) or seasonal niches (Merryweather and Fitter, 1998).

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**Fig. 1** AM hyphal growth of *G. hoi* into the patches for the treatments: hyphal access to the second compartment (OM – AMF), hyphal access to the second compartment with an organic patch (OM + AMF) or only a control patch of sand (S + AMF). Data are means ± SE (OM – AMF, *n* = 11, OM + AMF and S + AMF, *n* = 12). More hyphal growth occurred in the organic patches (*H* = 25.45, d.f. = 2, *P* < 0.001). Statistical differences among treatments as determined by Bonferroni corrected Mann-Whitney U tests are indicated by different letters. Note the log scale on the Y-axis.

**Fig. 2**. Relationship between total plant 15N content and hyphal length density (m g-1 patch DW) in the organic patch for the OM +AMF treatment. Grey circles: 29 days, white circles: 56 days and black circles: 98 days. The data are fitted with a best fit linear regression line, and the *P* value refers to the significant, positive relationship between hyphal density in the patch and total 15N content of the mycorrhizal host plant (mg15N = 0.164 + 0.107 m hyphae g-1 patch dry weight (DW), R2 = 76.3 %, *F*1,10 = 32.28, *P* < 0.001).

Fig. 1



Fig 2

