**Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material**

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1.    Nitrogen (N) capture by arbuscular mycorrhizal (AM) fungi from organic material is a recently discovered phenomenon. We investigated the ability of two *Glomus* species to transfer N from organic material to host plants and if the ability to capture N is related to fungal hyphal growth.   
2.    Experimental microcosms had two compartments, either containing a single plant of *Plantago lanceolata* inoculated with *G. hoi* or *G. intraradices* or a patch of dried shoot material, labelled with 15N and 13C. In one treatment hyphae, but not roots, were allowed access to the patch; in the other access by both was prevented.   
3*.*When allowed, fungi proliferated in the patch and captured N but not C, although *G. intraradices* transferred more N than *G. hoi* to the plant. Plants colonised with *G. intraradices* had greater N concentration than controls.   
4.   Up to a third of the patch N was captured by the AM fungi and transferred to the plant while ~20% of plant N may have been patch derived. These findings indicate that uptake from organic N could be important in AM symbiosis for both plant and fungal partners and that some AM fungi may acquire inorganic N from organic sources.

Key words: Arbuscular mycorrhizal fungi, soil heterogeneity, nitrogen, organic material, *Glomus hoi*, *Glomus intraradices*, stable isotopes 13C and 15N**Introduction**

Arbuscular mycorrhizal (AM) fungi are symbiotically associated with the roots of approximately two-thirds of all species of land plant (Smith & Read, 2008). All are obligate biotrophs which benefit the plant in several ways, such as increasing nutrient capture (Mosse *et al.,* 1973), drought resistance (George *et al.,* 1992) and pathogen protection (Newsham *et al.,* 1995). In return, plants provide the fungus with photosynthetically fixed carbon. Enhancement of phosphorus (P) uptake is the most widely studied of the AM fungal benefits to their associated host (Smith & Read, 2008). Phosphate ions are poorly mobile in soils (Tinker & Nye, 2000) and can limit both plant and microbial growth (Mosse *et al*., 1973). Over time, depletion zones form around the roots because phosphate ions diffuse through soil towards the root surface more slowly than the rate of uptake across root cell membranes (Tinker & Nye, 2000). The fungal hyphae act as an extension of the plant root, bypassing the root’s P depletion zone, and are an efficient way to explore large volumes of soil.

Nitrogen (N) is mainly captured by plants in inorganic form (i.e. as nitrate or ammonium). These N forms are more mobile than phosphate and nitrate in particular diffuses rapidly through the soil matrix (Tinker & Nye, 2000). However, in the majority of natural ecosystems most N is in complex organic form and consequently unavailable for uptake by the majority of plants (Swift *et al.,* 1979). Furthermore, these complex N forms are distributed heterogeneously in the soil matrix. Soil fungi play a major role in N mineralization, particularly where the N occurs in recalcitrant materials rich in lignin (Kjøller & Struwe, 1982; Cook & Rayner, 1984). Enzymes are secreted by saprotrophic fungi, causing extracellular breakdown of complex organic material, the products of which are subsequently taken up. There is no direct evidence for saprotrophy by AM fungi (Smith & Read, 2008), but their hyphae colonise soil extensively, increasing the chances of encountering a nutrient-rich “patch” of organic material undergoing decomposition. In environments where competition for nutrients is high, increasing the effective absorbing surface area could improve plant uptake of N and other nutrients such as phosphate (Hodge *et al*., 1999).

AM fungal involvement in plant N uptake is not as clearly defined as that of P (Smith *et al.,* 1985; Azcόn *et al.,* 1992). Govindarajulu *et al.* (2005) demonstrated substantial N uptake by cultured mycorrhizal roots but could not measure N transfer to the plant. Similarly, microcosm experiments using whole plants have generally shown that while AM fungi can increase N uptake by plants, there are only minimal effects on plant N concentration (Ames *et al.,* 1983a; Johansen, 1993; Tobar *et al.,* 1994; Hawkins *et al.,* 2000; Hodge *et al.,* 2001). The N uptake of an AM plant is apparently not affected by colonisation, which can have neutral or negative results on hosts, particularly if P is not limiting (Reynolds *et al*., 2005). However, studies in natural ecosystems have found a shift in AM fungal communities following application of inorganic N to the soil (Egerton-Warburton & Allen, 2000; Bradley *et al.,* 2006) suggesting that AM fungi are sensitive to soil N availability and have a role in N uptake in natural systems when there is competition for N. Whether organic material is an important source of N for either partner in the AM symbiosis is unknown and teasing apart the dynamics of N uptake in complex natural environments would be a difficult task. Instead, the use of microcosms with separate compartments which allow access to hyphae but not roots have been used extensively to observe the fundamental niche of AM fungal hyphae (Jakobsen *et al*., 1992; Mader *et al*., 2000; Hodge *et al*., 2001)

Using compartmented microcosms to separate the fungal hyphae from the roots, one AM fungal species, *Glomus hoi*, was shown to grow aggressively towards an organic patch, labelled with 15N and 13C, in one compartment to which plant roots had no access, rather than to a second potential host plant, also in a separate compartment, even though the latter is a known source of nutrition for an AM fungus. In addition, the fungus was shown to capture inorganic N from organic material and transfer it to the host plant (Hodge *et al.*, 2001). The uptake of N correlated with fungal extraradical mycelium (ERM) growth into the patch. To explore the significance of N capture from organic material and subsequent transfer to the host by AM fungi we undertook an experiment with a similar microcosm design but in the absence of a second plant and using two AM fungal species. We compared *G. hoi* with *G. intraradices*, which has been shown to produce a more extensive ERM (Drew *et al.,* 2003), to determine whether the correlation of N uptake with ERM length density shown by Hodge *et al*. (2001) was consistent between the two fungi.

We therefore tested the hypotheses that AM fungi could capture N from the organic material and transfer it back to their host plant, and that the two species of fungi differed in their ability to capture N, due to differences in growth of the ERM: specifically, we expected *G. intraradices* to produce more ERM and to capture and transfer more N from the patch to the plant than *G. hoi*.

**Materials and Methods**

Two microcosm experiments were set up in successive years. Experiment 1 was started on 15 August 2005. Experiment 2 began on 21 July 2006. In experiment 1, comparison was made of the two fungal species *Glomus hoi* and *G. intraradices* in symbiosis with *Plantago lanceolata* L.; in experiment 2, an additional non-mycorrhizal (NM) treatment was added as a further control.

*Microcosm design*

Microcosm units (see Hodge *et al.,* 2001) were constructed by joining two plastic boxes, measuring 14 x 14 x 14 cm, via a double mesh barrier through which hyphae but not plant roots could grow (20 μm mesh; John Stanier & Co., Whitefield, Manchester, UK) or which prevented both root and hyphal growth but allowed diffusion (0.45 μm mesh; Anachem, Bedfordshire, UK). Both boxes were filled with a sand and Terragreen® (a calcinated attapulgite clay soil conditioner, Oil-Dri, Cambridgeshire, UK; see Hodge, 2001) mix (1:1 v/v). The plant compartment received 30 g of AM fungal inoculum (see below) and 0.25 g L-1 bonemeal (Vitax, Leicestershire, UK) in 2 L sand/Terragreen substrate. Bonemeal was also added to the patch compartment in experiment 1, but not in experiment 2. The microcosms were set up in a heated, lit glasshouse and re-randomised every two weeks to prevent environmental gradients influencing growth. Each experiment therefore comprised two access treatments (with and without hyphal access) and two fungal treatments with an added non-mycorrhizal control (NM) in the case of experiment 2. In experiment 1 there were 5 replicates, giving a total of 20 microcosms, and in experiment 2 there were 4 replicates giving a total of 24.

The daily mean temperature during experiment 1 was 17oC (± 0.1) and the photosynthetically active radiation (PAR) flux measured weekly at midday averaged 250 (± 51) μmol m-1 s-1 over the duration of the experimental period. Additional lighting, in the form of 400 W halogen bulbs, was used from 28 September 2005, early morning and evenings, to extend the photoperiod to 16 hours. In experiment 2, the temperature was 19oC (± 1.2) and the PAR flux averaged 400 (± 179) μmol m-1 s-1 at plant level; additional lighting was used from 2 October 2006 as above.

*AM fungal inoculum and plant species*

Cultures for *G. hoi* (isolate number UY 110) and *G. intraradices* (isolate BB-E; Biorhize, Dijon, France) were established at least 3 months before use, in pots with *Plantago lanceolata* and *Trifolium repens* L. (both from Emorsgate Wild seeds, Nottingham, UK) in a sand and Terragreen mix with 0.25 g L-1 bonemeal. Either *G. hoi* or *G. intraradices* inoculum (roots and growth medium; 30 g) was mixed well into the plant compartment of the experimental microcosms. In the NM control treatment an autoclaved mix of both species was added. To equalise the starting microbial community in all microcosms, 50 ml of inoculum washing solution was added to the plant compartment of each microcosm (Koide & Li, 1989). This was produced by suspending 1 g live inoculum in 10 ml deionised water and filtering through a 1 μm nylon mesh (John Staniar & Co., Manchester, UK). *P. lanceolata* seeds were surface sterilised in 100% bleach then rinsed in water before pre-germination on moist filter paper. Three seeds were planted per microcosm and thinned to one within two weeks.

*Nutrient availability*

Terragreen contains approximately 0.84% P2O5 (0.37% P). However this phosphate is in largely insoluble form; when 1 g Terragreen was shaken with 10 ml water, only 0.1 mg (< 3% of the total) was recovered in solution.

Two weeks after planting the plant compartment of each microcosm was fed twice a week with 50 ml of a complete nutrient solution adapted from Thornton and Bauswine (2000) to 1/10 the original N and P concentrations (2.5 mmol l-1 NH4NO3) and 0.034 mmol l-1 NaH2PO4.2H2O). The pH was adjusted to 7.0 with KOH. Plants were watered as necessary to avoid drought stress and water was also added to the patch compartment.

*Patch material*

*Lolium perenne* L. shoots were grown in a sand soil mix (1:1, v/v) for at least 6 weeks in 2 L boxes. 15N was applied in two pulses as a 0.06 M solution of NH4NO3 (15N2, 98%) 4 and 2 days before harvesting, and 13C as a single pulse of 13CO2 on the day of the second N pulse. For the latter, plants were placed in an airtight cabinet with 5 g NaH13C03, 8 ml orthophosphoric acid and 24 ml water (Hodge *et al.,* 1998). The harvested shoot material was dried at 65oC and then milled to a fine powder. Continuous flow-isotope ratio mass spectrometry (CF-IRMS) was used to measure the atom percent (At%) of 15N and 13C in the material (Table 1). For each experiment, 1 g of material was mixed with 19 g of sterilised sand and added one month after planting at a depth of 8 cm to the patch compartment, 2 cm from the mesh barrier.

*Harvest and sample analysis*

Forty-two days after patch addition microcosms were destructively harvested. Fresh weight of shoots and roots were recorded, sub-samples of root and soil were taken for measurement of mycorrhizal colonisation. The remaining root and shoot material was dried at 65oC and the dry weight (DW) was recorded.

Root sub-samples were cleared with 10% KOH, acidified with 1% HCl and stained with acid fuchsin as described by Kormanick & McGraw (1982) but omitting phenol. The percentage root length colonisation (%RLC) by the fungus was assessed at 200 x magnification, along with the percentage arbuscule and vesicle presence. At least 100 root intersections were observed for each sample using the method described by McGonigle *et al.* (1990). AM fungal ERM density was extracted from soil following the membrane filter technique and at least 50 views were counted at 125 x magnification using the gridline intercept technique (Staddon *et al.*, 1999). Although hyphal recovery is possibly incomplete with this method, it does allow cross comparisons of ERM growth among replicates. Substrate samples from the plant compartment after mixing and from directly within the patch were taken for ERM analysis.

Dried plant material was milled to a fine powder for analysis of 13C and 15N by CF-IRMS. Measurement of phosphorus was carried out after triple digestion of milled plant material using the molybdenum blue method (Allen, 1974).

*Data analysis*

All results were analysed on SPSS 15.0 by a two way analysis of variance (ANOVA) with Levene’s test to assess equality of variance. Data were log transformed where necessary. The two experiments were analysed separately. A one way ANOVA with an LSD post-hoc test was used to determine significant differences among means. A paired t-test was used to compare the amount of 15N in shoots versus roots of plants.

In three cases in experiment 1 and one in experiment 2 fungi breached the 0.45 μm mesh, observed when samples from the patch were analysed for ERM growth (see above). These replicates were excluded from the analysis. There was no intra- or extraradical hyphal growth detected in the NM treatments.

**Results**

*G. intraradices* colonised host plant roots more intensively than *G. hoi* in experiment 1 (Table 2) but other than this, the extent of colonisation was similar in the two experiments. Permitting hyphal access to the patch compartment decreased AM fungal colonisation and lowered the frequency of arbuscules only in the case of roots colonised by *G. hoi* in experiment 1. In both experiments there were many more vesicles present in the roots of plants colonised by *G. intraradices*. ERM growth into the patch by *G. intraradices* was higher than by *G. hoi* in experiment 1 but not in experiment 2 (Table 2). ERM length density in the plant compartment (1.3 m hyphae g-1 soil dry weight) did not differ among treatments or experiments, and was lower than in the patch.

In experiment 1, plants colonised by *G. intraradices* were smaller than plants colonised by *G. hoi* (Table 3). There was no difference in mass among plants in experiment 2, which were similar to those grown with *G. intraradices* in experiment 1.

Nutrient content of the shoots was affected by AM fungal species and by patch access in both experiments (Fig. 1). In experiment 1 the P content in shoots of plants was lower when *G. intraradices* was prevented from accessing the patch compartment but not when the AM fungus was *G. hoi* (Fig. 1a). The P concentration of plants colonised by *G. intraradices* with patch compartment access was significantly higher (4.0 mg g-1 in each case) in both experiments than in the other treatments, which did not differ amongst themselves and had mean values of 2.3 and 2.6 mg g-1 respectively (experiment 1: *F*3,13 = 7.4, *P* = 0.004; experiment 2: *F*5,17 = 5.8, *P* = 0.003). The shoot N content was not affected but the N concentration increased significantly in plants colonised with *G. intraradices* when patch compartment access was allowed (Fig. 1b). Plants colonised by *G. intraradices* and with patch access had a higher N concentration than the other 3 treatment groups in both experiments (Fig. 1c).

Neither P nor N content of the plants was correlated with hyphal length density in the patch, but in both experiments AM fungal access to the patch compartment increased the plant 15N enrichment and there was an interaction between fungal species and patch access (Table 4). When the AM fungi were permitted access to the patch in experiment 1, plants colonised with *G. intraradices* contained more 15N than those colonised with *G. hoi* (Fig. 2a; *F*1,13 = 11, *P* = 0.006). However 15N content of control plants was high and not different to that in plants when *G. hoi* had access to the patch compartment. In contrast, uptake of 15N from the patch in experiment 2 was very small when patch access was denied and both fungi increased 15N capture by their host plant when access was permitted (Fig. 2b; *F*1,11 = 87.8, *P* < 0.001); this enhancement was significantly greater than that seen in experiment 1 (*F*1,14 = 128, *P* < 0.001).

The 15N in plants represented a small percentage of total plant N content: 0.8 and 1.2% for *G. hoi* and *G. intraradices* respectively in experiment 1, and 2.1 and 3.7% in experiment 2. However, when the 15N in plants is expressed as a percentage of that in the original patch, it emerges that plants colonised with *G. hoi* and *G. intraradices* captured 5.0 and 9.3% of patch 15N respectively in experiment 1 and 16 and 32%, in experiment 2. However there was no relationship between 15N content of the shoots and ERM length density in the patch in either experiment.

The 15N captured and transferred to the plant was not distributed evenly between roots and shoots (Fig. 2): in both experiments, plants colonised by *G. hoi* with patch compartment access had more 15N in roots than shoots (Experiment 1: t4 = -3.5, *P* = 0.026, Experiment 2: t3 = -3.6. *P* = 0.038). However in plants colonised with *G. intraradices* that had access to the patch compartment, the 15N in roots and shoots was not different (Experiment 1: t4 = 0.32, *P* = 0.77, Experiment 2: t3 = 0.38, *P* = 0.73).

There was an average of 0.14 ± 0.01 mg 13C in plants in experiment 1 and 0.07 ± 0.01 mg in experiment 2 which in both cases is less than 1% of the patch C. There was no significant effect of hyphal access on the amount of 13C in plants.

Shoot N:P ratio (Fig. 4) was lower in experiment 1 than in experiment 2 (F1,30 = 10.6, P < 0.003) and also when AM fungi had access to the patch compartment (Experiment1: *F*1,13 = 4.7, *P* = 0.050; Experiment 2: *F*1,17 = 5.5, *P* = 0.03), but there were no differences among the fungal treatments, except that NM plants had a higher N:P ratio than AM plants in experiment 2 (*F*2,17 = 6.03, *P* = 0.01). N capture by plants was not related to N:P ratio.

**Discussion**

AM fungi have been shown to acquire N from organic sources and to transfer it to their associated host plant (Hodge *et al.,* 2001), but the amounts transferred were small and it has been suggested that arbuscular mycorrhizas are not an important route for N uptake by plants (Reynolds *et al.,* 2005). In the experiments reported here, AM fungi did not increase total plant N capture. However, they did capture large amounts of N from organic material and transfer it to the plant. Not only was a substantial fraction of patch N present in the plant, but this N accounted for a biologically significant part of total plant N content in the case of plants colonised by *G. intraradices,* in which it significantly enhanced plant N concentration in two experiments. The difference in plant N concentration between plants colonised with *G. hoi* and *G. intraradices* is perhaps due to with a decrease in plant size, particularly in experiment 1, rather than a significant enhancement of total N uptake by *G. intraradices*. The results therefore support those of Hodge *et al.* (2001) that AM fungi can acquire N from organic material, and show that the N is likely to be captured in inorganic form, as no 13C was found in the plant material. In addition, we found that *G. hoi* is not unique in its ability to capture N: in fact it was relatively ineffective, and *G. intraradices* transferred a substantially greater amount of patch N to the host plant.

The uptake and use of the N by the AM fungi differed between experiments, possibly because of large differences in the C:N ratio of the organic material used; this finding could help to explain inconsistent results reported in the literature. The patch material of experiment 2 was rich in N and had a similar C:N ratio (<10:1) to the one used by Hodge *et al.* (2001) when the uptake of N by AM fungi from organic material was first demonstrated. In contrast, the C:N ratio of the material in experiment 1 was high (27:1). The C:N ratio of the material being decomposed directly affects the rate of mineralization in soil and the amount of N that is subsequently liberated (Melillo *et al*., 1982), and which is then presumably available for AM fungal uptake. As the C:N ratio of a material is increased, the rate of mineralization and therefore percentage of N captured will decrease (Hodge *et al*., 2000). At a C:N ratio of 27:1 we would expect slow release of N or even immobilisation, in contrast to the rapid decomposition of the material used in experiment 2. There will also have been some inorganic N present in the patch material, and its availability may have been enhanced by drying and milling the material.

Although the patch C:N ratio may explain the difference in absolute uptake of 15N between the two experiments, it does not help to explain the diffusion across the mesh barrier of so much 15N in the first experiment (Fig. 2a). Only inorganic N ions or small organic N compounds could have diffused the distance required to cross the barrier in the time available (Tinker & Nye, 2000). As there were no hyphae present in the patch compartment of control treatments, the 15N must have moved across the mesh barrier by mass flow of water containing soluble products from the patch, mainly as nitrate, into the plant compartment, or because watering led to flow from the patch compartment to the plant. The high level of 15N in control plants of experiment 1 indicates there was more labelled soluble N available, perhaps even before any decomposition took place. However, because we cannot firmly explain these high background levels of 15N in plant material in experiment 1, the following discussion of N capture is based on experiment 2.

The relative availability of N and P differed between the two experiments: in experiment 1 the patch had a high C:N ratio but bonemeal was added to the patch compartment, whereas in experiment 2 there was no bonemeal in the patch compartment but the patch itself had a low C:N ratio. These differences were reflected in the N:P ratio in plant material, which was much higher in experiment 2, showing that N was relatively more available there. Giving access to patch compartment by hyphae reduced N:P ratio in both experiments, suggesting that hyphae were more effective at acquiring P than N, but the only significant increase in P content due to access was with *G. intraradices*. As there is P in the Terragreen used as the growth medium, the extra P taken up when fungi had access to the patch compartment was likely to be from here, rather than the organic material, though we cannot be sure as the P was not labelled in this experiment. In experiment 1, bonemeal in the second compartment was also a source of P.

One third of the 15N in patch material was transferred to the plant by *G. intraradices* when access was allowed in experiment 2, although this is an estimate of gross, not net transfer. This fraction is substantially more than has been observed in other studies (Hawkins *et al*., 2000; Hodge *et al*., 2001). We cannot be sure that the 15N label in N captured by the fungus was in proportion to that in the organic material, because of likely differential labelling of soluble and structural materials. Nevertheless, given that there will also have been capture of 14N through the course of the experiment, we conclude that a significant amount of N was taken up by the AM fungi and transferred to the plant by both *G. intraradices* and, to a lesser extent, *G. hoi*. This leads us to conclude that where an N rich patch of organic material is supplied, AM fungi can capture a significant amount of N from that material. Earlier studies of AM fungi showed extensive proliferation in and around soil organic material (Nicolson, 1959; Mosse, 1959). Their small size (hyphal diameter 2 - 20 μm; Nicolson, 1959; Friese & Allen, 1991) will enable effective penetration of organic material, reducing the distance for diffusion of the products of decomposition. There is no direct evidence for saprotrophy by AM fungi, but they may compete effectively with other micro-organisms in the soil for the N made available by mineralization of organic material.

The amount of 15N transferred to the plants was only a small percentage of the total plant N. However, if we extrapolate the total amount of N from the 15N captured and assume that 14N and 15N were taken up in the proportions present in the patch, then up to 13 ± 4.4 % (*G. hoi*) or 22 ± 7.6 % (*G. intraradices*) of plant N could have come from the patch. These values are comparable with or even larger than what might be expected were roots alone allowed to colonise a similar organic patch (ca. 10%; Hodge, 2004). This calculation could be an overestimate: because 15N was supplied shortly prior to harvest of the grass material, it is likely to have been over-represented in more labile N fractions. Nevertheless, some 14N will also have been captured and it seems likely that that a large proportion of patch N was captured by the fungus and transferred to the plant.

The presence of 15N in the shoots demonstrates unequivocally that patch N was transferred to the plant. However, the proportion of 15N in the shoots was higher when plants were colonised by *G. intraradices* rather than *G. hoi,* indicating that *G. intraradices* transferred more N to the host at the plant/fungal interface. In previous studies where AM fungi have been shown to capture N from organic material, there was very little change to the nutrient status of the plant (Hodge *et al*., 2001; Hawkins, 2000). Here we have shown that *G. intraradices* can enhance the N concentration of its host plant, apparently by uptake of N from a decomposing patch of organic material. In contrast, though *G. hoi* acquired N from the patch, less was transferred to the plant and consequently plant N concentration was unchanged.

The two AM fungi therefore differ in their capacity to take up N and transfer it to their host, but this difference does not apparently arise simply as a consequence of differences in ERM development. In experiment 2, hyphal growth into the patch by the two fungi was similar, yet 15N transfer to the plant was very different. Although we did not measure the hyphal 15N content and so cannot draw conclusions about total fungal uptake, the results show that *G. intraradices* was more efficient at capturing N from the organic patch than *G. hoi* and transferring it to the host plant, which could have consequences for host selection of AM fungal partners. We know that *G. hoi* can influence the decomposition of organic material (Hodge *et al*., 2001), but not the mechanisms for N capture from organic material or the reasons for the difference we have found between the two fungal species.

In soil, where there is competition with other plants and organisms, the ability to proliferate roots near a nutrient rich patch of soil is known to be advantageous (Hodge, 2004). Given the extensive mycelial network that can be formed by AM hyphae through the soil (Smith & Read, 2008), there is a high probability that hyphae will encounter such material before roots. The ability to capture nutrients from it would put the fungus at an advantage, particularly one such as *G. intraradices* which produces large amounts of external hyphae. Ames *et al.* (1983b) suggested that there were different soil N pools available to mycorrhizal and non-mycorrhizal plants. Our data indicate that the identity of the AM fungi involved may determine which N pools are accessible to the symbiosis. That there are differences in P acquisition among fungi is known and a recent challenge has been to look for functional complementarity among fungal species (Violi *et al.,* 2007, Koide, 2000). Perhaps colonisation by more than one species of fungus can be advantageous to the host plant, not only for P (Jansa *et al.*, 2008) but also for N uptake, thus highlighting an important area for future work.

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**Figure 1:** Shoot phosphorus (P) and nitrogen (N) content (mg). No access (white bars) and patch access (black bars) treatments. Bars represent Means ± SE. The two experiments were analysed separately. Letters denote the results of an LSD post hoc test within an experiment.

**(a)** Mean P content in shoots (mg). In experiment 1, species, access and interaction effects were significant (*F1,13*= 12.6, *P* < 0.01; *F1,13*= 13.9, *P* < 0.01; *F1,13*= 9.0, *P* = 0.01). In experiment 2, access (*F1,17*= 11.28, *P* = 0.004) and species (*F2,17*= 8.685, *P* = 0.003) had a significant effect.

**(b)** Mean N content in shoots (mg). In experiment 1 the species (*F1,13*= 22.2, *P* < 0.001) and interaction terms (*F1,13*= 10.5, *P* = 0.007) were significant. In experiment 2 there were no significant differences.

**(c)** The N concentration of total plant material (mg g-1 DW). There interaction term was significant in both experiments (Experiment1: *F1,13*= 31.9, *P* < 0.001; Experiment 2: *F1,17* = 5.8, *P* = 0.008).

**Figure 2**: Amount of 15N in plant shoot (black) and root (white) material in experiments 1 (Fig. a) and 2 (Fig. b). Bars represent Means ± SE. The amount of 15N in shoots versus roots of *G. hoi* was significantly different (Experiment1: *t4* = -3.455, *P* = 0.026; Experiment2: *t3* = -3.572 *P* = 0.038) but plants colonised with *G. intraradices* contained equal amounts in roots and shoots. Letters denote significant differences in mg 15N in total plant (shoots and roots together).

**Figure 3**. The shoot nitrogen to phosphorus (N:P) ratio was higher in experiment 2 than experiment 1 (*F*1,30 = 17.8, *P* < 0.001). Letters denote differences between AM treatments within an experiment. Access decreased the N:P ratio in both experiment 1 (*F*1,13 = 4.7, *P* = 0.05) and experiment 2 (*F*1,17 = 5.5, *P* = 0.003). **Table 1: Patch material composition**: patch material consisted of milled *Lollium perenne* shoots labelled with 15N and 13C.

|  |  |  |
| --- | --- | --- |
|  | Experiment 1 | Experiment 2 |
| % Nitrogen (N) | 1.5 % | 4 % |
| Atom % 15N | 38 At% | 21 At% |
| Total 15N in 1 g patch | 5.7 mg | 8.4 mg |
| % Carbon (C) | 41 % | 38 % |
| Atom % 13C | 2.6 At% | 5.2 At% |
| Total 13C in 1 g patch | 10.7 mg | 19.8 mg |
| C:N ratio | 27:1 | 9.5:1 |

**Table 2**: Percentage colonisation of host plant roots in total and by arbuscules and vesicles, AM hyphal length densities in the organic patch and plant dry weight (DW) in experiment 1 (E1) and experiment 2 (E2).

A significant difference was found between %RLC by fungal species in experiment 1 (*F*1,13 = 80, *P* < 0.001); arbuscule colonisation in experiment 1 (*F*1,13 = 5.5, *P* = 0.035); vesicle colonisation in experiment 1 (*F*1,13 = 844, *P* < 0.001) and experiment 2 (*F*1,11 = 85, *P* < 0.001); extraradical mycelium (ERM) density in the patch in experiment one (*F*1,8 = 5.4, *P* = 0.048);. Different letters denote significant differences within a column except for extraradical mycelium (ERM) density where differences between access treatments only are shown. Letters denote differences within an experiment.

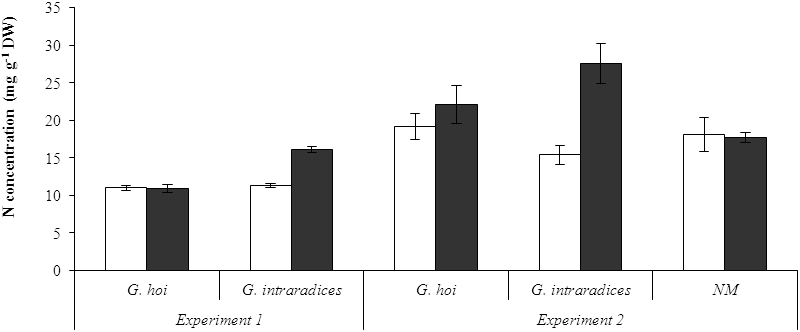
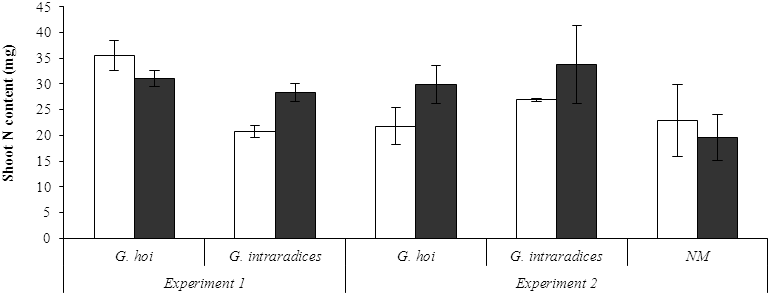
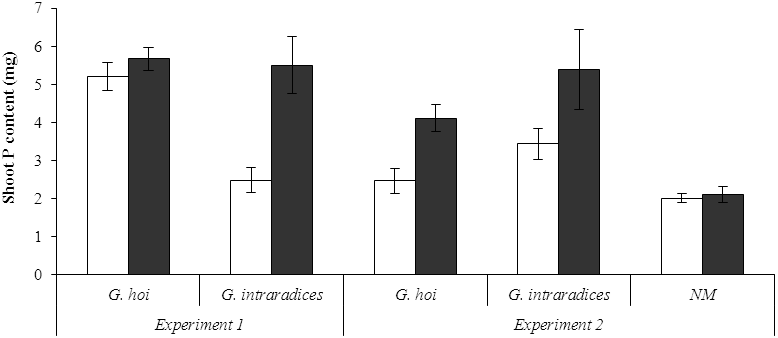
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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | RLC (%)  E1 E2 | | Arbuscules (%)  E1 E2 | | Vesicles (%)  E1 E2 | | Patch ERM density  (m g-1 soil)  E1 E2 | |
| *G. hoi* | No access | 22b | 36a | 4a | 12a | 0.2b | 0.9b | 0.01 | 0.02 |
| Patch access | 14c | 45a | 1b | 11a | 0.1b | 0.5b | 1.5b | 2.7a |
| *G. intraradices* | No access | 46a | 44a | 10a | 9a | 26a | 24a | 0.03 | 0 |
| Patch access | 51a | 63a | 4a | 13a | 35a | 34a | 3.1a | 3.1a |

**Table 3**: Plant dry weight in experiment 1 (E1) and experiment 2(E2). Different letters within a column denote significant differences within an experiment.

|  |  |  |  |
| --- | --- | --- | --- |
|  | | Plant  DW (g)  E1 E2 | |
| *G. hoi* | No access | 5.5a | 2.6a |
| Patch access | 4.5a | 3.2a |
| *G. intraradices* | No access | 3.2b | 3.3a |
| Patch access | 3.0b | 2.8a |
| Non mycorrhizal | No access | - | 2.7a |
| Patch access | - | 2.5a |

**Table 4**: Two-way ANOVA results for mg 15N in total plant material in experiments 1 and 2. The amount of 15N was higher in experiment 2 than experiment 1 (*F*1,17 = 86.7, *P* < 0.001). NM treatments from experiment 2 were not included in the analysis, but were not significantly different from the no access treatments.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Experiment 1 | | Experiment 2 | |
| F | P | F | P |
| AM fungal species | 0.5 | 0.509 | 0.01 | 0.915 |
| Patch access | 10.5 | 0.006 | 264 | < 0.001 |
| AM fungal species x patch access | 7.7 | 0.016 | 5.6 | 0.037 |
| Degrees of freedom | 1, 13 | | 1, 11 | |



a

a

b

a

z

xy

yz

x

z

z

a

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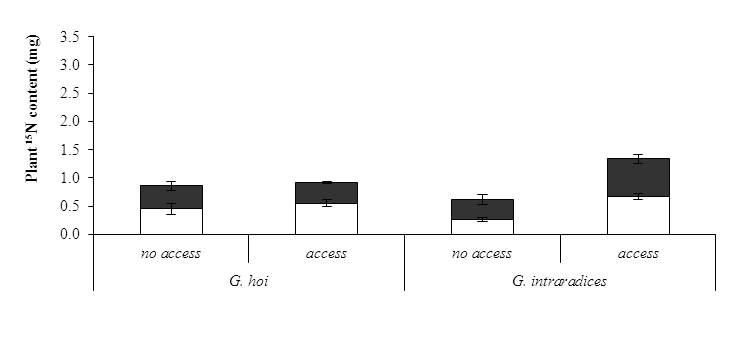
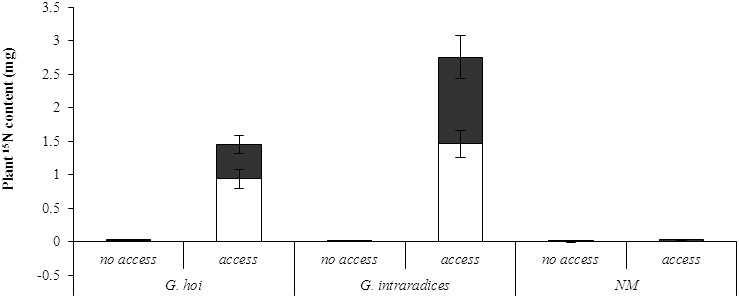
z

**a**

**b**

**c**

**Figure 1**



b

b

b

a

**a**

b

a

c

c

c

c

**b**

**Figure 2**



x

x

y

y

y

a

a

a

a

y

**Figure 3**