Title: Aphids can acquire the nitrogen delivered to plants by arbuscular mycorrhizal fungi.

Thomas D.J. Wilkinson1\*, Julia Ferrari1, Sue E. Hartley1,2 and Angela Hodge1,

1 Department of Biology, University of York, Wentworth Way, York, YO10 5DD, U.K.

2 York Environmental Sustainability Institute, University of York, YO10 5DD, U.K.

\*Author for correspondence: Thomas D. J. Wilkinson, Department of Biology, University of York, Wentworth Way, York, YO10 5DD, U.K. Email: tw645@york.ac.uk

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Summary

1. Above and below ground organisms can interact by altering the quality of shared host plants. Arbuscular mycorrhizal fungi (AMF) influence plant nutrient uptake, including nitrogen (N) acquisition. Under low N and phosphorus conditions, AMF delivery of N from organic sources not immediately available to the plant can have large impacts on plant N status, a limiting nutrient in the aphid diet.
2. This study investigated the effect of AMF colonisation upon aphid number and determined the consequences of AMF directly accessing an organic nutrient patch that the plant cannot. We hypothesised that AMF colonisation of plants will increase plant and aphid N status, plant performance and aphid number, but only when the AMF had direct access to the added organic patch.
3. Barley plants hosting the grain aphid *Sitobion avenae* were colonised by the AMF, *Funneliformis mosseae*,or no AMF. A two compartment microcosm was used to separate the plant roots from a 15N labelled organic patch in a second compartment. AMF colonised plants, but without access to the second compartment, were used to examine the effect of AMF colonisation on aphid number. In a separate treatment, and to determine whether AMF access to a plant inaccessible N source modified the effect of AMF colonisation on aphid number, AMF hyphae were permitted access to the second compartment containing an organic patch. As a control for AMF accessing a larger substrate volume, AMF were allowed access to a second compartment without an organic patch.
4. When the AMF accessed the organic patch, more N from the patch was delivered to the plant resulting in a higher grain N concentration although plant growth was depressed. More N from the patch was also delivered to the aphids, but the N status of the aphid remained unchanged. Regardless of the level of access to the organic patch, AMF colonisation did not affect aphid number.
5. Our data show that by accessing N sources not readily available to plants, AMF can indirectly deliver N to above ground organisms, a finding which has major implications for N transfer between higher trophic levels.

Key words

Arbuscular mycorrhizal fungi, *Funneliformis mosseae*, *Hordeum vulgare*, multitrophic interactions, 15N, nutrient acquisition, *Sitobion avenae*, stable isotopic labelling.

**Introduction**

Via the conduit of a shared plant host, multitrophic interactions can occur among a wide range of below and above ground organisms including bacteria, fungi, invertebrates and vertebrates (Gehring & Bennett 2009; Pineda *et al.* 2012; van der Heyde *et al.* 2017). Due to the diverse organisms that exhibit interactions across the above-below ground interface, these interactions have large implications in key ecological processes such as nutrient cycling (Hodge & Fitter 2010; Lau 2011; Grabmaier *et al.* 2014), potent greenhouse gas emissions (Bender, Conen & van der Heijden 2015; Storer *et al.* 2018) and both population and community structure (Gehring & Bennett 2009).

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that form a mutualistic relationship with the roots of around two thirds of plant species (Hughes *et al.* 2008). AMF benefit their associated plant host in a number of ways including, aiding nutrient acquisition (Harrison, Dewbre & Liu 2002; Leigh, Hodge & Fitter 2009) and resistance to biotic (Koricheva, Gange & Jones 2009; Yang *et al.* 2014; Mauch-Mani *et al.* 2017) and abiotic pressures (Zhang *et al.* 2005; Talaat & Shawky 2012) in return for fixed carbon (C) from the plant. AMF colonisation can also alter the performance of many foliar-feeding herbivores including aphids (Hartley & Gange 2009). Impacts of AMF on aphid performance can be positive (Gange & West 1994; Simon *et al.* 2017), negative (Guerrieri *et al.* 2004; Hempel *et al.* 2009) or have no effect (Williams, Birkhofer & Hedlund 2014; Karley, Emslie-Smith & Bennett 2017). It is currently not fully understood what mechanisms cause such context specific outcomes, but varying plant-AMF-aphid species, genotypes or environmental conditions may play a role. Although not in direct contact, aphids and AMF could affect the performance of the other via altering the quality of the plant host through modulating defence responses, and/or the plant’s phosphorus (P) (Smith & Read 2008) and nitrogen (N) (Thirkell, Cameron & Hodge 2016) status.

N is a limiting factor in an aphid’s diet of phloem sap; increased N fertilisation has been shown to increase the performance of *Rhopalosiphum padi* on barley (*Hordeum vulgare* L.) (Ponder *et al.* 2000) and *R. padi* and the English grain aphid (*Sitobion avenae* (Fabricius)) on wheat (Triticum aestivum) (Aqueel & Leather 2011). Thus, if AMF altered the plant’s N concentration, and hence, the amount of N an aphid had access to per unit time of feeding, this could significantly impact upon aphid performance. However, how increased N fertilisation affects aphid performance may depend on the plant species (Bogaert *et al.* 2017) and aphid morph (Khan & Port 2008). Moreover, the effect of changes to plant host N by AMF upon aphids shows no general trend in the literature (Hempel *et al.* 2009; Babikova *et al.* 2014; Williams, Birkhofer & Hedlund 2014; Tomczak & Muller 2017), perhaps due to concurrent changes to aphid feeding behaviour, how the N is allocated in the plant tissue or whether it is partitioned into plant defence responses.

The distribution of nutrients in soil is both spatially and temporally heterogeneous. Thus, nutrients do not occur in a uniform manner but as discrete zones or ‘patches’. Plant root systems have to cope with this heterogeneity in order to acquire the nutrients they need (Hodge 2004), while AMF hyphae, by virtue of small size and large surface area, may aid the plant in this nutrient acquisition role through being able to physically access nutrient sources the plant cannot (Hodge & Storer 2015; Hodge 2016). Under poor nutrient conditions, AMF facilitated N-transfer to their associated host plant contributes substantially to the plants N supply, especially when the AMF are able to access an organic nutrient patch that the roots cannot (Leigh, Hodge & Fitter 2009; Thirkell, Cameron & Hodge 2016). Accessing these organic nutrient patches may also affect plant P status (Barrett, Campbell & Hodge 2014; Thirkell, Cameron & Hodge 2016), and although the benefit of P fertilisation on aphid performance is conflicting (Jansson & Ekbom 2002; Venter *et al.* 2014), this could contribute to the growth response of host plants to AMF colonisation.

However, AMF associations with barley in pot systems often induce plant growth depressions (Smith & Smith 2011). Although growth responses may depend on many plant and AMF partner traits, this is potentially in part due to the fine root structures of cereals resulting in efficient nutrient acquisition. Hence, the AMF symbiosis may not provide a nutritional benefit to the plant that outweighs the C cost of maintaining the symbiosis (Smith & Smith 2011), which may be further exacerbated in pot systems where the plant roots are able to explore the majority of the soil volume. Therefore, to fully understand if AMF directly alter aphid performance via nutrient acquisition, we need to determine the effects of AMF access to otherwise plant inaccessible nutrient sources on aphid performance.

In this study, we investigated the effect of AMF colonisation of barley upon plant host biomass and above ground N and P nutrition, and on the number of *S. avenae* sharing the plant host, as a surrogate for aphid performance. We investigated whether these plant and aphid characteristics depended upon the AM fungus being able to directly access N contained in organic nutrient patches. Finally, we used stable isotope labelling to assess, for the first time, whether AMF indirectly transfer this N to trophic levels higher than the plant. Specifically the following hypotheses were tested: 1) when the AMF are denied direct access to the organic nutrient patch, AMF colonisation will not enhance nutrient transfer from the patch to the plant and consequently will have no effect on plant N and P status or aphid number. Instead, plant growth will be depressed by the AMF acting as a C drain on the plant for no nutritional benefit. Conversely, 2) when AMF are able to directly access the organic patch, the increased nutrient availability will increase plant biomass, plant N and P status, and aphid number. Finally, 3) when AMF are able to directly access the organic patch, more N will be transferred, as determined by 15N movement, from this below ground patch to the above ground tissues of the plant and then to its associated aphid herbivores.

**Materials and methods**

**Experimental design**

A two compartment microcosm design was used to manipulate three aspects of the plant-AMF symbiosis: 1) whether the plant was colonised by AMF (+AM) or not (-AM), 2) the impact of access to increased physical volume on the nutrient foraging availability of the AMF (+Access/ -Access) and 3) whether this access led to direct AMF access to an organic nutrient patch (+Patch/-Patch) (Fig 1). Due to the nature of the hypotheses being tested the design was not fully factorial (Table 1). Comparison of non-AMF plants (-AM) with AMF plants where both roots and AMF access were confined to the plant compartment (+AM, -Access) allowed hypothesis 1 to be tested. As the ‘+Access’ treatment only refers to the access by the AMF hyphae and not plant roots, and neither ‘Access’ treatment impedes the movement of small solutes, an ‘Access’ treatment with respect to the ‘-AM’ treatment is redundant. Hypothesis 2 was investigated by comparing non-colonised plants (-AM) with plants colonised by AMF allowed to directly access the organic patch in the second compartment (+AM, +Access). To minimise differences in nutrient availability in the plant compartment due to the mass flow or diffusion of solutes from the organic patch, an organic patch was added to the ‘-AM’ and ‘+AM, -Access’ as well as the ‘+AM, +Access’ treatment. As the effect of AMF access to, rather than the presence of an organic nutrient patch was investigated, ‘-Patch’ treatments were not combined with ‘-AM’, and ‘+AM, -Access’ treatments. However, when the AMF has access to the second compartment (+AM, +Access) it also has access to a larger volume of growth substrate. Thus a ‘Volume Control’ was generated by allowing AMF access to the second compartment, but with no organic nutrient patch present.

**Microcosm design**

Microcosms were adapted from the design by Hodge & Fitter (2010), and comprised of two identically sized polypropylene compartments each measuring 14 x 14 x 13 cm. A 6 x 4 cm window was cut into the adjoining sides of the compartments and covered with a double layer of either 20 µm (John Stanier and Co., Whitefield, Manchester, UK) or 0.45 µm (Santa Cruz Biotechnology, Dallas, Texas, US) nylon mesh used to control the access of AMF hyphae to the second compartment. The 20 µm mesh permits AMF to penetrate from the plant compartment into the second compartment (used in AMF ‘+Access’) treatments whereas the 0.45 µm does not (used in AMF ‘-Access’ treatments).

**AMF inoculation and plant growth**

Silica sand was mixed 1:1 (v/v) with Agsorb® (a calcinated, attapulgite clay particle soil conditioner (Oil-Dri, Cambridgeshire, UK)) washed twice with deionised water and inoculated with AMF to produce the growth substrate added to the plant compartment of AMF treatments (+AM).

The AMF inoculum was *Funneliformis mosseae* (Plantworks UK Ltd. Kent, UK), added as colonised roots of *Plantago lanceolata* L. and *Trifolium pratense* L. in a sand: Agsorb® medium (1:1 (v/v)). Bonemeal (0.25 g L-1; Vitax Leicestershire, UK) was also added as a complex N and P source to encourage AMF development (Hodge & Fitter 2010).

The growth substrate for the plant compartments of non-AMF treatments (-AM) were set up in the same manner except the AMF inoculum was autoclaved twice at 121°C. In an attempt to equalise starting non-fungal microbial communities between +AM and -AM treatments, prior to autoclaving the AMF inoculum was washed with dH2O, filtered through 20 µm nylon mesh to remove large particles, then subsequently filtered through Whatman No.1 filter paper (pore size 11 µm, Whatman plc, Maidstone, Kent, UK) and 10 mL of the resulting filtrate added to -AM treatment plant compartments (as Hodge 2001).

Sand and Agsorb® was washed and mixed as previously described and added to the second, unplanted, compartment except no AMF inoculum, filtrate or bonemeal was added. Aluminium foil was used to cover the surface of the second compartment to reduce evaporation and algal growth.

Three barley seeds (cultivar: Xanadu, SAATEN-UNION GmbH, Isernhagen, Hanover, Germany) were surface sterilised in 4% (w/v) sodium hypochlorite solution before being germinated in the centre of the plant compartment. Seedlings were thinned to one per pot after one week and fed weekly with 50 mL of a low N and low P nutrient solution (see Leigh, Hodge & Fitter 2009) to produce a low N and P system where the effects of N acquisition from an organic patch via AMF may be substantial (Thirkell, Cameron & Hodge 2016). Microcosms were arranged in a randomised block design in a lit and heated glass house with a 16 hour day length on the 27/04/16. Plants were treated and harvested in block order and watered with up to 100 mL dH2O twice weekly as required.

**15N-labelled organic patch material generation and addition**

Barley seedlings (cultivar: Optic, Syngenta UK Limited, Cambridgeshire, UK) were labelled with 15N to produce labelled organic material for use as the organic ‘patch’. Briefly, barley seedlings were grown in sand and Agsorb® medium and fed with nutrient solution (Thornton & Bausenwein 2000) containing 1:1 14NH414NO3 : 98 atom % 15N 14NH415NO3 (Sigma Aldrich, St. Louis, Missouri, United States). The resulting labelled shoot material was dried at 70°C, homogenised in a ball mill (Retsch MM400, Retsch GmbH, Haan, Germany) and analysed by Isotope Ratio Mass Spectrometry (I.R.M.S) (PDZ 2020, Sercon Ltd, Crewe, UK). The shoot material contained 0.52% P, 2.1% N of which 4% was 15N.

A 7 cm deep, 1 cm diameter core of growth substrate, 2 cm away from the centre of the mesh window was removed from the second compartment two weeks after the seedlings were thinned to one seedling per pot. For ‘+Patch’ treatments 2.2 g of the 15N-labelled milled shoot material was added into the resulting space, as a discrete layer or ‘patch’ and the cored substrate replaced on top of the patch material. The organic material added contained 45.98 mg N, of which 1.82 mg was 15N. The ‘-Patch’ treatments were treated in the same manner, although no organic material was added and the removed substrate was simply replaced.

**Aphid rearing and treatment**

After 8 weeks growth, one week prior to aphid addition, the outline of all experimental plant live leaves were traced on 100 g m-2 paper and subsequently weighed to give a non-destructive estimate of leaf area. Adults of a single genotype of *S. avenae* (genotype K, originally supplied by Koppert Biological Systems, Netherlands) raised on barley (cultivar: Optic) at 20°C were transferred to 2 week old seedlings with roots fixed in 2 % agar in water in a Petri dish, and allowed to birth for 48 hours before being removed. The resulting offspring were allowed to grow on the seedlings for 6 more days. Three of these 7 day ± 1 day aphids were transferred to the base of the stem of the main tiller of each experimental plant and a muslin mesh supported by bamboo canes was placed over all plants.

**Harvest and analysis of plant, aphid and AMF traits**

Two weeks after aphid addition and 11 weeks after planting, the aphids were removed from plants with a fine paintbrush and the number found on the stem and leaf fraction, or on the grain fraction recorded, before flash freezing and storage at -80°C. A 1 cm diameter core was taken at the site of the ‘patch’ to determine the hyphal length density (HLD) in this zone. Plants were separated into root, stem, leaf and grain fractions. Sub fractions of roots were stored in 40 % ethanol for AMF staining. The remaining plant material was oven dried at 70°C for at least 72 hours.

Aphids were lyophilised for 24 hours using a Lyotrap (LTE Scientific Ltd, Oldham, UK) and analysed via I.R.M.S for bulk and 15N using a Finnigan DELTA plus XP (ThermoFischer Scientific, Waltham, Massachusetts, USA). Plant material was milled to a fine powder before being pelleted for X-ray fluorescence (XRF) to assess P concentration using a Thermo Scientific TM portable X-ray fluorescence analyser (as described in Reidinger, Ramsey & Hartley (2012)) and also for bulk N and 15N analysis via I.R.M.S. Growth substrate cores were processed for HLD according to Staddon, Fitter & Graves (1999), and HLD estimated using the gridline intercept method (Hodge 2003). However, the dried growth substrate was lost and so the HLD was calculated per fresh weight rather than dry weight. Roots were stained for AMF colonisation assessment using the acetic acid-ink staining method (Vierheilig *et al.* 1998) but with the following modifications: roots were rinsed with dH2O then incubated in 10 % KOH at 70°C for 45 min or until the roots became translucent. Roots were thoroughly rinsed with dH2O again, before being stained with 5% acetic acid, 5% Pelican 4001 Brilliant Black ink® (Pelikan International, Feusisberg, Switzerland) and 90% dH2O for 30 minutes. Root length colonisation (RLC) was assessed using the line intercept method under 200 x magnification using a Nikon eclipse 50i upright microscope (Nikon UK Ltd, Surrey, UK) and a minimum of 100 intercepts (Hodge 2003).

**Statistical analysis**

Analysis was carried out using R version 3.3.2 (2016-10-31) (R Core Team 2016), and the packages, "lme4", “lmerTest”, “lsmeans”, “car”, “userfriendlyscience, “multcomp” and “multcompView”. A Levene’s test was used to test data for equal variances and where this assumption was correct, data were analysed using linear models with treatment (as detailed in Table 1) as the explanatory variable. Block was added as a random factor if the model containing block had a significantly lower AIC value. A Shapiro-Wilks test was used to test for the normal distribution of model residuals and when these were not normally distributed, percentage/proportion data were logit or square root transformed and concentration data log transformed, which allowed the assumptions of the linear model to be met. A Tukey *post hoc* test was used where appropriate. A Welch’s ANOVA with a Games Howell *post hoc* test was used to analyse data with unequal variances. For the analysis of AMF traits the ‘-AM’ data was not included, and for 15N data ‘Volume control’ treatment data was not included in the model as data followed a baseline and were thus distributed similar to zero values. Initially plants were set up with 10 replicates per treatment although for one replicate of the ‘Volume control’ treatment aphids did not reproduce and this replicate was removed from the subsequent analysis.

**Results**

**AMF characteristics**

Plant roots of +AM treatments were well colonised (*c*. 49 ± 2.9 S.E % RLC; 13 ± 1.6 % vesicles and 15 ± 1.6 % arbuscules; see Fig S1 in Supporting information ), whilst no AMF colonisation was found in the ‘-AM’ treatment. Neither access to, or the presence of, an organic patch in the second compartment influenced RLC, or the percentage of vesicles and arbuscules (*F2,26* = 0.16 , *P* = 0.82; *F*2,26 = 0.24, *P* = 0.79; *F*2,26 = 0.62, *P* = 0.55 respectively). However, AMF access to the second compartment, and the presence of the organic patch influenced HLD in the ‘patch zone’ (*F*2,25 = 6.4, *P* < 0.01). When the AMF had access to the second compartment with an organic patch (+AM, +Access), the HLD values were significantly greater (0.41 ± 0.07 m hyphae g-1 fresh substrate) than when no AMF access was permitted (+AM, -Access: 0.13 ± 0.06 m hyphae g-1 fresh substrate), whilst HLD in the treatment with AMF access to the second compartment without an organic patch (Volume control) did not significantly differ from either of these treatments (0.29 ± 0.08 m hyphae g-1 fresh substrate).

**Plant characteristics**

To investigate the effects of AMF colonisation on plant biomass and above ground nutrient status when the AMF cannot directly access the organic nutrient patch, a treatment where the AMF was restricted to the plant compartment (+AM, -Access) was included in the experimental design. Compared to non-AMF plants (-AM), this treatment (+AM, -Access) resulted in a reduction of live plant leaf area one week prior to aphid addition (38.9 ± 5.7 cm2 c.f. 53.9 ± 3.9 cm2; *F*3,26.1 = 4.7, *P* <0.01). In contrast, the other +AMF colonisation treatments did not differ from the non-AMF plants. The +AM, -Access plants also showed reduced total dry weight at harvest compared to non-AMF plants, but increased grain, stem and leaf P concentrations (Table 2). However, neither the grain N concentration, or the amount of grain N originating from the organic patch, of the +AM, -Access plants differed significantly from that of the non-AMF plants (Fig 2.; Table 3), but the *proportion* of grain N derived from the patch for these plants was lower than any other treatment (Fig 3a). No treatment altered the N concentration of the stem and leaf (Table 2), the stem and leaf N:P ratio (1.16 ± 0.05), or the grain N:P ratio (3.01 ± 0.05) (*F*3,32 = 1.1, *P* = 0.37, and *F*3, 33 = 1.1, *P* = 0.37, respectively).

To investigate the effect of AMF colonisation when the AMF can directly access more nutrients than the plant hosts roots, the AMF were allowed to access a second compartment containing an organic nutrient patch (+AM, +Access). This did not alter total plant biomass compared to non-AMF (-AM) plants, but increased above ground P concentrations (Table 2). Moreover, the grain N concentration was increased (*c*. 28%) compared to non-AMF plants (Fig. 2), and more grain N was derived from the organic patch (Table 3). Consequently, the proportion of grain N that originated from the organic patch was highest in this treatment (7.8 %± 0.93; Fig. 3a).

The volume control was included and allowed AMF access to the second compartment but without an organic nutrient patch present. This did not alter grain N or P concentrations compared to the other treatments, but, unlike when the AMF was completely denied access to the second compartment (+AM, -Access), did not induce a reduction of grain biomass compared to non-AMF (-AM) plants (Table 2). Moreover, the grain biomass of the volume control’ was higher than the other AMF colonised treatments, although the mean grain biomass was highest when plants were not colonised by AMF (-AM). However, a Games Howell *post hoc* test did not identify this as being significantly different from that of the ‘+AM, +Access’ treatment (*P = 0.051*) (Table 2).

**Aphid Characteristics**

The majority of the aphids were found feeding upon the grain (80% ± 0.03 S.E), and the mean number of aphids feeding on each plant (51.8 ± 2.7, *F*3,35 = 0.09, *P* = 0.97), total aphid biomass (7.1 ± 0.5 mg, *F*3,35 = 0.24, *P* = 0.87), aphid N concentration (62.3 ± 1 mg g-1, *F*3,35 = 1.1, *P* = 0.38) and the total N content in the aphid population (0.44 ± 0.03 mg, *F*3.35 = 0.005, *P* = 0.99) did not differ amongst any treatments. However, allowing the AMF to access the organic patch material (+AM, +Access) resulted in more N being delivered to the aphid population (Table 3) and more of the N in the aphid being derived from the organic patch (7.21 % ± 0.95 S.E., Fig. 3b).

**Discussion**

This study used a two compartment microcosm design to investigate the direct effect of AMF access to a complex N source on barley and its associated aphid herbivores under low N and P conditions. We reconfirmed that by directly accessing nutrient patches that the plant cannot access, AMF may deliver previously unavailable N from such patches to the host plant (see Barrett, Campbell & Hodge 2014). We also demonstrate, for the first time, that this may also be true for herbivores sharing the host plant. It was hypothesised that the delivery of this N would lead to an increase in plant performance and aphid number. This was reflected in some aspects of the plant’s performance, but interestingly, not in the aphids feeding upon it.

**Effects of direct AMF access to organic nutrient patches on plant growth and nutrition**

We hypothesised that when the AMF were denied access to the second compartment containing an organic patch, the cost of the AMF symbiosis to the plant would outweigh its nutritional benefits. Supporting this hypothesis, plants colonised by AMF restricted to the plant compartment derived the least amount of N from the organic patch, did not increase above ground N concentrations, and displayed the most severe growth depression compared to non-AMF plants (Tables 2 and 3). Whilst the above ground P concentrations were higher in these ‘+AM, -Access’ plants than in non-AMF plants, this could be due to reduced plant biomass. Conversely, we hypothesised that increased nutrient transfer to the host plant would occur when the AM fungus could directly access the organic patch.

We cannot disentangle whether the increased above ground P concentrations in plants colonised by AMF accessing the organic patch is due to reduced biomass or increased P transfer from the patch, but as expected, these plants were the most highly enriched with the 15N used to label the patch material. This suggests that the increase in grain N concentration above that of the non-AMF plants was not due to reduced plant biomass, and as the live leaf area of these treatments did not differ, it is likely that differences in 15N enrichment are due to AMF mediated delivery and not driven by mass flow.

Contrary to expectations, this AMF mediated delivery of nutrients from the organic patch did not positively impact plant biomass. Moreover, the grain biomass was reduced in comparison to when the AMF were allowed to access the second compartment but without a nutrient patch present. AMF may become more beneficial to the plant host with increasing pot size (Audet & Charest 2010; Zangaro *et al.* 2015), and the current study provides evidence that this is due to the AMF being able to explore growth substrate beyond the reach of the plant roots. However, AMF have relatively high N demands and can act as a sink for patch derived N (Hodge & Fitter 2010; Herman *et al.* 2012), hence, under low N conditions, as used in this study, AMF may become less mutualistic (Johnson *et al.* 2010; Puschel *et al.* 2016), perhaps due to AMF fulfilling their own nutritional requirements first, before passing nutrients to the host. Thus, it is possible, given that AMF proliferate their hyphae extensively upon organic patch interception (Ravnskov *et al.* 1999; Hodge, Campbell & Fitter 2001), that this may have drawn more carbon from the plant host without an equal return in N, resulting in the growth depression found when the AMF had access to the organic patch present in the second compartment.

This plant biomass reduction contrasts to previously reported effects in a low N and P context (Thirkell, Cameron & Hodge 2016). Evidence suggests that AMF rely upon the plant host for the energetically expensive process of fatty acid synthesis (Keymer & Gutjahr 2018), although nutritional and plant–AMF partner genetic contexts also play roles in whether plant growth depression occurs (Jin *et al.* 2017). This imbalance may have been exacerbated by above ground herbivory leading to increased C allocation to the roots (Wamberg, Christensen & Jakobsen 2003).

**The effect of direct AMF access to nutrients on aphid number and N status**

We hypothesised that the nutritional benefit of AMF directly accessing an organic patch would be passed on to the plant’s associated herbivores. The amount of N derived from the patch in the aphid closely relates to that of the grain (i.e. 7.2 %; aphid v. 7.8%; grain), which reflects where the majority of aphids were found. This suggests that the AMF transfer of N from the patch to the grain, leading to increased grain N concentration, made more N available to the aphid per unit time. This change in concentration (*c.* 28 % between non-AMF plants and AMF plants accessing the organic patch) is similar in magnitude to changes in leaf N concentrations of *Miscanthus* species in which altering N regimes impacted aphid performance (Bogaert *et al.* 2017). However, in the present study, aphid number and N status remained unchanged, suggesting that the aphids did not make use of the extra N made available to them by the AMF, and that aphid and plant N status can be uncoupled. One caveat of our experimental design is that we measured aphid numbers rather than more detailed components of aphid performance (such as survival or individual fecundity), which may might reveal more subtle effects of increased N concentration.

During their life cycle, aphids can compensate for initial reductions in their biomass that are caused by AMF induced changes to plant nutrition (Tomczak & Muller 2017). The ratio of essential to non-essential amino acids in the phloem is a driver of the host plants nutritional quality to aphids (Douglas 2006), with the symbiotic bacteria *Buchnera aphidicola* synthesising essential amino acids lacking from this diet (Guenduez & Douglas 2009). Thus, the composition of essential amino acids in the phloem, the synthesis of amino acids by *B. aphidicola,* and the aphid’s ability to alter its length of feeding (Ponder *et al.* 2000), could all influence any effects of altered host plant tissue nutrient concentrations on aphid performance. Although we were not able to measure amino acid availability to the aphid (Johnson, Ryalls & Karley 2014), by tracking and measuring the N status of the aphid alongside that of their host plant, we show that, at least in certain cases, aphids can adjust their overall N intake when the nutritional quality of a plant host is altered by AMF colonisation. This may aid in explaining the contrasting effects reported of AMF-induced changes to plant host N nutrition upon aphid performance (Hempel *et al.* 2009; Babikova *et al.* 2014; Williams, Birkhofer & Hedlund 2014), alongside potential simultaneous synergistic or confounding effects of AMF-induced changes to plant defence (Mauch-Mani *et al.* 2017). For example, a significant defence for barley, as for many grasses, is silicon (Hartley & DeGabriel 2016) and levels of foliar silicon have been shown to be increased by the presence of AMF (Anda, Opfergelt & Declerck 2016). Silicon uptake is likely to be relatively unaffected by any changes in plant growth-defence trade-offs resulting from colonisation (Simpson *et al.* 2017), though reductions in levels of other barley defences in relation to nutrient content and growth have been observed (Norbaek *et al.* 2003).

Although the nutritional status of the growth medium in our microcosm was low compared to a conventionally managed agriculture systems, our results demonstrate that via the shared host plant, AMF can transfer N derived from complex N sources not directly available to the plant to higher trophic levels. This supports our hypothesis that direct AMF access to organic nutrient patches will result in more N being transferred to above ground organisms and highlights the importance of AMF in below-above ground N cycling.

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**Author’s contributions**

All authors contributed to the experimental design of the study and writing of the manuscript. T.W. conducted the experiment and analysed the data.

**Disclosure**

The authors declare no competing interests.

**Data Accessibility**

Data will be archived in the Dryad Digital Repository; entry doi: xx.xxxx/dryad.xxxx (TBC)

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Figure S1 a) and b) Photomicrographs of the arbuscular mycorrhiza (AM) fungus *Funneliformis mosseae* in roots of barley.

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| Figure 1. Illustration of the two compartment experimental microcosm unit. Plant roots are confined to the plant compartment only, while arbuscular mycorrhizal fungi (AMF) hyphae (blue lines) are permitted (20 µm mesh) or denied (0.45 µm mesh) access to the second compartment. The second compartment contained a 15N labelled organic nutrient patch of milled barley shoot material except in the ‘Volume control’ treatment which was included to examine the impact of AMF access to increased physical space on the AMF-plant-aphid interaction. The system investigates the effect of direct access of AMF to nutrients by allowing the AMF, if present, access to the second compartment containing an organic patch. |

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|  |
| Figure 2. Mean grain N concentration of AMF colonisation and AMF access treatments at harvest. Different letters indicate significant differences at *P* = 0.05 based on a Tukey *post hoc* test. Error bars are ± standard error of the mean (n = 10, except for ‘Volume control’ treatments where n = 9). AMF colonisation and access to the organic nutrient patch in the second compartment significantly increased the grain N concentration (*F3,35* = 7.2, *P* < 0.001). |

|  |  |
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Figure 3. Mean percentage (%) of nitrogen (N) in the A) grain and B) aphid tissues derived from the added organic patch of AMF colonisation and AMF access treatments at harvest. Different letters indicate significant differences at *P* = 0.05 based on a Tukey *post hoc* test. Error bars are ± standard error of the mean (n = 10). AMF colonisation with access to the organic nutrient patch in the second compartment significantly increased the proportion of N derived from the organic nutrient patch contributing to the grain and the aphid (*F2,27* = 21.7, *P* < 0.001; *F2,27* = 8.7, *P* < 0.01; respectively).

Table 1. AMF colonisation, AMF access to the second compartment and patch presence treatment combinations. Arrow direction represents the hypothesised effect of the treatment compared to the non-colonised plant. ‘=’ represents no effect, ‘NA’ represents no hypothesis in comparison to the ‘-AM’ treatment.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment name | Treatment design | | |  | Purpose of treatment |  | Hypothesised effect compared to ‘-AM’ | | | |
|  | AMF colonisation | Potential AMF access to second compartment | Organic patch presence |  |  |  | Plant biomass | Plant N and P | N delivery from the patch above ground | Aphid number |
| -AM | None | +Access | +Patch |  | Control for hypotheses 1), 2) and 3).  As plant roots are always restricted to the plant compartment by the use of meshes, a ‘-AM, -Access, -Patch’ treatment is redundant. |  | NA | NA | NA | NA |
| +AM,  -Access | Yes | -Access | +Patch |  | Tests hypothesis 1) and 3). Compare with (-AM) to test the effect of AMF colonisation when AMF cannot directly access more nutrient sources than the plant. |  | ↓ | = | = | = |
| +AM, +Access | Yes | +Access | +Patch |  | Tests hypothesis 2) and 3). Compare with (-AM) to test effect of AMF colonisation when the AMF can directly access nutrient sources the plant cannot. |  | ↑ | ↑ | ↑ | ↑ |
| Volume control | Yes | +Access | -Patch |  | ‘Volume control’ for hypothesis 2). Compare with (+AM, -Access) to test for the effect of AMF exploring more physical volume (growth substrate). If the volume of growth substrate has no effect, plant and aphid nutrient responses will not differ from ‘+AM, -Access’. If volume does have an effect, then comparison with ‘+AM, +Access’ enables the effects of the nutrient patch to be studied. |  | ↓ | = | NA | = |

Table 2. Plant biomass (dry weight; dw) and above ground tissue nutrient responses (mean ± 1 standard error) to AMF colonisation, AMF access to the second compartment and organic patch presence at harvest. Different letters within rows indicate significant differences at *P* = 0.05 using a Tukey, or a Games Howell *post hoc* test and are shown in bold. D.F = degrees of freedom.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | AM, access and organic patch presence or absence | | | | | Test statistics | | |
|  | -AM | +AM, -Access | +AM, +Access | Volume Control |  | D.F. | *F* | *P* |
| Root dw (g) | 0.35 ± 0.04 | 0.23 ± 0.02 | 0.27 ± 0.04 | 0.28 ± 0.04 |  | 3,35 | 1.92 | 0.145 |
| Stem and leaf dw (g) | 1.20 ± 0.16**b** | 0.65 ± 0.06**a** | 0.79 ± 0.09**a** | 0.94 ± 0.07**ab** |  | 3,35 | 5.12 | **0.005** |
| Grain dw (g) | 1.43 ± 0.15**cb** | 0.91 ± 0.07**a** | 0.95 ± 0.03**b** | 1.34 ± 0.08**c** |  | 3,17 | 8.45 | **0.001** |
| Total plant dw (g) | 2.98 ± 0.33**b** | 1.80 ± 0.15**a** | 2.01 ± 0.13**ab** | 2.56 ± 0.17**b** |  | 3,19 | 5.67 | **0.006** |
| Stem and leaf P concentration (mg g-1) | 2.09 ± 0.13**a** | 2.69 ± 0.19**b** | 3.21 ± 0.18**b** | 2.80 ± 0.17**b** |  | 3,33 | 8.45 | **<0.001** |
| Grain P concentration (mg g-1) | 3.09 ± 0.12**a** | 3.61 ± 0.14**b** | 3.77 ± 0.10**b** | 3.52 ± 0.09**ab** |  | 3,34 | 6.18 | **0.002** |
| Stem and leaf N concentration (mg g-1) | 2.55 ± 0.30 | 2.92 ± 0.53 | 3.76 ± 0.49 | 3.61 ± 0.42 |  | 3,35 | 2.23 | 0.103 |

Table 3. Above ground plant and aphid 15N tissue responses (mean ± 1 standard error) to AMF colonisation and AMF access to the second compartment containing an organic patch at harvest. Note aphid 15N enrichment is expressed as ng whilst other 15N enrichments are expressed as µg. Different letters within rows indicate significant differences at a level of *P* = 0.05 using a Tukey *post hoc* test and are in bold. DF = degrees of freedom.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | AM colonisation and access | | | | Test statistics | | |
|  | -AM | +AM, -Access | +AM, +Access, |  | D.F. | *F* | *P* |
| Total shoot 15 N enrichment (µg) | 16.23 ± 2.27**b** | 5.73 ± 2.02**a** | 43.83 ± 7.24**c** | 2,27 | 22.05 | **<0.001** |
| Stem and leaf 15 N enrichment (µg) | 1.87 ± 0.30**b** | 0.71 ± 0.25**a** | 6.77 ± 3.05**b** | 2,27 | 13.90 | **<0.001** |
| Stem and leaf N derived from patch (%) | 1.88 ± 0.35**ab** | 0.89 ± 0.30**a** | 4.12 ± 0.73**b** | 2,27 | 11.32 | **<0.001** |
| Grain 15 N enrichment (µg) | 14.36 ± 2.15**a** | 5.02 ± 1.97**a** | 37.06 ± 5.28**b** | 2,27 | 22.36 | **<0.001** |
| Aphid 15 N enrichment (ng) | 0.50 ± 0.12**a** | 0.45 ± 0.18**a** | 1.36 ± 0.28**b** | 2,18 | 11.68 | **<0.001** |