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An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material

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Arbuscular mycorrhizal fungi (order Glomales), which form mycorrhizal symbioses with two out of three of all plant species[1], are believed to be obligate biotrophs that are wholly dependent on the plant partner for their carbon supply[2]. It is thought that they possess no degradative capability and that they are unable to decompose complex organic molecules, the form in which most soil nutrients occur. Earlier suggestions that they could exist saprotrophically were based on observation of hyphal proliferation on organic materials[3,4]. In contrast, other mycorrhizal types have been shown to acquire nitrogen directly from organic sources[5–7]. Here we show that the arbuscular mycorrhizal symbiosis can both enhance decomposition of and increase nitrogen capture from complex organic material (grass leaves) in soil. Hyphal growth of the fungal partner was increased in the presence of the organic material, independently of the host plant.

Nitrogen (N) is a critical limiting nutrient in many ecosystems[8]. Plants capture N largely in inorganic form, relying on microbes to release inorganic N (as NH_4^+) during decomposition of organic material. However, most N in soils is in organic form, often occurring in complex molecules. Some plants can take up simple, soluble organic N compounds[9,10] and others can use organic N sources directly by association with specialist mycorrhizal fungi[11]. To date, this mycorrhizal short cut in the N cycle has been shown only for ectomycorrhizas[5] (about 5% of plant species, all woody) and ericoid[7] mycorrhizas (about 1% of plant species). In contrast, fungi in the arbuscular mycorrhizal symbiosis promote plant growth principally by increasing uptake of immobile resources (notably inorganic phosphate), as they can acquire these beyond the depletion zone surrounding a root[12]. They can also interfere with pathogens[13], increase micronutrient uptake and alter drought resistance[2]. It is thought that these fungi have little ability to increase plant uptake of more mobile ions (for example, NO_3^-) as these diffuse rapidly to roots in soil[14], although they do transport the less mobile NH_4^+ (ref. 15). However, where mobile ions such as NO_3^- or NH_4^+ are being produced in decomposing patches of organic material, the ability of the fine hyphae of arbuscular mycorrhizal fungi to penetrate into the material and to compete with other microbes could lead to increased N acquisition by the plant. We tested the hypothesis that plant N uptake would be increased by giving the arbuscular mycorrhizal fungal hyphae access to a decomposing patch of organic matter from which roots were excluded.

We grew plants of *Plantago lanceolata* in microcosms (Fig. 1). Each microcosm comprised two rows of three compartments, four of which contained a single plant and two a patch of dual-labelled ($^{15}N/^{13}C$) *Lolium perenne* leaves containing 1.7 mg ^{15}N and 5.0 mg ^{13}C. The plant in the central compartment in each strip was inoculated with the mycorrhizal fungus *Glomus hoi*. The compartments containing plants were separated by a double layer of 20-μm mesh, which was permeable to hyphae but not roots—in one row (experimental) the compartment containing a patch of decomposing organic matter (hereafter referred to as patch) was separated by a double 20-μm mesh on one side; in the other row (control) separation was by a 0.45-μm mesh, through which hyphae cannot normally pass but which allows solute diffusion to occur.

Decomposition of the experimental patch was faster than the control: after 28 and 42 days (with no difference between these two sampling times) the mean ^{13}C content of the experimental patch samples was $0.21 \pm 0.08\ mg\ g^{-1}$ soil compared with $0.56 \pm 0.11\ mg\ g^{-1}$ for the controls ($F_{1,16} = 8.24$; $P = 0.011$), representing 4% and 11% retention, respectively. ^{15}N content showed a similar pattern ($F_{1,16} = 5.23$; $P = 0.036$) with 12% (experimental) and 21% (control) retention. However, the microbial community as described by the phospholipid fatty acid (PLFA) profile was unaffected by treatment. The increased rate of decomposition was therefore not due to qualitative changes in the microbial community caused by the fungi, but was probably a direct result of the presence of arbuscular mycorrhizal fungal hyphae.

The hyphae of *G. hoi* proliferated extensively within both the inoculated plant compartment and the experimental patch compartment (Fig. 2a), eventually reaching a similar density in each. In contrast, after 42 days there was less hyphal growth into the compartment that contained the uninoculated plant (Fig. 2a), and there was no difference between experimental and control rows in that compartment (Fig. 2b). The roots of the uninoculated plant were only weakly colonized in both experimental and control rows, 5% and 8% root length, respectively, compared with 47% and 37% for the inoculated plants. The preferential growth of hyphae into the organic patch rather than into the uninoculated plant compartment was unexpected: if the fungus acquires all its carbon (C) from plant hosts, it might be expected to benefit from maximizing the number of hosts it colonizes, which in this case would result in preferential growth into the uninoculated plant compartment. In contrast, growth into a patch of organic matter will potentially benefit its existing plant partner.

The benefit to the plant was significant in terms of N capture from the patch. Although experimental plants (in which the hyphae had access to the patch) were no different from controls in their dry weight, N content or N concentration, they captured three times as much patch N (15 ± 1.9%) as control plants (5 ± 1.2%; Fig. 3a). In

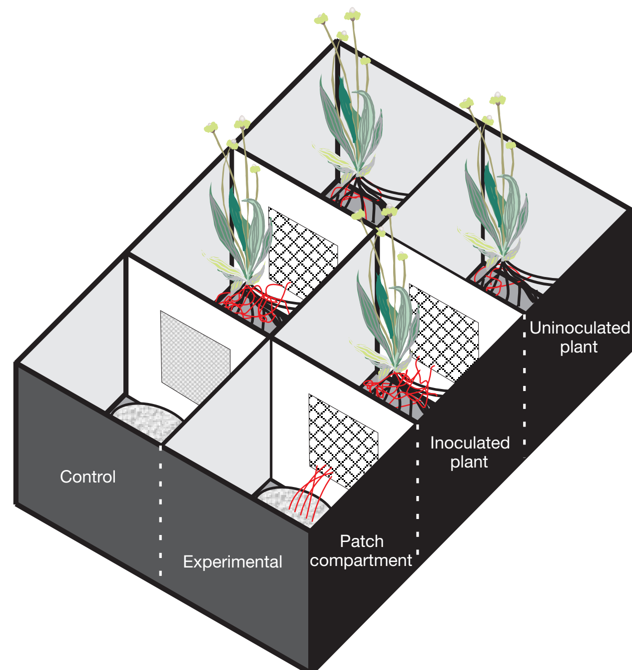

Figure 1 Microcosm unit design. The red lines in the compartments indicate arbuscular mycorrhizal hyphal mycelium; black lines indicate roots. Arbuscular mycorrhizal hyphae were denied access to the organic material on the control side of the microcosm unit.

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contrast, uninoculated plants captured only 0.7% of patch N, irrespective of treatment. The ^{15}N in control plants must have been acquired by mass flow or diffusion; the additional N in experimental plants must have crossed the membrane that separated patch and plant compartments by means of arbuscular mycorrhizal fungal hyphae. The quantity of ^{15}N in the experimental plants was a simple function of hyphal length density in the patch (Fig. 3b), emphasising that arbuscular mycorrhizal fungal hyphae here had a role in N capture, and demonstrating that the enhanced ^{15}N capture by experimental plants was not simply due to an increased rate of diffusion across the membrane. There was no difference in shoot mass between experimental and control plants, which could have led to differences in convective water (and nitrate) flux; in addition, the double layer of mesh would minimize such fluxes. Arbuscular mycorrhizal fungal hyphae have been shown to transport N compounds in situations where diffusion and mass flow have been prevented by the use of hydrophobic barriers[16].

The close relationship between N capture and hyphal density (Fig. 3b) is in marked contrast to the lack of a relationship between N uptake and root length density in other experiments[17–19]. Even a low density of root length can capture all of a mobile resource in a patch; why therefore is this not true of hyphae? A possible explanation is that the hyphae were able to compete with other microbes for N released from the decomposing patch. As they are very fine (less than 5 μm diameter), they can more easily penetrate throughout the material; more importantly, as they actively promoted decomposition—possibly by promoting the activity of hyphosphere bacteria—the hyphae must have been present at the sites of N release. Spatial proximity to the process of N release may make them effective scavengers for N.

The discovery that arbuscular mycorrhizal fungal hyphae may preferentially colonize organic patches rather than new host plants is surprising, even though an association between hyphal growth and organic matter is long established[3]. Current theory on the evolutionary strategy of the arbuscular mycorrhizal symbiosis[20] suggests that the best way for fungi to acquire C is to maximize the number of hosts that they colonize. In our data, the fungal growth pattern was more likely to enhance the performance of the existing host, even when no organic patch was made available, suggesting that promoting an existing host may be a more profitable strategy.

The increase in plant uptake of N from the patch demonstrates that arbuscular mycorrhizas may have a hitherto unappreciated role in plant N uptake in naturally heterogeneous soils where decomposing organic patches are crucial N sources[21]. In our experiment, the arbuscular mycorrhizal fungi both promoted decomposition and subsequently acquired the N that was released. They must also have used the products of decomposition, because they showed

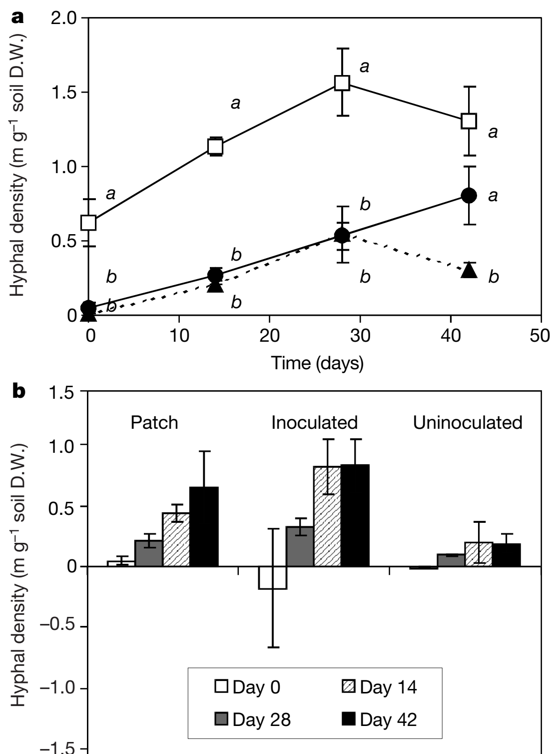

Figure 2 Arbuscular mycorrhizal hyphal densities. **a**, Hyphal densities in compartments of the experimental row of the microcosm. Inoculated plant, open squares; organic material, filled circles; uninoculated plant, filled triangles. Differences among compartments were significant ($F_{2,48} = 38.10$; $P < 0.001$). The letters beside data points represent significant differences at each collection date. Data were $\log_{10}$ transformed before statistical analysis. **b**, Difference between experimental and control values in each compartment. There was an increase in hyphal density when the arbuscular mycorrhizal fungus was allowed access to the organic material. Data are means with standard error bars. Analysis of variance showed that experimental and control rows differed in the patch ($F_{1,28} = 32.55$; $P < 0.001$) and inoculated plant ($F_{1,28} = 5.81$; $P = 0.023$) compartments, but not in the uninoculated plant compartment ($F_{1,28} = 0.82$; $P = 0.372$). D.W., dry weight.

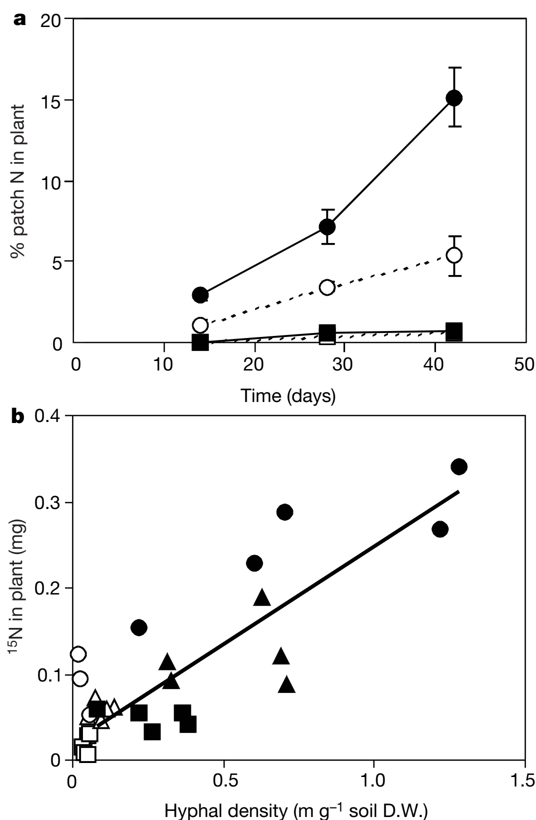

Figure 3 Nitrogen capture from the patch. **a**, Percentage N added in the organic patch detected in inoculated experimental plants (filled circles), inoculated control plants (open circles), uninoculated experimental plants (filled squares) and uninoculated control plants (open squares) over time. Data for uninoculated plants are superimposed. **b**, Relationship between the quantity of ^{15}N in the inoculated plant and the arbuscular mycorrhizal hyphal density in the organic patch compartment. There was no relationship for the control plant (open symbols) where arbuscular mycorrhizal hyphae were excluded from the patch. The experimental (filled symbols) plant data were fitted by a significant regression (mg $^{15}N = 0.0208 + 0.226$ m hyphae g^{-1} soil; $P < 0.001$, $F_{1,13} = 24.23$, $r^2 = 65\%$). Data for each collection are distinguished by symbols (that is, day 14, squares; day 28, triangles; day 42, circles). D.W., dry weight.

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markedly increased hyphal growth, both in the patch and in the inoculated plant compartment, despite the lack of any growth response by the plant partner in the symbiosis. These results show that the arbuscular mycorrhizal symbiosis can have saprotrophic capability, although the mechanism remains unknown and it is not yet established whether other arbuscular mycorrhizal fungi behave similarly. □

Methods

Microcosms

Twenty microcosm units were established under initially sterile conditions in containers measuring $39.5 \times 30 \times 16.3\ cm^3$, each divided into six compartments measuring $12 \times 13 \times 16\ cm^3$ and filled with a 1.8 kg mixture of autoclaved quartz sand and autoclaved (121 °C; 30 min) loam garden soil (1:1 v/v). The compartments were separated by double-thickness mesh barriers sealed to the sides with adhesive (see above). The two middle compartments of the microcosm unit each received 100 g inoculum of the arbuscular mycorrhizal fungus *G. hoi* (isolate number UY 110) as colonized roots of *P. lanceolata* in a sand and Terra-Green (a calcined attapulgite clay soil conditioner; Turf-Pro) growth medium. All compartments of the microcosm units received 50 ml filtered washing of the arbuscular mycorrhizal inoculum, but without arbuscular mycorrhizal propagules, to correct for differences in the starting microbial community[22,23]. The experiment was set up in a randomized block design in a heated, lit glasshouse. The daily mean temperature over the duration of the experiment was 19.3 °C (standard error ± 0.08). Photosynthetically active radiation (PAR) flux was recorded weekly and averaged 464 $\mu mol\ m^{-2}\ s^{-1}$ at plant level. All compartments of the microcosm units were watered daily. Four compartments of each of the microcosm units were planted with single *P. lanceolata* L. seedlings on 12 July 2000. The two remaining compartments received 0.55 g organic material 26 days later. The organic material added was milled *L. perenne* L. shoots that were dual-labelled[19] with ^{13}C and ^{15}N such that 35 mg N (1.7 mg ^{15}N) and 223 mg C (5.0 mg ^{13}C) was added to each unplanted compartment. Grass leaves are typical of the range of organic materials added to soil in heterogeneous distributions. The material was milled to ensure that the organic material added in the patch compartment was uniform and homogenous. The organic material was added at a depth of 10 cm as a thin layer of material in a circle of diameter 6.5 cm and at a distance of 2.5 cm from the mesh to the patch perimeter. Units were collected 0, 14, 28 and 42 days after addition of the organic patch. At the final collection (42 days) roots from two of the plants in the control row had entered the patch by breaking through the adhesive that sealed the barrier between compartments to the box; the mesh was intact in all cases. These replicates are therefore excluded from the analysis. There were no significant differences in shoot, root or total dry weights between inoculated control and experimental plants and the uninoculated control and experimental plants. At day 42 the inoculated plant dry weight was 7.1 ± 0.41 g and the uninoculated plant dry weight was 7.7 ± 0.57 g.

Analyses

We used a subsample of root material for mycorrhizal assessment[24]. Arbuscular mycorrhizal fungi hyphal length was measured using a modified membrane filter technique[25]. Shoot, root and soil samples from the unplanted compartments were dried (70 °C; 48 h) and milled in a ball mill to a fine powder for analysis by mass spectrometry performed on a continuous-flow isotope ratio mass spectrometer (CF-IRMS). Data presented are for ^{13}C and ^{15}N in excess of the natural abundances of ^{15}N and ^{13}C. PLFA profiles were extracted using the modified Bligh and Dyer method[26] and analysed by gas chromatography[27]. Individual PLFA, expressed as mol %, were analysed by multivariate analysis to determine changes in the overall microbial community structure. For statistical analysis, data were checked by a one-sample Kolmogorov–Smirnov test and transformed appropriately to ensure the data did not differ from a normal distribution before parametric analysis. Data were analysed using the general linear model (GLM) factorial design command in SPSS v. 7.0 for factorial analyses, or by one-way analysis of variance (ANOVA) or linear regression in Minitab v.12.1.

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The rhythm of microbial adaptation

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The evolutionary biologist "studies the steps by which the miraculous adaptations so characteristic of every aspect of the organic world have evolved"[1]. But the general nature of such adaptive steps is still unclear. Evolution is often thought to be random and dependent on unpredictable events[2]. In this light, one might expect the steps taken by adaptation to be completely random, both biologically and temporally. Here I present a mathematical derivation to show that, on the contrary, adaptive steps can have fairly strong rhythm. I find that the strength of the adaptive rhythm, that is its relative temporal regularity, is equal to a constant that is the same for all microbial populations. As a consequence, numbers of accumulated adaptations are predicted to have a universal variance/mean ratio. The theory derived here is potentially applicable to the study of molecular evolution.

Populations of organisms adapt to their environment through the

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