Foraging for Resources in Arbuscular Mycorrhizal Fungi: What is an Obligate Symbiont Searching for and How is it Done?

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1 Introduction

In this review, we will present (1) a brief summary of basic concepts in foraging theory and an update on recent progress in our understanding of foraging by mycorrhizal fungi, (2) an overview of the results of some experiments testing hypotheses and models presented in a previous review on this subject (Olsson et al. 2002), and (3) an evaluation of new directions and promising approaches and methodological developments for further research on this topic. Basic processes, models and theories as they stood until year 2000 were explained in the review by Olsson et al. (2002), which also made a comparison of the foraging strategies in ectomycorrhizal and arbuscular mycorrhizal fungi.

1.1 What is Foraging?

Foraging is the most widely used term in the scientific literature when referring to the search for resources by various organisms. The theory was first developed for animals, but it has been adapted to microbes and plants although many of these organisms do not strictly look for forage. The noun forage is in most dictionaries defined as animal food from plant origin, but the verb (to forage) is used in a much wider context implying the act of looking or searching for food or provisions, or collecting diverse supplies, not necessarily defined as forage. Resources and supplies for living organisms may include (1) energy, as radiation or energy substrates, such as carbon compounds, (2) nutrients, and (3) water. The cost-benefit associated to the search for new resources has been extensively studied and modeled in animals (Stephens and Krebs 1986). Having a good strategy for the search and selection of prey, plants or nectar was recognised as an essential component of animal fitness.

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A. Varma (ed.) *Mycorrhiza*,

1.2 Foraging Theory

Foraging strategies are widely studied in all mobile terrestrial and aquatic macroscopic organisms, such as mammals, birds, reptiles, fishes, and large invertebrates. The concepts of optimal foraging and success as a consequence of being good at finding high quality food became highly popular in animal foraging research (MacArthur and Pianka 1966). Foraging has also been studied in sessile organisms such as plants, including both above- and belowground resources. In plants, however, there are restricted possibilities to perform a search, and most studies have used measurements of phenotypic plasticity and resource allocation to understand how plants optimize the use of their resources (Hodge 2005; Kembel et al. 2005; Yang and Midmore 2005; De Kroon and Mommer 2006). Plants can also modify their physiology, architecture and biomass distribution to make an efficient search for light, nutrients and water (Jansen et al. 2006; Walk et al. 2006; Wang et al. 2006). Clonal plants have been a popular group in foraging studies since they have the possibility of moving towards a new resource by growing in its direction, as long as the resource is available within a reasonable distance (Kelly 1990; Streitwolf-Engel et al. 1997; Cole and Mahall 2006). Some studies of plant foraging have even incorporated symbionts or interactions with soil microbes to understand how these may alter plant strategies (Cui and Caldwell 1996a; Streitwolf-Engel et al. 1997; Farley and Fitter 1999; Hodge et al.1999, 2000a, 2000b; Hodge 2001), in particular because some of them may compete with the roots for the same resources and some may provide other resources for the plant. Mycorrhizal fungi, for example, may compete with the roots for carbon, but may also provide them with phosphorus, and both actions have the potential to alter root foraging.

Foraging strategies have been studied in saprotrophic fungi, as summarized in a recommended review by Boddy (1999) and in other recent papers (Donnelly et al. 2004; Harris and Boddy 2005). Saprotrophic growth is extensive enough to allow elegant studies in which fungal foraging can be quantified through its growth pattern in heterogeneous environments (Ritz et al. 1996). Mycorrhizal fungi are more difficult to study because the symbiosis needs to be established with a host plant. Ectomycorrhizal fungi are easier to culture in this sense and have been studied more (Bending and Read 1995; Lindahl et al. 2001; Jentschke et al. 2001; Donnelly et al. 2004; Wallander et al. 2006) than arbuscular mycorrhizal fungi (AMF) (Cui and Caldwell 1996b; Olsson et al. 2002; Gavito and Olsson 2003; Nakano-Hylander and Olsson 2007).

However, culturing problems are not the only reason for the lack of studies in AMF. Most mycorrhizal research has centered on studying the capacity of AMF to transfer nutrients to the plants. The focus on nutrient exchange has distracted our attention from the fact that plants and fungi in this association are independent organisms, mainly working for their own benefit and finding resources for themselves, not for their partners (Fitter et al. 2000). Even within the plant-centered perspective, the importance of the external mycelium to explore the soil and take up mineral nutrients in AMF is obvious, but most literature is focused on nutrient

delivery to the host plant, not on the searching process. We still know very little about the capacity, plasticity, and strategies of these fungi to find their resources.

2 Resources for an Arbuscular Mycorrhizal Fungus

2.1 Carbon

Host plants are temporary sources of carbon for the fungal symbionts, and provide them with carbon, especially during growth seasons. Host plants are the only energy source for AMF (Smith and Read 1997; Nakano et al. 1999) and the roots that host them are mainly produced during vegetative growth periods. Root life span varies widely, even within a species, from days to weeks and years, depending mainly on the phenological stage. Fine roots are usually replaced and rarely last an entire growing season (Gill and Jackson 2000). Furthermore, once plants enter their reproductive stages, flowers and fruits become competing sinks for C, and C allocation belowground is usually reduced (Marschner 1995; Farrar and Jones 2000). Root production declines and many roots senesce as a consequence of the C shortage resulting from the investment in reproductive tissue (Pritchard and Rogers 2000). In addition to this, herbivory, shading, water stress induced stomatal closure, leaf shedding, etc., are potential obstacles for a continuous C flow belowground. If we add the continuous variation in C allocation belowground to root turnover and to C competition between shoot, roots and other microbes feeding from the same pool, it seems likely that plant C is an important limiting resource for AMF in most environments. AMF should, in theory, be searching continuously for alternative sources to support growth and metabolism (Olsson et al. 2002). AMF seem to be good competitors and C scavengers, as indicated by the typical reductions in root growth with increasing degree of mycorrhizal colonization (Gavito et al. 2002). Furthermore, sometimes when plants are colonized by C-demanding fungi considerable growth depressions can be seen (Eissenstat et al. 1993; Peng et al. 1993). However, once C export belowground is reduced, the fate of roots and endophytes is defined. Roots die, but AMF have the possibility of a finding a new host. If a new host is not found immediately, energy reserves are needed to persist until a host is found.

Some important new findings have strong implications for AMF C metabolism and, as a consequence, also on foraging. The complete dependency of AMF on their hosts in terms of C (Nakano et al. 1999), and evidence indicating that the extraradical mycelium relies on the export of triacylglycerides and glycogen synthesized and packed in the intraradical mycelium (Bago et al. 2000, 2003), links the foraging ability of these fungi to the continuous translocation of C compounds from the intraradical to the extraradical mycelium. The physiological differentiation of the intraradical and extraradical phases of the mycelium has strong implications for the foraging strategies of an AMF. If the extraradical phase depends on the internal

phase (Bago et al. 2002), foraging requires that the hyphal network maintains its integrity and is efficient in exporting the energy required to sustain the search and the exploitation of resources in distant parts of the mycelium. It is, therefore, extremely important to consider both host C availability and C translocation, from the intraradical to the extraradical mycelium, when studying how AMF perform the search for new resources. Pfeffer et al. (2004) have also demonstrated that the previous belief of C transfer between mycelium interconnected plants, which had already been questioned, was not correct and that the fungal symbionts did not deliver any C to other host plants. C circulates in the mycelium network in all directions and is retained and used within the mycelium.

In an experiment testing C allocation through mycelial networks from an established donor to receiver plant species, it was shown that C was not directed towards receiver seedlings to any higher degree than towards other directions (Nakano-Hylander and Olsson 2007). C was evenly distributed in the extraradical mycelium with similar labeling in extraradical mycelium as in intraradical mycelium in newly colonized seedlings. This was surprising since optimal foraging predicts that allocation of resources should increase in response to the encounter of a new resource (foraging precision). However, a test conducted with data from numerous plant species showed no support for the widespread assumption that foraging precision increases the benefit gained from growth in heterogeneous soil (Kembel et al. 2005). We are still far from having enough information to test a similar hypothesis in AMF, and clearly many more studies are needed to understand if optimal foraging, foraging precision, or foraging scale, exist in AMF and are similar to root foraging.

2.2 Water

Water is a limiting resource in dry environments and during dry seasons, and some AMF are resistant to high levels of water stress. The capacity of AMF to access water in distant and small-sized soil pores is well documented, as well as their ability to alleviate water stress in plants (Augé 2001). Water is a very little studied resource in AMF, and basically all research in mycorrhizal water relations has focused on the plants. Plants exhibit a complex set of signals and protection mechanisms to desiccation, and morphological and physiological adaptations for water search, that have not yet been explored in AMF (Ruiz-Lozano 2003; Ruiz-Lozano et al. 2006). Indirect evidence from the highly variable effects of AMF isolates on plant–water relations suggests that AMF isolates differ in their mechanisms to alleviate plant water stress (Marulanda et al. 2003). Some of these mechanisms likely involve fungal foraging and fungal plasticity, besides other complex molecular mechanisms that are currently being elucidated. Saprotrophic fungi show, for example, morphological adaptations to reduce desiccation and increase water search efficiency, such as cord formation and hyphal aggregation (Mijail and Bruhn 2005, and references therein). AMF foraging for water is becoming an increasingly relevant study area in the global climate change context.

2.3 Mineral Nutrients

The external mycelium of AMF can be stimulated by various amendments, such as organic matter (St. John et al. 1983a; Joner and Jakobsen 1995; Albertsen et al. 2006), specific organic compounds such as yeast and bovine serum albumin (Larsen and Jakobsen 1996; Ravnskov et al. 1999), or mineral nutrient-rich patches (St. John et al. 1983b). Cui and Caldwell (1996b) showed that AMF were equally efficient at acquiring P and delivering it to the roots when nutrients were located in a few enriched patches or uniformly in the soil. This implied that either hyphal proliferation or nutrient uptake capacity, or both, must have increased substantially in the nutrient-rich patches. Mycelium growth responses and nutrient uptake capacity were not measured, but the results suggest a similar plasticity to that widely reported in roots (Grime and Mackey 2002) and discussed above.

AMF foraging for mineral nutrients was studied by Gavito and Olsson (2003, 2008) in two different experimental systems, showing that mycelium responses may vary depending on how the nutrients are presented. This is in accordance to previous observations in root foraging (Grime and Mackey 2002) and to theories about AMF foraging (Tibbett 2006) that are just starting to be tested. The two foraging models will be discussed in detail in the Sect. 4 "Testing Foraging Models in AMF".

3 Foraging Activity

3.1 Growth Strategies

AMF are nonresource-unit-restricted foragers' *sensu* Boddy (1999), since they are not confined within the organic resource they are living of, and their extraradical mycelium can extend considerably beyond the host roots. The search implies risks and/or costs with no warranty for a balanced or outweighed return for the investment. Growth strategies are of fundamental importance to foraging, and studies with clonal plants can be useful to understand fungal strategies. There are two main growth strategies in plants (Schmid and Harper 1985). One is formed by plants that send out stolons on which new ramets are formed at long distances from the mother ramet. This strategy is termed guerilla type and can be found for instance in *Trifolium repens*. Other clonal plants form dense tussocks and this is called the phallanx type. It can be found for example in *Deschampsia cespitosa*. The growth strategy has critical importance for the interactions of clonal plants in communities, where the phallanx type is very stable once established in a spot, while the guerilla type has greater possibilities of spreading. The same concepts can be adopted in fungal competition, and it is clear that AMF foraging fits into the "runner" type described by Bell (1984) or the "guerrilla" pattern from Schmid and Harper (1985). Many saprotrophic fungi with dense hyphal fronts have a growth strategy more similar to the phallanx type.

No mycelium growing fronts are observed in AMF and the search is performed by long and sparse runner hyphae, with little branching. This type of foraging seems to be associated with successful foraging in heterogeneous environments with scattered resources (Donnelly and Boddy 1997). Although difficult to visualize in soil, this type of foraging is easy to observe in vitro in root organ cultures where mycelium develops in a Petri plate with nutrient rich medium. Growth *in vitro* on solid medium is likely to be different from growth in soil, but these cultures can help us to understand general strategies for how AMF might be searching in soil.

3.2 Growth and Function of AMF Extraradical Mycelium Networks

Many recent papers using monoxenic cultures convey very useful information to predict how AMF might be searching. The following is a summary of our and other researchers' observations on mycelium development *in vitro* and in soil.

Hyphae grow from roots or pieces of mycelium by sending a few runner hyphae in different directions to search the medium or the soil (Friese and Allen 1991; Bago 2000).

Runner hyphae with an adequate supply of carbon (C) from transformed roots grow several centimetres without branching until they reach the edge of the plate, with BAS (branched absorbing structures), and sometimes even spores forming directly on the runner hyphae (Fig. 1). Later, new profuse branching from the first

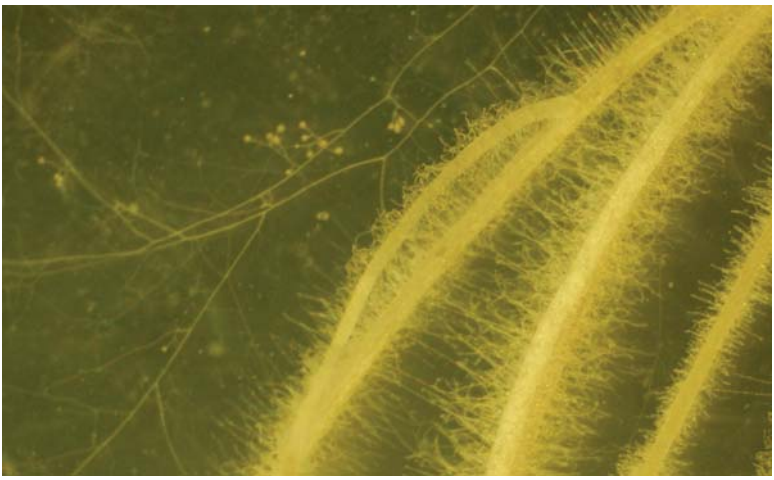


Fig. 1 Extraradical AMF mycelium networks established in monoxenic whole plant cultures between *Trifolium subterraneum* and *Glomus intraradices*. The symbiosis develops in solid Minimum medium in Petri plates. Roots are surrounded by runner hyphae connected by hyphal bridges in a young mycelium network. *Photo* Mayra E. Gavito

order group and hyphal anastomosing bridges begin to establish a loose hyphal network. Most AMF show this pattern of extraradical mycelium growth, with slight variations among the different genera in morphogenesis, architecture, anastomoses, and healing mechanisms (De Souza and Declerck 2003; De la Providencia et al. 2006; Voets et al. 2006). Some features do change when AMF grow in more heterogeneous substrates (Dodd et al. 2000), but patterns seem to be mainly the same in soil.

Extraradical hyphae with adequate carbon supply grow on average a few mm per day (Jakobsen et al. 1992; Giovanetti et al. 2000). Mycelium growing in nutrient rich medium in plates is likely foraging for carbon, not nutrients or water. When new roots are not found, the fungus keeps feeding on the medium until roots exhaust their carbon source and stop supplying carbon to the fungus. Mycelium stops growing, and when this happens BAS disappear gradually, followed by spore formation and maturation. Neutral lipids move constantly into maturing spores and, after some days, cytoplasm begins to retract from distant parts of the mycelium forming septa to close large parts of the previously active network (Giovanetti et al. 2000). In *Glomus* species, cytoplasm contracts first in distant hyphae and later in runner hyphae until most extraradical mycelium is closed (Gavito, personal observations). The cytoplasm fragmentation in the extraradical AMF mycelium when septa are formed indicates that it has only short-lasting capacity as a propagule and probably also as a foraging structure.

New evidence suggests that anastomoses and healing of injured hyphae may play an important role in the structure of hyphal networks (Voets et al. 2006) and in the foraging abilities of AMF. Mechanisms to reconnect severed sections and to maintain cytoplasm flow when mycelium is injured or cytoplasm begins to retract are essential for an efficient flux in the network, and for survival of the remaining parts of a senescing network.

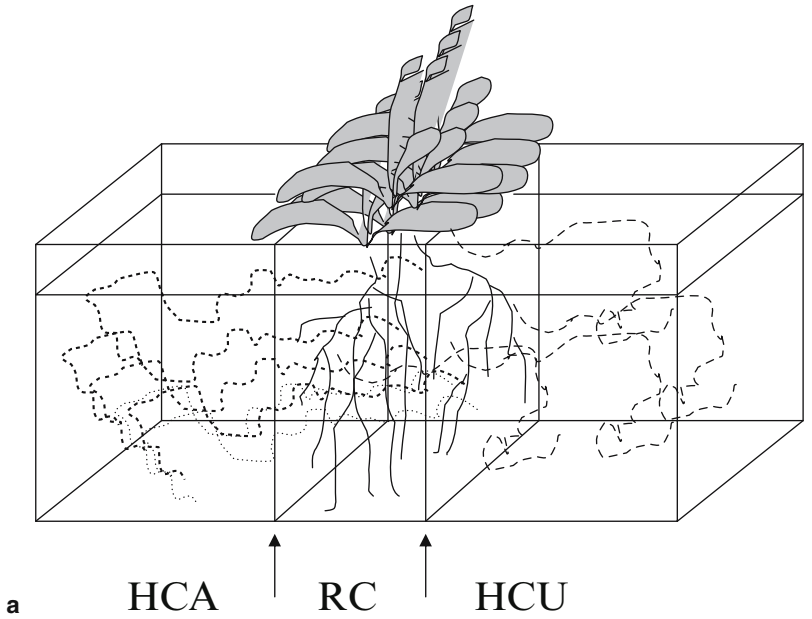
Carbon reallocation from distant parts of the mycelium to support foraging and exploit new resources found has been reported in some saprotrophic fungi (Boddy 1999). Carbon reallocation within the AMF mycelium is unknown, but some indirect evidence (Cui and Caldwell 1996b) suggests that it may exist.

4 Testing Foraging Models in AMF

Olsson et al. (2002) presented two alternative models to study foraging strategies of the AMF mycelium.

4.1 Two Dimensional Model

In the two-dimensional model, a plant colonized by AMF grows in a central compartment and two choices are presented in mycelium compartments on the right and left sides of the pot (Fig. 2a) as described in Gavito and Olsson (2003) and in the PVC cross-tube system used by Ravnskov et al. (1999) and Albertsen et al. (2006). This



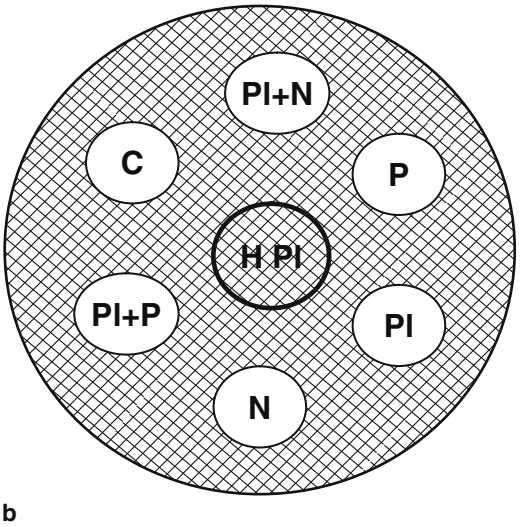


Fig. 2 Diagrams illustrating experimental models to study foraging in mycorrhizal fungi proposed by Olsson et al. (2002). **a** Two-dimensional. The host plant develops in a central compartment with one option on the right and one on the left side of the pot, so mycelium is forced to grow into the side compartments. Usually one side is amended and the other side is a control. From Gavito and Olsson (2003), printed with permission from *FEMS Microbiology Ecology*. **b** Multidimensional. The host plant grows restricted to a compartment in the middle of a large pot; mycelium develops freely and encounters the different choices after growing some distance from the plant compartment. Mycelium does not form a dense front and may choose entering or not the patches with choices. From Gavito and Olsson (2008) with permission from Applied Soil Ecology

kind of system is simple and easy to handle because it gives the possibility of sampling and changing the contents in the lateral compartments with minimum disturbance for the rest of the pot. It has the disadvantage that mycelium development is unrealistically high in the limited volume of the plant compartment and leads to the establishment of an artificial mycelium front towards the choices that are presented right in contact with dense hyphal fronts. The bidimensional model is likely to overestimate the response of the AMF. Hyphal lengths measured in these kinds of systems are higher than those observed in large pots with unrestricted root volume with the same AMF isolate used by Ravnskov and Albertsen (Gavito, personal observation), or in field measurements testing amendments (Gryndler et al. 2006).

Results from tests of this model have been useful to identify mycelium responses to inorganic and organic nutrient forms, and organic matter. They tell us little, however, about the magnitude of the response of the mycelium when it grows in a more natural manner and searches in a heterogeneous substrate with more choices available and possibilities to disregard the option presented. Gavito and Olsson (2003) used this model and found large responses to organic and inorganic nutrients provided in different combinations, in comparison with an unamended compartment located in the opposite side of the pot (Fig. 3). We found

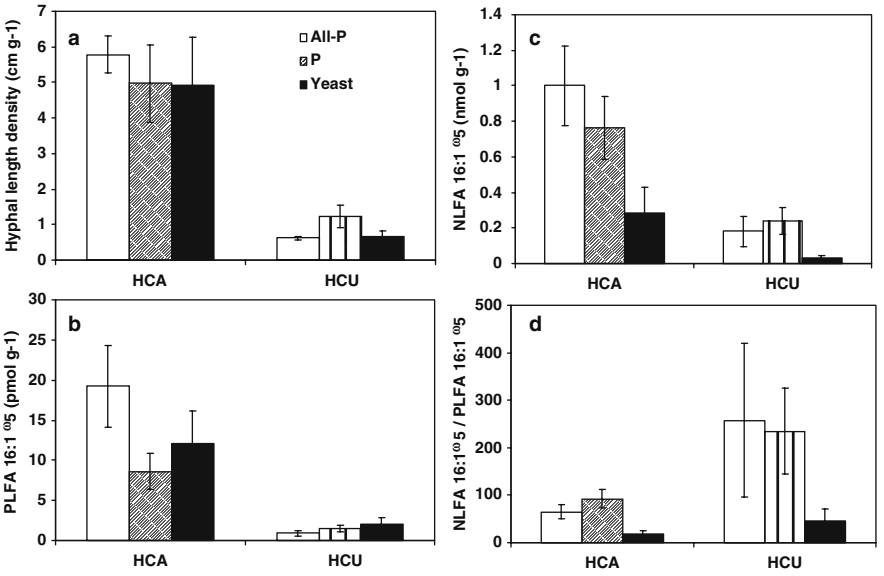


Fig. 3 Two-dimensional model. Measurements of long-term extraradical mycelium development as **a** hyphal length, **b** content of phospholipid fatty acid (PLFA) 16:1ω5, indicator for AMF biomass, **c** content of neutral lipid fatty acid (NLFA) 16:1ω5, indicator for AMF reserves. One compartment was left unamended and the other was amended with either continuous nutrient solution minus P (*All-P*), continuous only P (*P*), or a single organic amendment (*O*, as dry yeast) application. **d** PLFA/NLFA ratio as a measure of C allocation to biomass (rather "hyphal growth". Since neutral lipids is such a large part of the biomass) (PLFA), or to reserves (NLFA). From Gavito and Olsson (2003) printed with permission from *FEMS Microbiology Ecology*

that the investment made in a compartment with no nutrients was only exploratory and that most resources were sent to the amended compartment. However, the most important finding was perhaps that resources had been allocated differently within the same mycelium (Fig. 3). In the amended compartment where nutrient addition had occurred only at the start of the experiment, there had been high mycelium development while the resource was available but, when this was exhausted, and after weeks with no other supply, the mycelium began to allocate more carbon to reserves as indicated by a high neutral lipid to phospholipid ratio (Fig. 3). In the amended treatments with continuous nutrient supply, resources were used mainly for growth and exploitation of nutrients in the compartment, not for reserves. A novelty in this experiment was the use of ^{13}C to investigate short-term C allocation to AMF by measuring ^{13}C enrichment directly in the signature fatty acid 16:1ω5. The stable isotope allowed us to confirm that most C allocation prior to harvest was being used for growth in the treatments with continuous nutrient supply and for reserve lipids in the treatments with no nutrient supply or with nutrients only in a single initial dose.

4.2 Multi Dimensional Model

The second, multidimensional, model proposed by Olsson et al. (2002) implies the simultaneous exposure of the extraradical mycelium to patches of different quality. This model was tested in another study by Gavito and Olsson (2008), where a host was grown in a restricted compartment in the centre of a pot and the extraradical mycelium of *Glomus intraradices* or *Scutellospora calospora* grew radially from the central compartment to the edge of the pot. Six choices were presented at the same time in mesh bags inserted around the plant compartment (Fig. 2b). A control (no amendment), nitrogen, phosphorus, host plant, host + N, and host + P treatments were introduced and mycelium was allowed to develop for 3 weeks in the mesh bags containing the choices. The most striking result was the small response of both isolates to all choices, confirming the overestimating values obtained when using the two-dimensional model (Fig. 4).

In the two-dimensional system, however, both roots and AMF could sense the application of the amendment since there was no barrier. In the multidimensional system, there was a buffer zone to confine the choice to strictly mycelium contact. *Glomus intraradices* showed very little response to all choices, whereas *Scutellospora calospora* grew better in patches containing a host plant, especially a plant with no other amendment. All new host plants became colonized by the foraging mycelium of both isolates. However, the mycelium of *G. intraradices* was substantially weakened when the original host plant senesced and mycelium aged. *G. intraradices* colonized much less root length and formed almost no new hyphae in the patches when its mycelium was old and presumably C limited. Even in a nutrient poor substrate, the isolates did not proliferate in the N or P patches, indicating that these nutrients were not limiting and that creating a poor environment for the fungi is

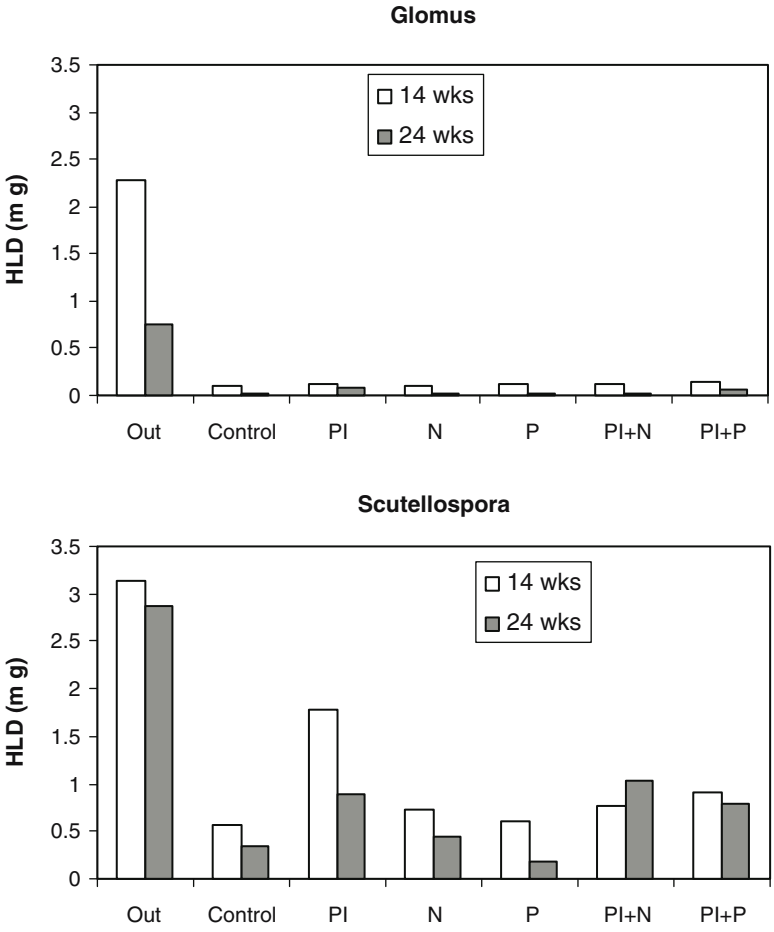


Fig. 4 Multidimensional model. The extraradical mycelium of *Glomus intraradices* and *Scutellospora calospora* was established in the pots for 11 or 21 weeks before introducing the bags with choices and measured 25 days after the choices had been introduced. Hyphal length density values in meters per gram soil at the time of harvest (14 weeks for the "young" mycelium or 24 weeks for the "old" mycelium. *Out* Sand surrounding the mesh bags as a reference, *control* unamended, *P* phosphorus, *N* nitrogen, *Pl* plant, *Pl+N* plant plus nitrogen, *Pl+P* plant plus phosphorus. From Gavito and Olsson (2008) redrawn with permission from Applied Soil Ecology

rather difficult. According to these results, the AMF isolates were mainly looking for a new source of C in a host plant, and *S. calospora* was searching more actively than *G. intraradices*.

The two models have high potential to convey very meaningful information concerning foraging strategies in AMF. They have been used very little and there are still many questions to answer in this topic.

5 Main Challenges and New Approaches in Foraging Studies

Stable and radioactive isotopes have high potential in fungal foraging studies. Tracking the movement of resources is essential to understand the response of the mycelium to resource variability in the environment. AMF foraging differs from plant foraging in the sense that roots are mostly vegetative structures with the only function of absorbing and transporting water and nutrients, whereas AMF mycelium performs both vegetative and reproductive functions. Resources in the extraradical AMF mycelium are therefore shared between both functions, and allocation is a trade-off between growth and persistence. This means that AMF are more similar to whole plants than to roots only. In plants, root to shoot ratios and leaf, flower and fruit proportional biomass can be used as indicators of resource allocation. Movement of resources between these pools shows what the plant is doing. In AMF, a spore/mycelium ratio would not be an adequate measure of C allocation because mycelium has both vegetative and reproductive functions. This implies that the only way to know what AMF are doing is through direct measurements of resource flow within mycelium and spores, and this can only be done by marking the resource in question with an isotope and following the label to a signature molecule like a fatty acid (Boschker and Middelburg 2002; Treonis et al. 2004; Evershed et al. 2006). The incorporation of photosynthesis assimilated ^{13}C into the signature fatty acid 16:1ω5 as a biomarker of AMF (Olsson and Johansen 2000) has been a useful measurement in several experiments. ^{13}C enrichment in the neutral lipid fatty acid 16:1ω5 was positively correlated with ^{13}C enrichment in total C, and this relationship was not found in a biomarker for other fungi, indicating preferential incorporation of C to the AMF biomarker (Olsson et al. 2005).

A recent methodological approach based on stable isotopes is the incorporation of ^{13}C to DNA or RNA (stable isotope probing, SIP). This method gives the advantage of distinguishing the enrichment in different AMF species in a sample (Whiteley et al. 2006), which is not possible with the fatty acid method (Treonis et al. 2004). The fatty acid method is more reliable though in terms of accurate quantification of ^{13}C incorporation. It is cheaper and does not require such strong labeling as needed for gradient centrifugation of DNA or RNA (Wellington et al. 2003). Long-pulse labeling allowed Rangel-Castro et al. (2005) to get enough label to study the influence of liming on microbial communities in grassland soils, but they failed to detect labeled mycorrhizal fungal sequences (expected to show in ^{13}C RNA). SIP has not been used in studies specifically investigating mycorrhizal symbionts and needs to be improved to solve insufficient labeling and primer bias, but has a large potential in AMF foraging studies since species identification is critical for testing functional variation. Extraradical mycelial networks in AMF species are diverse under several criteria (Avio et al. 2006), and are usually intermingled in soil. The main challenge to improve our resolution in foraging studies is the accurate identification and quantification of multiple interacting AMF mycelia in soil.

6 Conclusions

The process of searching for resources is critical for the performance of all organisms, but foraging studies in AMF are still to scarce in the literature. Recent evidence suggests AMF mycelial networks might be highly variable in growth and functional traits that make them weak or strong under certain conditions. Also, some AMF seem more active in foraging than others. Understanding why an intensive foraging activity is advantageous for some isolates is fundamental for our basic knowledge of how AMF interact with their environment and how AMF communities are established.

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