# Life Histories of Symbiotic Rhizobia and Mycorrhizal Fungi

Review

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Research on life history strategies of microbial symbionts is key to understanding the evolution of cooperation with hosts, but also their survival between hosts. Rhizobia are soil bacteria known for fixing nitrogen inside legume root nodules. Arbuscular mycorrhizal (AM) fungi are ubiquitous root symbionts that provide plants with nutrients and other benefits. Both kinds of symbionts employ strategies to reproduce during symbiosis using host resources; to repopulate the soil; to survive in the soil between hosts; and to find and infect new hosts. Here we focus on the fitness of the microbial symbionts and how interactions at each of these stages has shaped microbial life-history strategies. During symbiosis, microbial fitness could be increased by diverting more resources to individual re-production, but that may trigger fitness-reducing host sanctions. To survive in the soil, symbionts employ sophisticated strategies, such as persister formation for rhizobia and reversal of spore germination by mycorrhizae. Interactions among symbionts, from rhizobial quorum sensing to fusion of genetically distinct fungal hyphae, increase adaptive plasticity. The evolutionary implications of these interactions and of microbial strategies to repopulate and survive in the soil are largely unexplored.

## Introduction

Research on rhizobial and mycorrhizal symbioses has emphasized fitness benefits to plants. Here, we take a different vantage point, focusing on the fitness of the micro-bial symbionts themselves and how symbiosis has shaped microbial life-history strategies. Past research has revealed much about the infection and active-symbiosis phases of the microbial life-cycles. Other phases, such as repopulation and survival in the soil, are less understood but equally crit-ical to the evolution and ecological persistence of rhizo-sphere symbioses.

Rhizobia are soil bacteria best known as root-nodule symbionts of legumes. Globally, the amount of nitrogen fixed by rhizobia is similar to that from synthetic ammonia production [1]. Symbiosis is not obligate for either partner: some rhizobia can grow endophytically in nonlegumes [2], and non-symbiotic rhizobia sometimes outnumber symbiotic genotypes in soil [3]. Our discussion, however, will be limited to rhizobia that retain the potential for sym-biosis with legumes. When a host is present, a lucky few (of the vast number of rhizobial cells in the soil) infect host-plant roots and proliferate to millions of cells inside each root nodule (Figure 1A). Once inside a nodule, some rhizobia differentiate into bacteroids: a modified form that

can convert atmospheric $N_2$ into nitrogen forms their host can use. When the nodule senesces, many of the rhizobia inside apparently escape to the soil [4,5], but this process has not been studied in detail. In some hosts, bacteroids lose the ability to reproduce, so the soil is repo-pulated by their undifferentiated clonemates from the same nodule. This can have interesting evolutionary implications [6–8].

An estimated 70–90% of plant species are involved in mycorrhizal symbioses [9]. We shall focus on arbuscular mycorrhizal (AM) fungi, which are obligate symbionts, dependent on plant roots for reduced carbon, and provide various benefits in return, including — but not limited to — nutrient uptake [10]. Plants can allocate 4–20% of their photosynthate to supporting AM fungi — this equates with the consumption of roughly five billion tonnes of carbon per year by AM fungi [9,11].

The life cycle of mycorrhizal fungi begins when a fungal spore germinates and hyphae grow toward a host root (Fig-ure 1B). Fungal signals drive physiological changes in the hosts [12], counteracting the plant immune program [13]. The plant cell actively prepares its intracellular environment [9]. The fungus penetrates the host's parenchyma cortex and forms branches, called arbuscules, or coils, where nutrient exchange occurs (Figure 2). External hyphae colo-nize the soil and take up nutrients. Phosphorus and nitrogen are the most prominent, and these, along with a number of micronutrients, are transferred to the hosts. In return, host-derived carbon is transferred to the fungi, and stored either in energy-rich vesicles to support vegetative growth or spores [14]. Hyphae that grow from both spores and from host roots can colonize new plants.

Across the plant species tested (a small fraction of potential host species), individual plants have been found to be infected by multiple strains of rhizobia and/or mycorrhizal fungi [15–18]. For example, individual clover (*Trifolium pratense*) plants averaged 11 rhizobial strains each [19]. Similar within-plant diversity was seen in pea (*Pisum sativum*), where the proba-bility of two adjacent nodules containing the same strain was only about twice that expected from random sampling of the bulk soil population [20]. However, a very young seed-ling with only one nodule might have only one strain per plant, although even single nodules can contain multiple strains. In one study, 12–32% of field-grown soybean nodules were found to contain two strains [21].

With two or more strains per plant, collective benefits to the symbionts from increasing host-plant growth has the potential to aid a focal strain's most likely competitors for future hosts. This within-plant diversity can therefore select for individual strains to 'free-load', exploiting the host growth and photosynthesis facilitated by other strains [22]. For the levels of AM fungal and rhizobial diversity typically found within a single host plant, theoretical models predict that these symbionts should invest nothing in costly activities that benefit the host, unless the hosts preferentially favor more-beneficial symbionts [23]. Models that incorrectly assume one symbiont strain per host [24,25] can under-esti-mate the ease with which cheating symbionts can invade and disrupt mutualisms [26].

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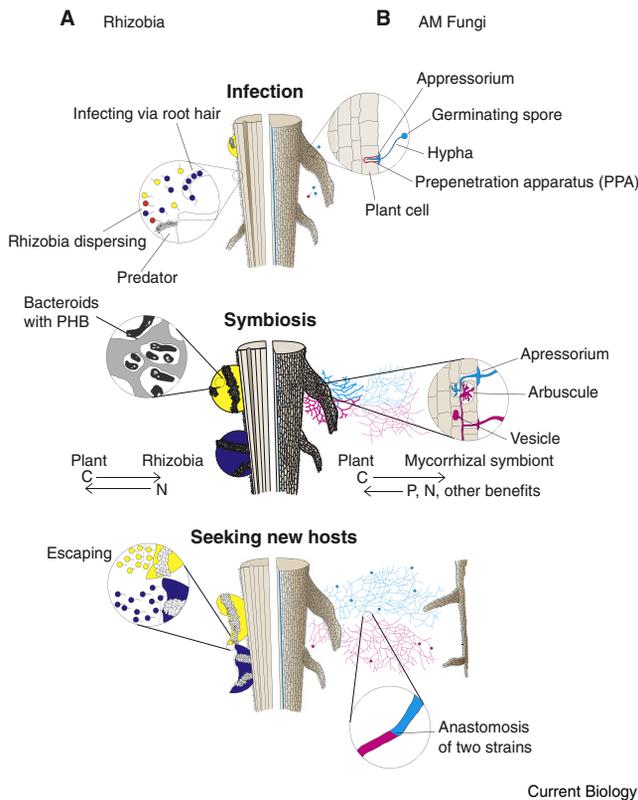E-mail: denis036@umn.edu (R.F.D.), toby.kiers@vu.nl (E.T.K.)

Figure 1. Life cycles of rhizobia and AM fungi.
Different colors for each strain are used to highlight infection by multiple strains per plant. (A) Top: rhizobia typically infect hosts via root hairs, but chemical cues from predators and high competitor population densities may cause some to disperse or form persisters. Middle: inside root nodules, rhizobia that have transformed into bacteroids use plant carbon to power $N_2$ fixation; excessive carbon diversion to hoarded polyhydroxybutyrate granules (a storage compound that can increase rhizobial fitness) may trigger host sanctions. Bottom: different strains escaping from the same plant are likely future competitors, which may undermine cooperation. (B) Top: germinating spores of AM fungi respond to signals that result in hyphal growth and hyphal branching. The plant cell actively prepares a prepenetration apparatus (PPA) to guide the fungus into the cell. The fungus enters the cell via a fungal appressorium. Middle: AM fungal hyphae branch repeatedly to produce the arbuscule, an important site of nutrient transfer. Vesicles, potentially important fungal storage structures, are developed by some AM fungal species. During active symbiosis, host carbon (usually in the form of hexose) is exchanged for nutrients (for example, phosphorus and nitrogen). Bottom: fungal 'individuals' can simultaneously interact with multiple host plants. Hyphae of genetically different (denoted by different colors), but typically closely-related fungi, can fuse (anastomose). New spores are typically formed at the leading tip of individual fungal hyphae. Plants can be infected by both infecting hyphae and spores.

For both kinds of symbionts, persistence depends on the ability to reproduce using host resources, repopulate the soil, survive in the soil between hosts, and find and infect new hosts. We shall discuss rhizobial and mycorrhizal strategies separately, in this sequential order, before returning to some common life-history themes.

## Rhizobial Life History

The potential fitness benefits for rhizobia of entering into symbiosis are striking. A rhizobial cell can reproduce a million-fold or more in a legume root nodule, so why is there often little or no increase in rhizobial populations in soil over years? First, we will examine fitness benefits to rhizobia from symbiosis, then consider each subsequent stage in the life cycle of symbiotic rhizobia, up to infection of the next host. We suggest that the main limitation on rhizobial population growth is a lack of nodulation opportunities, relative to their numbers. For rhizobia, symbiosis is like a lottery, with enormous fitness rewards for a very few lucky winners.

### Symbiosis and Fitness Benefits to Rhizobia

A single rhizobial cell that founds a root-nodule population is likely to have many more descendants than if it had remained in the soil. Lab and field experiments give mean values from $10^8$ to $10^9$ culturable *Bradyrhizobium japonicum* cells per soybean (*Glycine max*) nodule [27,28], while a siratro (*Macroptilium atropurpureum*) nodule may contain more than $10^9$ reproductively viable rhizobia [29], all descended from one or a few founding cells.

The opportunity to reproduce inside a nodule presumably imposes strong selection in favor of symbiosis, but there are other potential benefits. Rhizobial cells can also accumulate resources inside nodules that may increase future survival, including energy-rich polyhydroxybutyrate (PHB) and phosphate. *Sinorhizobium meliloti*, which nodulates alfalfa (*Medicago sativa*), can accumulate enough PHB per cell to support a tripling in population size without an external carbon source [30]. Similarly, *B. japonicum* cultured at phosphorus levels similar to those in nodules can store enough phosphate to support up to five generations in phosphorus-free culture [31]. Given these benefits, there must be strong selection to nodulate, given a clear opportunity.

It is important to remember that rhizobial fitness benefits from nodulation depend on the rhizobia's ability to reproduce inside a nodule, and only indirectly on how much they benefit their host. Once inside a nodule, how does rhizobial allocation of resources between reproduction and $N_2$ fixation (Table 1) affect rhizobial fitness? The interests of legumes and rhizobia overlap somewhat: a nodule containing more rhizobia can fix more $N_2$, perhaps supporting more plant growth and photosynthesis, which might, in turn, support more rhizobia. But typical levels of rhizobial strain diversity within individual plants would create a tragedy of the commons, undermining cooperation, unless legumes impose fitness-reducing sanctions on nodules that fix less nitrogen [6,23,32,33]. Sanctions against less-beneficial rhizobia have been documented both in hosts where all rhizobia retain the potential to reproduce [27,34] and in hosts where only rhizobia that have not yet differentiated into bacteroids can reproduce [35].

Nodules that fail to fix any nitrogen usually receive fewer host resources, as shown by their smaller size (but see [36]), although this may not always limit rhizobial reproduction [37,38]. Rhizobial interference in host signaling [29] may forestall sanctions and resource-limited rhizobia may consume nodule tissue [39]. However, escaping sanctions in mixed nodules may be a more common explanation for the persistence of rhizobial "cheaters", which benefit by diverting resources from $N_2$ fixation, and defective mutants, which fix less $N_2$ without benefiting from their defection [33,40]. Furthermore, mediocre rhizobial performance may not trigger sanctions consistently [41,42].

Even when sanctions are imposed, a rhizobial cell that founds a nodule will have many more descendants — millions more — than if it had remained in the soil. That benefit should
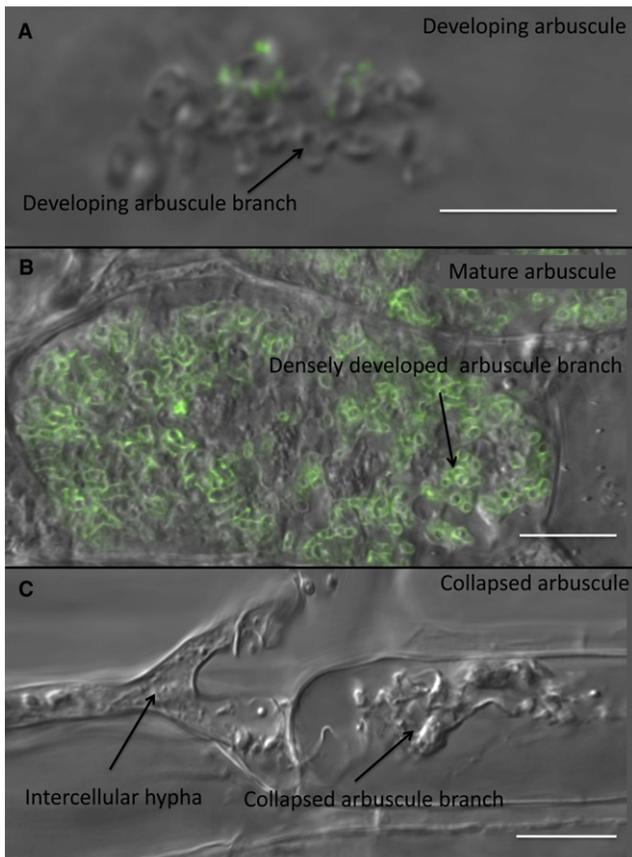
Figure 2. Development and collapse of a *Glomus intraradices* arbuscule in a rice root.
Confocal microscope images show: (A) developing; (B) mature (18 days after inoculation); and (C) collapsed arbuscules. The arbuscule shape was visualized using green fluorescent protein (GFP; A,B) merged with differential interference contrast (DIC; A–C) microscopy. Techniques described in [85]. Bar = 10 μm. (Courtesy of Y. Kobae.)

help maintain nodulation genes, even in strains that have lost genes for nitrogen fixation. But the fitness consequences of nodulation do not depend only on reproduction inside nodules. Escape from nodules and subsequent survival in the soil are also critical, as we discuss below.

### Repopulating the Soil

Given a nodule containing millions of rhizobial cells, how many rhizobia will escape from each senescing nodule and become established in the soil? This may be the most-neglected question in the life-history of symbiotic rhizobia. Only rough estimates are possible from published data. Brockwell *et al.* [43] reported that rhizobial populations increased by $2.5 \times 10^5$ cells per g soil during nodule senescence of soybean. Increased soil populations are apparently due to rhizobial escape from nodules (Figure 1A), rather than stimulation of rhizosphere populations, because increases are less with cultivars that nodulate poorly with the focal strain [44]. Multiplying this increase by $2 \times 10^9$ g soil (to plow depth) per hectare [45] and dividing by $2 \times 10^5$ plants per hectare [43] gives an estimated release of $2.5 \times 10^9$ rhizobia per plant. With twenty-five nodules per plant [43] and one founding cell per nodule, we estimate that each

rhizobial cell that nodulates soybean will thereby generate an average of $10^8$ descendants in the soil, a few months later (this represents between 10 and 100% of the $10^8$ to $10^9$ rhizobia in each soybean nodule [27,28]).

In some hosts, including alfalfa, rhizobia lose the ability to reproduce when they differentiate into bacteroids. But a typical alfalfa nodule also contains $10^6$ or so reproductively viable still-undifferentiated rhizobia [35]. Even if we assume that only 10% of these rhizobia escape into the soil, that represents 100,000-fold or greater reproduction from symbiosis, plus any benefits from PHB or phosphate. So, even conservative estimates of rhizobial release from nodules imply strong selection for nodulation, although not necessarily for fixing nitrogen.

### Survival in the Soil

Like rhizobial escape from nodules, survival in soil and the adaptations responsible for that survival have received much less attention than interactions with hosts, so much of what follows is based on limited data. Elevated soil populations after rhizobial release from nodules are typically followed by a decrease over a few months. Predation by protozoa can be significant [46,47], perhaps especially near nodules releasing many potential prey, but abiotic factors can also be important [48]. After this initial decrease, rhizobial population size can remain high for years, even without host plants. When one year of bean was followed by three years of wheat, soil populations of bean rhizobia remained more than one-thousand times that in plots where only wheat had been grown [49]. In another field experiment, soil populations of pea rhizobia remained elevated for 5 years after peas were grown [50].

Such stability of soil rhizobial populations over years could reflect either a remarkable balance between rhizobial reproduction and death or else remarkable longevity of individual rhizobial cells. We tentatively favor the latter hypothesis, because we would otherwise have to explain why having grown pea once would increase reproduction or decrease death of pea rhizobia in the soil three years later.

How might individual rhizobia survive for months or years between legume hosts? Root exudates from nonhosts might support survival or even reproduction. Significant reproduction can occur as endophytes inside roots, stems, and leaves of nonlegumes, with some rhizobia leaving via plant stomata to potentially recolonize the soil [2]. But these non-symbiotic lifestyles probably make only small contributions to soil numbers (relative to massive releases from nodules) and variation in root exudation or endophyte release seem more likely to cause fluctuations than stability. Another intriguing possibility is that at least some nodules release rhizobia over a period of many months, perhaps even years. But what are the options for long-term persistence of rhizobia in the bulk soil?

Rhizobia can form multispecies biofilms on surfaces exposed to soil [51], although clusters of only a few bacterial cells may be more common in soil [52]. Joining a biofilm could offer protection against desiccation or adverse pH [48], but biofilm formation may provide at most a partial explanation for the long-term survival of rhizobia in soil. Rhizobial numbers in biofilms fell to one-eighth of their initial value over a period of just two weeks [51].

This rapid die-off contrasts with 70% survival, even after 528 days without external resources, of *S. meliloti*

Table 1. Life-history comparisons of rhizobial and arbuscular mycorrhizal fungal symbionts.

| Life history | Rhizobia | Arbuscular mycorrhizal fungi |
|---|---|---|
| Taxonomy | Various α- and β- proteobacteria | Fungi in the phylum Glomeromycota |
| Symbiosis obligate | No | Yes for fungal partner |
| Benefit to host plant | N | P, N, protection, others |
| Potential for conflict among symbionts (genetic heterogeneity) | Many strains per plant, multiple strains per nodule possible | Many strains per plant, many genotypes per fungal 'individual' |
| Persistence in the absence of host | Saprophytic growth using soil C or root exudates, starvation-resistant persisters, biofilms | Dormant spores, direct connection to compatible mycorrhizal networks if host roots are too distant |
| Strategies employed in soil | Quorum-sensing, persister-formation | Reversible germination of spores, reconnection of disrupted networks, anastamosis of genetically-different hyphae, variable proliferation of nuclei |
| Strategies employed during symbiosis | Resource allocation among $N_2$ fixation, hoarding, and rhizobial reproduction | Symbiosis with multiple hosts simultaneously, conditional allocation to soil vs. root colonization, hoarding of C, retention of P in hyphal network |

'persisters' [53], which have high PHB reserves and apparently low metabolic activity. Rhizobia in the persister state show resistance to ampicillin, which kills actively growing cells of the same genotype. Persisters also have less protein synthesis relative to actively growing cells of the same genotype [54]. When an *S. meliloti* cell divides under starvation, the old-pole cell retains most of the PHB and becomes a persister, while the new-pole cell is more active, with greater competitiveness under high-resource conditions. A single cell producing both a persister and a cell primed for active growth follows a bet-hedging strategy similar to individual plants with seed dimorphism. This is unlike the population-level bet-hedging previously reported in bacteria, which involves a small random subset of cells switching phenotype.

Persisters can survive long-term starvation, but they could still be consumed by predators. Crevices in soil particles may offer refuges too small to be invaded by predators [52,55]. Joining a biofilm 'selfish herd' [56,57] might provide protection from predation. A persister's antibiotic resistance might be particularly useful in biofilms, surrounded by so many potentially hostile neighbors, although persister resistance to the bacteriocins rhizobia use against each other [58,59] has not yet been determined. In summary, it is well-established that rhizobial populations can survive in soil for years without hosts, and individual cells can survive for over a year as persisters. What is less understood is the full range of rhizobial adaptations to survival in soil, and any tradeoffs that could occur with other life-cycle stages.

### Finding and Infecting New Hosts

Most individual rhizobial cells in soil never infect and nodulate a host. Bacteria that are classified as rhizobia — but that are unable to nodulate — sometimes outnumber symbiotic strains [3]. Still, there are many more potentially symbiotic rhizobia (typically $\sim 10^{13}$ ha$^{-1}$) than there are nodulation opportunities (typically $\sim 10^7$ ha$^{-1}$) [60]. When soil rhizobial populations are at steady state (not true for soils where a legume crop is being grown for the first time), the number of descendants a rhizobial cell produces via symbiosis is balanced by the low average probability of nodulating successfully. For example, if the average rhizobial cell in soil had a one-in-ten-thousand chance of nodulating in a given year, thereby producing one million descendants in the soil, then rhizobial populations would increase one hundred-fold each year. So, if rhizobial populations are not increasing, either nodulating results in far fewer than one million descendants in the soil, or the chance of nodulating is much less than one in ten thousand. Based on our estimate of rhizobial release above, the latter hypothesis seems more likely, but additional field data are needed.

But, however long the odds, is attempting to nodulate — for example, by chemotaxis towards a receptive root — always the best rhizobial strategy? Only if the chance of nodulating successfully, times the fitness benefit from doing so, outweighs any added risks. The risks from attempting to nodulate include exposure to phage or toxic bacteriocins, both of which are likely to be more abundant where greater numbers of rhizobia swarm around receptive legume roots. In peat, phage released by one rhizobial strain reduced the population of another by 98%, while a bacteriocin-producer decreased a bacteriocin-sensitive strain 99% [61]. Predatory protozoa may also be more abundant where rhizobia swarm around prey populations are greater, for example, near receptive roots (Figure 1A).

If a rhizobial cell is already near a receptive root, then the potential benefits of attempting to nodulate may greatly outweigh any added risk from bacteriocins, phage, or predation. Rhizobia that are slightly more distant, however, could swim into the danger zone around a receptive root, only to find that all nodulation opportunities have been taken before they arrive, especially if the local population density of competing rhizobia is high.

Rhizobial cells may use chemical cues to assess their individual chances of nodulating successfully, as well as any additional risks involved. Key variables include the density of predators, the distance to the root — or the distance along the root to a region root hair currently susceptible to nodulation [62] — and the local density of rhizobial competitors. Some bacteria detect chemical cues released by predators [63], but it is not known how rhizobia respond to such cues.

Root exudates from legumes and other plants attract rhizobia and other bacteria [64]. Generic growth substrates like amino acids stimulate chemotaxis and could presumably attract rhizobia to roots of either host or nonhost species. Host-specific signal molecules, like luteolin [65], cause chemotaxis at less than one-hundredth the concentration needed to activate rhizobial nodulation genes. Chemotaxis is weaker for luteolin than for organic acids or amino acids, however, perhaps reflecting the difficulty in detecting direction when concentration is so low [66]. If the luteolin concentration is too low to indicate direction, that may also indicate that the susceptible root is relatively distant, so the chance of reaching it before competitors do is small.

This chance of reaching a receptive root hair before the competition depends on how many competitors are nearby. Like many bacteria, rhizobia obtain information about the density of conspecifics from various chemical cues. Unlike quorum-sensing 'signals', the incidental release of chemical 'cues' does not depend on any benefits they may provide to receivers [67]. It has been suggested that quorum-sensing evolved as an individual adaptation, releasing and monitoring a chemical probe to predict how quickly expensive extracellular enzymes would be lost to diffusion [68]. But such probes may then be used as population density cues by eavesdroppers. Similarly, the rhizobial siderophore bradyoxetin is released to obtain iron, but it is also apparently used by other rhizobia to estimate competitor density. High concentrations of bradyoxetin can shut down nodulation genes [69]. Additional evidence that density cues can suppress nodulation attempts includes reduced nodulation at very high rhizobial densities [70,71] and greater nodulation by mutants defective in quorum sensing [72,73].

If chemical cues indicate that receptive roots are distant, competitors are abundant, and predation risk is high, then dispersing away from the crowd (Figure 1A) or staying safe inside a soil aggregate, perhaps as a starvation-resistant persister, may have higher expected fitness than attempting to nodulate immediately. As discussed above, a rhizobial cell or its descendants may survive for years in the soil, so waiting for a less-risky nodulation opportunity may be an option.

## Arbuscular Mycorrhizal Fungi Life History

The 450-million-year-old arbuscular mycorrhizal symbiosis is likely the world's most abundant mutualism. The plant–fungal partnership is responsible for massive global nutrient transfer, global carbon sequestration, and soil stabilization [9]. Recent research has taken a more mycocentric approach to the mutualism, and in doing so, the field has made rapid advances in understanding the life histories of these seemingly abstruse fungal symbionts.

One of the most important advances in the field is the finding that genetically different nuclei co-exist in individual AM fungal hyphae [74]. Because hyphal networks contain large numbers of genetically unique nuclei that potentially reproduce differentially, this means that selection is hypothesized to act within an 'individual' AM fungal network [75–78]. This has two important implications that require much more research, the first being that certain nuclei may be favored to proliferate, depending on local nutrient conditions or disturbance regimes. This has the potential to confer a unique dynamism to mycorrhizal networks, increasing their flexibility in both space and time [77], in ways not typical of asexual organisms. The second implication is that significant evolution is possible even without new mutations. There is a huge pool of functional variability even within single AM fungi species [79–82], perhaps partly due to differences among nuclei within individuals. This offers the intriguing possibility that genetic resources can be exchanged, similar to sexual reproduction, but in asexual organisms. This can complicate life-history studies. Because so much variation exists, at so many different levels, it can be difficult to draw more general conclusions about the symbionts themselves.

## *Symbiosis and Fitness Benefits to AM Fungi*

One of the key differences between rhizobia and AM fungi is that the latter are completely dependent on a plant host for growth and reproduction [9]. So benefits to the fungus from colonizing hosts are clear: AM fungi cannot obtain carbon without them. The structural interface, where nutrients are exchanged between plant and fungal partners, is therefore the epicenter of the mutualism.

To achieve resource exchange, the fungus must penetrate the root epidermis and form membranes, typically structures called 'arbuscules' (Figure 2). Mature arbuscules are characterized as intraradical hyphae that are highly branched with a high surface to volume area (Figure 2B). When arbuscules are formed, they are short-lived, functioning for only 4 to 5 days. Resource exchange is followed by rapid arbuscule collapse (Figure 2C), with structures degenerating within 2.5–5.5 hours [14], much more rapidly than the decline in $N_2$ fixation in nodules [83]. Stunted arbuscule morphology has been described when arbuscular-specific plant phosphate transporters had been knocked out [84]. This work is consistent with the idea that plants will decrease carbon provision, or directly digest arbuscules [85] when there is insufficient phosphate being transferred to the host across the colonized cell. However, we still lack direct evidence how (and where) carbon transport is controlled across interfaces. Understanding arbuscule collapse has, until recently, been a neglected area of research, but will likely aid in our comprehension of how plants and fungi enforce cooperation [9,86,87].

Like arbuscules, vesicles (fungal storage units) are potentially important AM fungal structures in defining fungal fitness for some AM fungal families (for example, Glomeraceae). Whereas a high frequency of arbuscules usually indicates effective nutrient exchange in both directions, high vesicular colonization is a potential indicator of fungal resource hoarding. The ratio of vesicular to arbuscular colonization is therefore often used as an estimation of symbiotic cost-effectiveness [88,89]. Allocating carbon into vesicles is similar to rhizobia storing carbon in PHB granules in nodules, as discussed above. High allocation to vesicles is particularly prominent under high external nutrient conditions, when hosts are less dependent on fungal partners for nutrient uptake. Some fungi, for example *Glomus intraradices*, are known to be tolerant to high phosphorus levels, while other species are apparently absent under these nutrient levels [90]. One recent study found that AM fungal investment (as a whole) in storage vesicles increased four-fold in fertilized compared to control plots [91], but whether this was due to a shift in species composition inside roots or changes in allocation strategy is unknown. From a fungal point of view, allocating more carbon to storage is likely the best strategy when nutrients are abundant. This is because host dependence on mycorrhizae is reduced by phosphorus fertilization, and evidence suggests that only recently assimilated plant carbon is allocated to the fungus; after that, carbon allocation stops [92].

High levels of available phosphorus have also been shown to have a suppressive effect on fungal colonization, leading to malformed arbuscules with reduced branching [93]. When plants reach a high phosphorus status, the mycorrhizal phosphorus uptake pathway can be almost completely repressed, with plant phosphorus transporter genes downregulated [94]. In these cases, high available levels of phosphorus appear to trigger an 'anti-symbiotic syndrome' which involves plant suppression of genes encoding critical enzymes in the symbiosis [93].

This result begs the question: have host plants evolved sanction-like mechanisms to control carbon allocation

patterns to their fungal symbionts, similar to what has been found with rhizobia [33]? Sanctions could arise either through fine-tuned suppression mechanisms involving gene regulation, via modification of carbohydrate relations, or both. It is reasonable that overall carbon availability for AM fungi will depend on the ability of the fungi to provide phosphate to the host, as previously suggested [95], although this would not automatically result in differential allocation of carbon resources among different strains. Research suggests that plants do have some control over carbon transfer to AM fungi. Reduced carbon transfer to AM fungi has been shown using plants with defective phloem loading or decreased root acid invertase activity. This resulted in reduced fungal colonization, suggesting that colonization can be controlled by plants via changes in sugar allocation [96].

Evidence from split-root experimental systems [97] suggested that hosts can identify and preferentially allocate carbon to the highest-quality mutualist, but only with extreme spatial structure: meaning only when colonized by two species per plant, on opposite halves of the root. In contrast, recent empirical work [98] has shown that strong spatial structuring of AM fungal communities may not be essential for cooperation to persist. A series of stable isotope probing experiments tracked carbon allocation of plant hosts into the RNA of fungal strains ranging in benefit from cooperative to less-cooperative. The RNA of this mixed fungal community was separated into heavy and light fractions via ultra-centrifugation. The abundance of each strain from each fraction was quantified using quantitative PCR. The heavier fraction, which had received more carbon from the plant, was dominated by RNA sequences corresponding to the more cooperative strains. This suggests that — even when symbionts intermingle on a single root system — an AM fungal strain that provides the host plant with more phosphorus will be rewarded with more carbon [98].

These results are consistent with host sanctions at a fine enough scale that host can identify 'cheaters' even when they are intermixed on the same root system with mutualists. Molecules such as lysophosphatidylcholine (LPc) may help hosts to sense phosphorus concentrations, potentially allowing hosts to evaluate the amount of phosphorus delivered via the mycorrhizal pathway [99]. However, the physiological details of how carbon and phosphorus fluxes are mediated at the cellular level have yet to be uncovered.

Although the AM fungal symbiosis is predominately characterized by the trade of carbon for soil nutrients, AM fungi confer a motley of benefits to host plants, including protection against biotic (pathogens, herbivores) and abiotic stresses (for example, drought, heavy metal uptake, salinity) [10]. In many cases, these functions appear to be the primary benefit a plant receives from the symbiosis (for example, [100]). Linking these 'auxiliary' host benefits with benefits to the AM fungi is difficult because the costs for the fungi of performing these actions are unclear. Are these benefits simply a byproduct of the nutritional exchange? For example, fungi in the Glomeraceae have been shown to confer greater protection against root pathogens such as *Fusarium* sp. and *Pythium* sp. [16], relative to the Gigasporaceae. It has been hypothesized that the greater protection comes as a result of high rates of internal root colonization, which decrease the potential infection sites available for pathogens (but see [101]). In contrast, fungi in the Gigasporaceae, characterized by high allocation to external, rather than internal, colonization show limited pathogen protection [16]. The extent to which AM fungi-induced changes in host physiology or morphology benefits plant and fungal fitness merits further research [33].

The good news is that mycorrhizal research is moving away from quantifying single functions provided by AM fungal partners and into an era of assessing their relative contributions to a diversity of functions. New methodologies, such as structural equation modeling, are providing tools to use existing data sets to determine which functions are most important in which AM fungal species/isolates [10]. This will allow us to refine our functional classification of AM fungi, and better relate multiple functions to life-history and benefits to fungal fitness.

### Finding and Infecting New Hosts
Even though they obtain little or no carbon there, the bulk of the AM fungal organism exists in soil and is subject to the shifting selection pressures of this environment. Unlike a rhizobial cell, AM fungi can infect new hosts while simultaneously engaged in an active symbiosis. This means the search for new hosts is constant. Fungal mycelia (hyphae) can grow up to 100 times longer than root hairs, providing a vastly more extensive nutrient foraging system than roots alone, and, from a fungal perspective, foraging provides a means to find new hosts.

The strategic (and perhaps conditional) allocation of resources by the fungus to soil colonization has interesting consequences for benefits conferred to the host [102–104]. But how does fungal strategy (for example, colonization intensity inside and outside the host) and architecture (for example, hyphal diameter, number of runner hyphae, absorptive hyphal networks and hyphal bridges [105,106]) relate to the fitness of the fungi themselves, including benefits from infecting new hosts?

Greater internal colonization (within host root) has the potential to enhance fungal carbon acquisition from, and phosphorus transfer to, the host. However, a large external hyphal network allows the fungi to better forage for nutrients and new hosts. Often AM fungal species supporting the greatest phosphorus acquisition incur the highest carbon costs, although there are clear examples in which large fungal carbon requirements result in negligible phosphorus uptake [107].

Internal *versus* external allocation strategy can be plastic (Table 1). For example, when a host plant is shaded, AM fungi will reallocate more carbon to external hyphae, potentially increasing the capacity to find a new host [92]. Likewise, in some but not all AM species, individual hyphae can fuse (anastomose, Figure 1B) and even help form connective networks between different plant species [108,109]. Hyphae of genetically distinct isolates have been shown to exchange genetic material [77,109,110], and evidence for recombination in local populations suggests that genetic exchange may be more common than previously thought [111].

The ability to anastomose has been linked with greater external soil colonization, and hence potentially greater fungal fitness [112]. However, exactly whose fitness increases in situations when fusion occurs between genetically different (but closely-related) AM fungi isolates — shown to occur with a frequency of 1–10% — will be an interesting line of future research [77]. For example, there may be a strategic 'optimum' for fusing frequency based on the benefits of spreading rapidly via a high fusion rate *versus* the proliferation of certain nuclei under environmental heterogeneity

increasing local adaptation. This is analogous to the way gene flow can increase or decrease local adaptation.

The ability to colonize several host plants simultaneously may likewise increase the potential for maximizing fungal fitness. From a fungal viewpoint, individual plants are theoretically dispensable, especially if they provide few carbon benefits relative to other hosts in the network [113]. Unlike rhizobia, whose options are limited once in symbiosis with a host, because they are fully encapsulated, AM fungi retain the potential to interact with different partners [33]. This could reduce potential host exploitation of the fungus, and raises the interesting possibility that fungi can abandon particular partnerships, for instance when an individual host becomes shaded and carbon supply is reduced [92]. Experiments utilizing *in vitro* root organ cultures found that AM fungi allocate significantly more phosphorus to root systems providing more C [98], suggesting that control is bidirectional. Now these type of experiments need to be scaled up to whole plants.

Although incredible variability can exist within single species, fungi in the Glomeraceae family are typically characterized by high allocation to colonization within the root [16]. In contrast, in the Gigasporaceae, the majority of the fungal biomass is allocated to external hyphae outside the root [16]. *Gigaspora margarita*, in particular, has remarkably high retention of phosphorus (as opposed to rapid transport to the host by *Glomus* sp.) in the external hyphae and this has been proposed as a strategy to maintain — and perhaps even stimulate — carbon transfer from the host [103]. Uptake and retention of phosphorus by AM fungi is thought to represent a hoarding strategy to make the host plant more reliant on the fungal partner for its phosphorus resources [87], though this effect could be under-cut if other fungal strains infecting the same plant continued to supply high phosphorus resources to the host.

A key question in the evolution of life-history strategies is whether there are important trade-offs in colonization of soil versus colonization of host [105]. One comprehensive study [114] found no support for trade-offs in internal versus external colonization, instead showing that root and soil colonization were positively correlated, even across different host species. However, such positive correlations can result from differences in total resource acquisition [115]; hosts with more carbon, for whatever reason, may support both more internal and more external growth.

Ultimately, fitness benefits will depend on the specific fungus/plant combinations and the environment in which the symbiosis is imbedded. While there may be strong conservatism of functional traits [114], trait expression (for example, the rate of spread) appears to be more plastic, varying for instance with host plant [116] or local environment [78,79]. One study of AM fungi grown in 'home' and 'away' soils found that the fungi produced more extraradical hyphae in their home soil, suggesting that locally adapted AM fungi were accessing greater amounts of carbon compared with nonlocal fungi [117]. This reiterates a fundamental aspect of AM fungi: the final outcome of the symbiosis, for both partners, is strongly context-dependent [118].

### Repopulating the Soil

Spore formation represents an important reproductive strategy of AM fungi, allowing them to propagate, recover from disturbance and survive the absence of a host, for more than 10 years, in some cases [119]. AM spores are
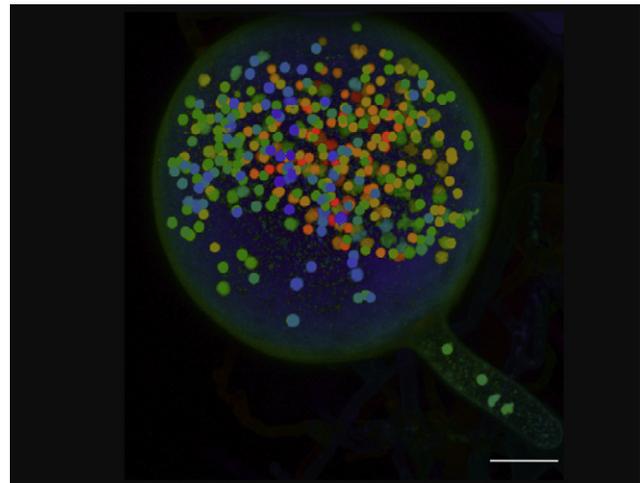


Figure 3. Typical multinucleated asexual spore of the AM fungus *Glomus diaphanum*.

The spore is visualized by confocal microscope showing nuclei stained with SytoGreen fluorescent dye. Spores of *G. diaphanum* range from 30–80 µm. Focal planes were colour-indexed on z-depth from red to violet (red colour on the bottom and violet on the top) to facilitate nuclear visualization. Figure is merged maximum intensity projection of 200 optical sections. Bar = 10 µm. (Courtesy of M. Hijri.)

surprisingly dynamic, sprouting hyphae that explore the soil but then arrest development and retract back into the spore, becoming dormant again, if they fail to meet a host root [14] (Table 1). In the absence of the host, spore germlings cease growth and retract within 8–20 days [119].

Even though AM fungi are completely reliant on host plants for carbon, their spores will germinate even in the absence of hosts, which is puzzling. However, spores can connect their germinating hyphae into larger compatible networks (via anastomosis), allowing them access to carbon from colonized plants [77,109], even if there is no direct access to the roots themselves. This is thought to lend a potentially critical fitness advantage at an early development stage.

The mechanics of spore formation remain largely a mystery. Cytoplasmic streaming translocates nuclei within hyphal networks and when spores are formed, they each contain hundreds to thousands of nuclei [120]. Nuclei are thought to come from two sources, those that migrate into the spore and those that arise by mitosis in the spore (Figure 2B) [121]. So unlike most other eukaryotes, AM fungi may not go through the genetic bottleneck of a single-nucleus stage.

There is a continuing debate on the nuclear composition of the AM fungi, with arguments that the genetic variation passed from generation to generation is the result of multiple chromosome sets (for example, high ploidy). This would mean that intracellular genetic variation is contained in each of the hundreds of nuclei that populate their cells and spores [122]. However, subsequent work has shown that even those AM fungi with larger nuclear DNA content are haploid [123].

It is proposed that multinucleate spores are initiated by a random sampling of nuclei from surrounding hyphae [77], potentially causing genetic segregation. Although this idea of segregation requires more research, the implication is that selection can act on populations of nuclei (nucleotypes) coexisting in fungal cytoplasm [78]. Recent work has

demonstrated that this type of segregation — for example, when new offspring of an AMF receive different complements of nucleotypes compared to the parent or siblings — can modify the function of the symbiosis, altering the transcription of certain plant genes, such as the expression level of phosphate transporters [75]. The effect of fungal segregation on host benefit has been found to be highly variable, depending strongly on host species identity. Although there has been progress in understanding how nucleotypes may cooperate and compete in other fungal systems [124,125], the exact mechanisms of how changes in AM fungal nucleotype frequency alters symbiotic interactions with plants remains a line of current cutting-edge research [77].

Importantly, carbon allocated to AM fungal reproduction represents a potential loss of resources to the plant host, and to other symbionts colonizing the same plant [33]. Differences in sporulation traits of AM fungi have been classified within the classic 'r–K' continuum [90,126,127]. For example, AM fungi in the Gigasporaceae produce few and large spores (> 200 μm) within a long life cycle, and tend to resemble K-strategists, while AM fungi in the Glomeraceae (Figure 3), particularly the globally cosmopolitan *Glomus intraradices*, display opportunistic behavior such as rapid colonization and production of many small spores (50–150 μm), typical of r-strategists.

Whatever the strategy, spore production, as well as the formation of auxiliary cells (clusters of cells important for reproduction in some AM fungal families) will be highly dependent on host carbon. When host carbon supply is terminated there is significant variation in AM fungal response [127]. For example, disconnection from hosts via strong, repeated disturbance appears to strongly select for AM fungi able to form spores quickly [128]. While the importance of sporulation should not be understated, it is likewise crucial to note that it is not a complete fitness measure, as some types of hyphae also infect plants, bypassing the spore stage (Figure 1B). Some fungi can exist as mycelium networks indefinitely, or nearly so, without making spores. Understanding variation in life-history strategies, from timing of sporulation to length of dormancy, is key to predicting how AM fungi persist and reproduce across an enormous diversity of host and environmental conditions, from deserts to wetlands, forests to agricultural fields.

## Conclusion

While there has been a clear past emphasis on how rhizobial and mycorrhizal symbioses affect plant fitness, research is now driving a new appreciation for how symbiosis affects the fitness of the microbial symbionts themselves. Significant progress has been made in understanding how symbiosis shapes microbial life-history strategies such as finding and infecting new hosts, and the ability to maximize use of host resources. What is still largely neglected is research into how symbionts repopulate the soil after symbiosis, and how they survive and adapt to challenges of the rhizosphere and bulk soil.

For rhizobia, escape from nodules and survival in the soil are two key phases of which surprisingly little is known. For example, delayed effects of host sanctions on these critical phases could conceivably reverse our current understanding of the benefits and costs of allocating energy to $N_2$ fixation versus rhizobial reproduction inside nodules. Similarly, can the risks of nodulation be quantified? An explicit test of the hypothesis that rhizobia may sometimes forgo long-shot nodulation opportunities (for example, when chemical cues indicate high predation risk) in ways that enhance survival until better opportunities are available would be worthwhile.

For AM fungi, research in recent years has demonstrated the occurrence of a sexual-like genetic system [129] involving hyphal fusion, biparental inheritance, recombination and even segregation (reviewed by [77]). But how does this potential plasticity allow individual fungi to maximize their own fitness? Research is needed to understand how fusion of genetically different isolates alters the reproductive success of fungal 'individuals'. Similarly, how important are rhizosphere selection pressures in shaping AM fungi strategies? Research has shown that manipulating factors such as external phosphate concentration can lead to small genetic changes in AM fungal isolates in just a few generations [78]. This work, among others, has stimulated an interest in the potential to generate (breed) novel AM fungal genotypes [75]. Our understanding of fungal life-history strategies — and our ability to breed for better strategies — could benefit greatly from research on how genetic polymorphism is distributed among nuclei, how nucleotypes compete and/or cooperate within fungal individuals, and how external selection pressures can be manipulated to direct this variation.

More generally, a greater understanding of such life-history strategies will increase our understanding of the evolution of cooperation and suggest new approaches for improving agricultural symbioses.

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