Community assembly and coexistence in communities of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi are asexual, obligately symbiotic fungi with unique morphology and genomic structure, which occupy a dual niche, that is, the soil and the host root. Consequently, the direct adoption of models for community assembly developed for other organism groups is not evident. In this paper we adapted modern coexistence and assembly theory to arbuscular mycorrhizal fungi. We review research on the elements of community assembly and coexistence of arbuscular mycorrhizal fungi, highlighting recent studies using molecular methods. By addressing several points from the individual to the community level where the application of modern community ecology terms runs into problems when arbuscular mycorrhizal fungi are concerned, we aim to account for these special circumstances from a mycocentric point of view. We suggest that hierarchical spatial structure of arbuscular mycorrhizal fungal communities should be explicitly taken into account in future studies. The conceptual framework we develop here for arbuscular mycorrhizal fungi is also adaptable for other host-associated microbial communities.

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developmental, genetic and ecological angles, the direct adoption of models for community assembly developed for other organism groups is not evident. There are several points from the individual to the community level where the application of modern community ecology terms runs into problems when AM fungi are concerned (Table 1). Especially in the area of coexistence, even for the definitions of such fundamental concepts as ‘fitness’ further research and discussion are needed (Table 2).

Here we introduce the elements of a community assembly and coexistence model by highlighting recent research on AM fungal communities. As examples for each element, we included studies that used DNA-based methods (preferentially, high-throughput sequencing approaches) to investigate AM fungal communities.

Factors affecting AM fungal community assembly: review of the elements of the proposed model

Regional pool
AM fungi have species pools with distinct composition according to paleocontinents, although endemic species are rare (Kivlin et al., 2011; Davison et al., 2015). Regionally, observed AM fungal communities

Figure 1 Applying the combination of a filter model of community assembly and neutral processes for AM fungi. The regional pool of AM fungi consists of species present in the soil and in the roots of the host community. Through local or long-distance dispersal and chance, species reach local habitats. The environmental filter prevents species whose environmental tolerances do not overlap with local conditions from entering the community. The host filter allows colonization only for compatible fungal partners, thus further removing species. The local community reflects the cumulative effects of these processes, and in turn influences them through feedbacks. Horizontal interactions within the symbiotic community and with other non-host species also affect local communities. Local communities in turn contribute to the regional species pools with autochthonous propagule input. The capital letters refer to different AM fungal species. Ellipses with different lines depict different root system communities. Details of the depicted community assembly and coexistence model elements can be found in the section 'Factors affecting AM fungal community assembly: review of the elements of the proposed model' in the main text.
<table>
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<th>Level of biological organisation</th>
<th>General definition</th>
<th>Problem with usage for AM fungi</th>
<th>Possible solutions</th>
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| **Individual**                  | In modular organisms, an individual can be defined:  
• as a physically continuous unit, which is separated from other such units (ramet)  
• a unit with uniform genetic composition (genet)                                      | In AM fungi, these two definitions do not delineate the same parts of the mycelium (Rosendahl, 2008):  
• genetically different AM fungi are able to anastomose with each other (Chagnon, 2014) and might form a continuous mycelium where nuclei of different genetic compositions mingle (Young, 2009)  
• a genetically uniform mycelium might be physically disrupted  
• because of asexual reproduction with no recombination, different mycelia with the same genetic composition can be found in large geographical distance from each other  
• ‘ramet’ and ‘genet’ are used by some researchers; however, many use the terms ‘clone’, ‘strain’ and ‘isolate’ as with other microbes to grasp different aspects of the concept of an individual | • DNA profiling of individuals using mitochondrial DNA, the mitochondrial genome appears to be genetically identical within mycelia (de la Providencia et al., 2013; Daubois et al., 2016) |
| **Species**                     | Some commonly applied species concepts for fungi are (Moore et al., 2011):  
• morphological: based on morphological similarity  
• biological: based on reproductive isolation  
• phylogenetic: defining OTUs based on genetic similarity | • Morphological: many AM fungal species are unculturables and their appearance in roots varies with the host  
• Biological: no evidence of sexual reproduction, so mating tests are not possible  
• Phylogenetic: It is not clear what level, if any, of genetic difference is a suitable proxy for species or other levels of biologically interacting units (Hao et al., 2011; Caruso et al., 2012b; Fawell, 2012) | • Morphological: traditional taxonomy of AM fungal morphotypes is based on the characteristics of spores  
• phylogenetic: – Fixed and named OTUs are available for the sake of comparability between environmental studies, based on the small subunit of the ribosomal DNA (Öpik et al., 2010)  
– Efforts are made to create a unified sequence-based species delimitation of Glomeromycota using multiple loci (Öpik et al., 2014) |
| **Community**                   | Species with similar ecology that coexist in the same spatial region (Chesson, 2000). Definitions often include that community members must be able to interact (for example, Whittaker, 1975) | At which spatial scale should the AM fungal community be defined? | Adapting a spatially explicit, hierarchical community system from parasitology (Figure 2, see also section ‘Scale dependency: different assembly rules for different spatial scales? An analogy borrowed from parasite communities’ in the main text):  
• AM fungi in a root fragment  
• infracomunity: AM fungi in an entire root system of one host  
• component community: AM fungi in the root systems of a population of one host species  
• compound community: AM fungi in the root systems of the host community (mixed root samples from a sampling site) |
| **Metacommunity**              | Metacommunities are spatially divided species assemblages, where dispersal among communities is limited (Morin, 2011) | The assemblage of AM fungal communities living in the root systems of a plant community cannot be easily described by the metacommunity theory:  
• instead of only dispersing between hosts by propagules, AM fungi in different hosts might interact or even be physically continuous with AM fungi living in other root systems, forming CMNs (Selosse et al., 2006), which are large, interconnected networks of fungal hyphae that are simultaneously connecting multiple hosts  
• hosts are not passive islands:  
– AM fungi can preferentially allocate nutrients to high-quality hosts connected to the same CMN (Fellbaum et al., 2014) and CMNs can provide means of infochemical transport between connected plants (Barto et al., 2011)  
– AM fungi can modify the fitness of their hosts depending on their identity. Fitness responsiveness of hosts to AM fungal colonization can change over time after the initial colonization (Veresoglou et al., 2012; Mihaljevic, 2012a, b) | Application of metacommunity theory would require modifications |

Abbreviations: AM, arbuscular mycorrhizal; CMN, common mycorrhizal network; OTU, operational taxonomic unit.
are spatially heterogenous, but temporarily stable, suggesting a fairly constant soil species pool from which mycorrhizae form during the season (Davison et al., 2012).

Dispersal and chance (neutral processes)
Propagules and vectors of dispersal in AM fungi. AM fungi disperse by autochthonous (local mycelium spread) and allochthonous propagules (spores and other inoculum, such as hyphal fragments or colonized root fragments from outside), with the allochthonous propagules being less important locally (Jumpponen and Egerton-Warburton, 2005). AM fungi often have large spores, and many species are distributed by zoochorhy (for example, through the guts of rats (Janos et al., 1995), earthworms (Shapiro et al., 1993) and collembolans (Klironomos and Moutoglis, 1999) or on the hooves of bison (Lekberg et al., 2011)) as opposed to wind, where their spores are detected rarely (Egan et al., 2014). Thus, AM fungal species are mostly limited to short-distance dispersal. However, over long timespans, these limited dispersal capabilities allow for a surprisingly efficient spread of taxa (Davison et al., 2015).

Spatial community structure, dispersal limitation and other stochastic processes. AM fungal communities are spatially structured, patchily distributed even in relatively homogenous local environments (Rosendahl and Stukenbrock, 2004; Mummer and Rillig, 2008), which suggests that there are other processes beyond environmental filtering that contribute to the structure of AM fungal communities, for example, dispersal limitation. The relative importance of dispersal to environmental filtering is scale-dependent and varies (soil type and dispersal ability, Lekberg et al., 2007; soil pH, C/N ratio, phosphorus and dispersal, Dumbrell et al., 2010a: soil temperature, plant bioiimes and dispersal, Kvlin et al., 2011). Dispersal and other neutral processes thus exhibit an effect size spectrum that can be completely masked by extreme environmental heterogeneity or anthropogenic disturbance, resulting in communities more dissimilar than expected under the assumptions of neutral theory. On the other hand, stochastic effects are also limited under very homogeneous environmental conditions because of niche effects (Caruso et al., 2012a).

Environmental filter
Niche partitioning along environmental gradients. The assembly process and the coexistence of AM fungi are influenced by various soil environmental variables, such as pH, soil type, soil chemistry and nutrient availability. As nutrient transport is a function of AM fungi, the effect of nutrient availability is well studied (reviewed in Johnson, 2010). The filtering role of the environment, when some species from the regional pool are not present under certain soil conditions, was shown in fertilizer addition experiments: AM fungal phylotype diversity decreased with increasing N and P availability and some AM fungi were only found in specific soil nutrient conditions (for example, Liu et al., 2012; Camenzind et al., 2014; Liu et al., 2015).

Seasonality. AM fungi show temporal niche partitioning over the course of the year. Previously rare types might replace the dominant species (Husband et al., 2002). As possible explanations for this shift, both changing environment, for example, changes in temperature and sunshine hours (which influence the soil carbon pool, Dumbrell et al., 2011), and the seasonal cycle of the plant community and phenology were suggested.

Disturbance. Increasing agricultural land-use intensity selectively removes rare AM fungal species from the local community (Helgason et al., 1998; Verbruggen et al., 2012). Heavy anthropogenic disturbance, such as plowing, tillage and fungicide treatment, can lower the number of AM fungal species, abundance and root colonization while favoring generalist species (Helgason et al., 1998; Hijri et al., 2006; Helgason et al., 2007; Schnoor et al., 2011). However, disturbance does not always shift communities in a predictable way (Lekberg et al., 2012) probably because of the dominance of stochastic effects (Caruso et al., 2012a)

Host filter
One of the particular features of AM fungal community assembly is the importance of the host filter compared with free-living or facultatively symbiotic organisms. Plants restrict AM fungal diversity in roots (Johnson et al., 2004) and they also differentially influence sporulation (Eom et al., 2000). Given the obligatory symbiotic AM fungal lifestyle, the existence of host effects could be obvious. The non-evident detail in the host–AM fungal relationship is the apparent lack of species-level specificity (there are many fewer AM fungal species than plant species, even though most land plants are mycorrhizal). As an explanation, it was proposed that being able to colonize and to be colonized by a wider range of partners has an evolutionary benefit, and that environmental conditions affect the ability of plants to differentially reward their symbionts (reviewed in Walder and van der Heijden, 2015). In the field, different plant species, and even plants of the same species at different growth stages, associate with different fungal communities from the same soil (Gollotte et al., 2004; Sykorová et al., 2007; Gosling et al., 2013) and some AM fungi do not colonize certain plants (Helgason et al., 2002). AM fungi differ regarding how beneficial they are for hosts (Helgason et al., 2007), and plants are able to reward better fungal partners with photosyntheses (Bever et al., 2009; Kiers et al., 2011). The solution might be that
host specificity does not happen at the species level, but on an ecological level, where generalist AM fungi interact with generalist plants while specialists tend to occur in the roots of specialist plants (Opik et al., 2010; Davison et al., 2011). Furthermore, pairings of hosts and symbionts with similar life history strategies (competitive, stress tolerant, ruderal, as described for AM fungi in Chagnon et al., 2013) are likely more beneficial. The functional traits defining these strategies are often conserved at a higher taxonomic level (Maherali and Klironomos, 2007; Chagnon et al., 2013). Not only the host itself but also neighboring plants (Hausmann and Hawkes, 2009) and plant species richness (Burrows and Pfleger, 2002; Engelmoer and Kiers, 2015) influence fungal communities. In addition, AM fungal preference regarding hosts also exists (Davison et al., 2011).

### Table 2 Problems and solution attempts in applying community ecology terms to AM fungi

<table>
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<tr>
<th>Community ecology term</th>
<th>Problems in using it in AM fungal community ecology</th>
<th>Current solution attempts and their issues</th>
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<tbody>
<tr>
<td>Fitness</td>
<td>The definition of fitness in other organisms usually includes a measure of reproduction. • As AM fungi are asexual organisms, how can their fitness be defined? • It is difficult to use a proxy for AM fungal fitness, which could be used to compare species, as: (1) higher propagule abundance does not necessarily translate to higher colonization (2) there are significant allocation differences among species in growth of spores versus hyphal network (Veresoglou and Halley, 2012) • AM fungal fitness always depends on plant carbon, as they do not have independent ways to take up carbon (Johnson, 2010)</td>
<td>• Spore production and root colonization rates are possible fitness measures • Marker gene copy numbers can be used as a proxy of root colonization (Thonar et al., 2014). Distinguishing some AM fungal species in co-colonized roots is now possible with species-specific quantitative real-time PCR</td>
</tr>
<tr>
<td>Traits</td>
<td>How to study AM fungal traits?</td>
<td>• Traits in culture: traits are assigned to strains and might not be representative of a species • Transcriptomes of a single species: the study of the transcriptomes of species (Tisserant et al., 2012) might explain perceived functional redundancy (Peay et al., 2008) and provide mechanical understanding of community assembly, but it suffers from the same problem • Metatranscriptomes: solving the annotation problem in the emerging field of metatranscriptomics might enable us to study traits in field communities</td>
</tr>
<tr>
<td>Niche</td>
<td>Dual niche of AM fungi in root and soil</td>
<td>• AM fungi are obligate symbionts, but not only are they required to colonize a root system to complete their life cycle, but also to forage in the soil for nutrients and water • Thus, they are affected by factors both within and outside the root system at the same time • The composition of AM fungal communities is different in the two compartments (Hempel et al., 2007), and it is likely that forces governing soil and root communities are different (Liu et al., 2012) • AM fungal species differ in functional traits regarding spatial niches (for example, to what extent do they colonize roots or soil), and these traits are also conserved (Hart and Reader, 2002; Powell et al., 2009)</td>
</tr>
<tr>
<td>Bipartite networks</td>
<td>How does the network theory describe host–AM fungal interactions (Chagnon et al., 2012)?</td>
<td>• AM fungal–plant networks regularly show nestedness (species interact with a subset of the species generalists interact with) and modularity (species tend to group into modules in which interactions are more frequent than with the rest of the community; Opik and Moora, 2012; Verbruggen et al., 2012) • These network characteristics may derive from overdominance of the founder AM fungus (Dumbrell et al., 2010b), habitat heterogeneity, specific selectivity in plant–AM fungal associations, plant–AM fungal overlapped phenology or AM fungal competition within the root (Montesinos-Navarro et al., 2012) • However, in order to correctly apply network theory to AM fungal–plant interactions, basic assumptions need to be verified, that is, detected co-occurrence must imply interactions (Caruso et al., 2012b)</td>
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Abbreviations: AM, arbuscular mycorrhizal; PCR, polymerase chain reaction.
**Non-host biotic interactions and feedbacks**

*Horizontal interactions between members of local AM fungal communities.* Past work has found intense competition for root space (Cano and Bago, 2005; Engelmoer et al., 2014) and even competitive exclusion (Hepper et al., 1988). As opposed to root colonization, the ability of AM fungal species to colonize soil did not influence coexistence (Maherali and Klironomos, 2012). Phylogenetic overdispersion promotes coexistence: communities of more distantly related and functionally different species showed higher realized species richness (Maherali and Klironomos, 2007). Conserved differences in other functional traits, such as timing of spore production and hyphal growth rate, metabolism of photosynthates, P and N uptake, might alleviate competition as well.

Despite the potential importance for commercial use of fungal inocula, the effect of arrival order in AM fungi is not well understood. Priority effects were shown (Mumhey et al., 2009); however, it was recently observed that the resident AM fungi did not suffer from reduced growth despite being invaded, which makes competition for space an unlikely explanation, and suggesting downregulation by the host instead (Werner and Kiers, 2015).

**Interactions with other non-host organisms.** Negative interactions with consumers (fungal grazers), pathogens and parasites could reduce competition between AM fungi. However, collembola feeding on AM fungi had no effect on the community composition (Gange, 2000), and parasitism has not yet been conclusively shown to exist in AM fungi (Purin and Rillig, 2008). Either these interactions are really not important for AM fungal communities or we are limited by data.

AM fungi harbor bacteria associated with their spores. These bacteria promote hyphal growth and stimulate nutrient biodynamics. They might facilitate not only the fungus, but the whole mycorrhizal system by contributing to the suppression of soil-borne plant pathogens and by adding nitrogen fixation to the benefits of the plant (Cruz and Ishii, 2011).

**Feedbacks: AM fungi as ecosystem engineers.** AM fungi significantly modify their habitat both in the soil and in the plant in a way that influences their own communities. In the soil they increase soil aggregation and the water stability of the aggregates by a variety of mechanisms, including hyphal enmeshment (Rillig et al., 2015). Greater particle size and pore space may in turn benefit hyphal growth (Rillig and Steinberg, 2002).

They affect plant diversity and composition by improving the nutrient status of their host plants and by facilitating their hosts, which was shown to induce shifts in plant communities (van der Heijden et al., 1998). To harness this effect, enhancing natural AM fungal communities is suggested as an environmentally friendly weed-control option in agricultural ecosystems (Cameron, 2010). On the other hand, plant community composition also has an effect on AM fungal communities, completing the feedback loop.

**Relative importance of different elements: possible explanations for the idiosyncratic response of AM fungi to biotic and abiotic variables**

Despite the considerable literature that exists on the host, abiotic environmental and neutral factors influencing AM fungal community composition, there is no consensus on their relative importance. AM fungi have an idiosyncratic response to these variables. We propose two hypotheses to explain this pattern.

‘Law of the minimum’: an idea from plant nutrition. In agricultural science, the ‘law of the minimum’ is an idea that the scarcest essential nutrient (the most limiting factor) is the most important in determining plant growth (Gorban et al., 2011). Similarly, but stepping away from only thinking about resources, the relative importance of assembly factors would depend on the most restrictive component, and the most limiting factor would explain the most variability. Under non-filtering environmental conditions, in an abiotically homogenous sampling area, host effects would be relatively more important. A strong environmental gradient that includes harsh conditions unsuitable for certain species would result in environmental filtering as the dominant structuring force.

**Scale dependency: different assembly rules for different spatial scales? An analogy borrowed from parasite communities.** Studies on AM fungal communities vary strongly in the spatial scale being addressed. Definitions range from AM fungi found in a root piece through an entire root system to a mixed root sample of an entire site. As different assembly factors act on different scales, explicitly considering the spatial structure of AM fungal communities could lead to a synthesis between contrasting responses to assembly factors (for example, host versus abiotic environmental filter). Parasitology defines a hierarchical, host-based, scale-dependent community system (Figure 2) and Table 1). In infra- and compound communities of fleas, which also have varying levels of host specificity, the relative importance of environmental and host effects depends on the spatial scale (Linardi and Krasnov, 2013; Krasnov et al., 2015). AM fungal communities have a similar host-based hierarchical spatial structure (Figure 2); therefore, it is a compelling idea that the relative importance of assembly factors depends on the spatial scale in AM fungi too. Maherali and Klironomos (2012) hypothesized that subplot-scale interactions (infracommunity) such as competition could determine coexistence, whereas the AM fungal composition of a whole site (compound community) would mostly depend on niche requirements or climate (environmental filter). Consequently, AM fungal communities are found to show phylogenetic
clustering within study sites (Kivlin et al., 2011), with sometimes negligible effects of the environment (Horn et al., 2014), which might indicate facilitation between species. At a global scale, the AM fungal community composition was shown to be best predicted by spatial distance, edaphic and climatic factors, and plant community type (Kivlin et al., 2011; Davison et al., 2015). To sum up, the scale dependency of the relative importance of the elements of community assembly and coexistence is well established in many organisms; however, explicit consideration of spatial scale in AM fungal community studies is still rare. An example of how the relative importance of the assembly processes might change with spatial scales is shown in Figure 2.

Conclusion: community ecology from the viewpoint of a microbial symbiont

We presented a conceptual framework of community assembly and coexistence adapted to a microbial symbiont group with a unique combination of characteristics. The importance of factors influencing obligate symbionts differs from those affecting free-living organisms, or even facultative symbionts (Linardi and Krasnov, 2013). The host–AM fungal relationship, similarly to parasites, exhibits a hierarchical spatial structure, which should be explicitly incorporated into future studies, to enable the study of the scale dependency of the relative importance of elements of community assembly. Adapting a symbiont-centered point of view in addition to considering how the host community is affected would help to fill the knowledge gaps of coexistence research, especially in the field of non-host interactions.

Outlook: how further research on AM fungal communities could advance the field of community assembly and coexistence theory

Owing to the advance of high-throughput molecular methods, researchers gained insight into the communities of specialized organisms, for example, the AM fungal communities in plant roots. With the number of AM fungal community studies rising, it is now possible to start to piece together the mechanisms influencing community assembly and coexistence. By considering the unique combination of characteristics in genetic makeup, physiology, niche and dispersal of AM fungi, and highlighting problems in applying community ecology concepts stemming from these, we are getting closer to adapting community assembly and coexistence models to them.

Taking levels of community organization related to the host into account (infracommunities, component communities and compound communities, see Figure 2) can help reconcile contrasting results regarding the relative importance of assembly factors.

In AM fungi, where the effect of the host filter is so significant, non-host biotic interactions, although they might not be able to act as a filter in community assembly, are still influencing community structure, and future studies in this currently neglected field might reveal more interesting relations.

Examining different assembly and coexistence factors in a multitude of specialized microbial groups would help advance the field of community ecology by increasing the external validity of its models and theories. Although it is important to
transfer concepts from general ecology, it is critical that these concepts be carefully evaluated before application (Table 1): two examples are the application of metacommunity concepts to symbiotic systems (Veresoglu et al., 2012) and the use of network theory in mycorrhizal ecology (Caruso et al., 2012b); in both cases it is important to verify the validity of assumptions lest analyses be misleading. Emerging concepts in community ecology, like metrics for quantifying intransitive competition (Sólymers et al., 2015) or community coalescence (Rillig et al., 2015), will require similar validation to apply them to specific microbial communities. Doing so can lead to new hypotheses in the AM fungal and broader community ecology, as in applying community phylogenetics (Webb et al., 2002; Vamosi et al., 2009) to AM fungi: after carefully proving that AM fungal traits related to spatial niche use are conserved at a higher taxonomic level (Maherali and Klironomos, 2007), this was used to generate hypotheses and a theoretical framework on the coupling of plant and AM fungal life history strategies (Chagnon et al., 2013).

Answers to the questions of community assembly and coexistence in AM fungi are increasingly required in order to more successfully manage AM fungi for application. Community composition influences ecosystem services, which is true also for AM fungi (van der Heijden et al., 1998). Better understanding of AM fungal communities could be a powerful tool in mitigating the effects of global change, for example, in agriculture and habitat restoration.

Conflict of Interest
The authors declare no conflict of interest.

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