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SPECIAL FEATURE – ESSAY REVIEW

THE TREE OF LIFE IN ECOSYSTEMS

A phenotypic plasticity framework for assessing intraspecific variation in arbuscular mycorrhizal fungal traits

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Summary

1. Statistical models of ecosystem functioning based on species traits are valuable tools for predicting how nutrient cycling will respond to global change. However, species such as arbuscular mycorrhizal fungi (AMF) have evolved high intraspecific trait variation, making trait characterization and inclusion in functional trait models difficult.

2. We present a five-part framework based on experimental designs from the phenotypic plasticity literature to quantify AMF intraspecific trait variation in a nutrient cycling context.

3. Framework experiments involve exposing AMF replicates to different environmental conditions and recording trait values to quantify the (i) degree of variation, (ii) reversibility of traits, (iii) relationships among traits, (iv) adaptive nature of traits and (v) potential for trait variation to evolve. We include a phenotypic trajectory analysis of a simulated data set to illustrate relationships among traits.

4. To focus future research, we provide a synthesis of AMF traits whose evolution is particularly relevant to nutrient cycling and environmental factors that induce variation in those traits.

5. *Synthesis*. Characterizing the depth and range of arbuscular mycorrhizal fungal trait variation is essential for predicting responses to natural and anthropogenic environmental changes, as well as understanding past and future fungal trait evolutionary trajectories in the Tree of Life.

Key-words: functional trait approach, global change, Glomeromycota, micro-organisms, multigenomic, mutualism, nutrient cycling, plant-soil (below-ground) interactions, trait variation

Introduction

In the current era of global change, it is increasingly important to understand the interactions and feedbacks between anthropogenic drivers of global change and ecosystem services. Nutrient cycles are of particular importance because they are directly affected by human activities, and human changes to nutrient cycles can be mitigated or exacerbated by natural systems. As nutrient cycles are being modified, organisms are evolving in response to changing conditions (Helgason & Fitter 2009; Pauls *et al.* 2013). Understanding the past and predicting the future changes in organismal traits is key to mediating nutrient cycle changes. Such trait variation can be studied at different resolutions on the Tree of Life, from clade and species levels, to finer resolutions, such as trait variation within species.

Functional trait statistical methods – where species traits are explicitly measured and incorporated into predictive models – have emerged as valuable tools in forecasting how an ecosystem process such as nutrient cycling might respond to environmental change (Suding *et al.* 2008; Webb *et al.* 2010; Mouillot *et al.* 2013). These have been particularly useful, for example, in predicting the effects of vegetation responses to climate change on carbon cycles (Van Bodegom *et al.* 2012). For functional trait methods to be implemented, it is necessary to know (i) species-specific trait values, (ii) the functional role of the trait with respect to the studied variable and (iii) the response of that trait to environmental perturbation (Suding *et al.* 2008). These methods are very applicable for well-studied organism groups such as plants, as there are many trait data bases available. However, for other less well-studied groups that are fundamental to nutrient cycles, such as soil micro-organisms, less is known about their traits, what traits are important, at what taxonomic resolution they should be studied or even how to measure them, making it challenging to incorporate these species into current functional trait model approaches.

Arbuscular mycorrhizal fungi (AMF) are soil micro-organisms that have evolved as major players in global

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nutrient cycling and are predicted to be important in terrestrial system responses to global change (Cheng *et al.* 2012; Bissett *et al.* 2013; Johnson *et al.* 2013; Verbruggen *et al.* 2013). AMF form nutrient exchange symbioses with 60–80% of terrestrial plant species and act as conduits for nutrient flux between above- and below-ground ecosystems. In these mutualisms, plants provide AMF with carbon-rich photosynthate in exchange for mineral nutrients that AMF take up from the soil. Nutrient exchange is regulated by both partners, and the level of nutrients exchanged is dependent on the traits of the plant species and AMF involved (Kiers *et al.* 2011; Hart *et al.* 2013; Treseder 2013).

While AMF traits are clearly important for nutrient flux, quantifying species-level trait values to incorporate into functional trait models has been hampered by several factors. First, simple species identification has been a challenge in the absence of molecular tools because AMF species do not differ substantially in morphological characters (Gorzelak *et al.* 2012). Molecular tools have been invaluable in species identification and revealing the extent of hidden AMF species diversity (Kivlin, Hawkes & Treseder 2011), but progress in obtaining new marker genes can be slow. Indeed, the entire species concept for glomeromycotan fungi has been wrought with difficulties (Stockinger, Kruger & Schussler 2010) due to the evolution of incredibly high functional diversity and intraspecific variation (Munkvold *et al.* 2004; see below). There are suggestions that the species concept should, like for some prokaryotes, be based on a number of characteristics including phylogenetic (rDNA), biochemical and physiological characteristics (van der Heijden, Scheublin & Brader 2004).

Secondly, although evolution has generated interspecific trait variation between AMF clades (Hart & Reader 2002), AMF have also evolved significant intraspecific variation in traits along the tips of the Tree of Life (Ehinger *et al.* 2012). AMF are unique among species because this intraspecific variation can be so high that it equals interspecific variation (Munkvold *et al.* 2004) rendering species identification based on trait values a challenge (van der Heijden & Scheublin 2007). Intraspecific evolution may be equally as important as evolution across deeper level nodes in generating the trait variation that affects ecosystem processes. To make things more complicated, this intraspecific variation can be further increased by environmental context (Hoeksema *et al.* 2010). For example, the concentration of nutrients in the soil can alter the allocation strategy of the fungus (e.g. amount of nutrients passed on from AMF to the plant; Bethlenfalvay 1983). This context-dependent intraspecific variation makes it difficult to assign a single trait value to a species. Furthermore, it makes it difficult to know which traits are important for nutrient flux when their functional roles are not constant.

Finally, the genetic basis of trait expression and intraspecific variation in AMF traits is not well understood because our understanding of AMF genetics is still developing (Sanders & Croll 2010). AMF spores and hyphae contain multiple and potentially genetically different nuclei within a common cytoplasm. Genetically different nuclei could provide standing genetic variation which permits fine-scale local adaptation of AMF to environmental variation (Angelard *et al.* 2013; Johnson *et al.* 2013), but more work is needed to understand the outcome of this within-individual variation.

Arbuscular mycorrhizal fungi traits are clearly important for nutrient cycling (Parrent *et al.* 2010; Opik & Moora 2012; Chagnon *et al.* 2013), but identifying which traits are most influential and determining the values of these traits is a herculean task. Experiments aimed at quantifying the intraspecific variation in traits relevant to nutrient cycling can elucidate the functional roles and values of important traits (Johnson *et al.* 2012). To structure this task, we present a framework of experimental designs (Fig. 1) inspired by the phenotypic plasticity literature. Phenotypic plasticity experiments aim to quantify trait variation and provide a useful framework that is applicable to assess trait variation in AMF. While the mechanisms generating intraspecific variation in AMF remain unknown, classic phenotypic plasticity experiments provide a useful approach to characterize this trait variation.

We start with a synthesis of the AMF traits relevant to nutrient cycling and review what is known about their intraspecific variation. These are traits that are likely candidates to include in functional trait models and thus should be the focus of future research. We then present our phenotypic plasticity framework outlining specific experimental designs that allow us to better quantify intraspecific trait variation. These experimental designs provide simplified conditions to investigate the biology of these complex organisms so we can understand their biological potential and future role in nutrient cycling.

AMF traits relevant to nutrient flux

Arbuscular mycorrhizal fungi individuals begin life as a spore in the soil. When conditions are right for germination, specialized hyphae grow out of the spore in search of a host root. Because AMF are obligate mutualists, they have a limited time to locate and colonize a host root before they run out of stored spore resources. Once a host root is located, there is a series of highly detailed molecular steps involved in colonizing the root (for a review see Bonfante & Genre 2010), which involves crosstalk with the plant's defence system. This allows the fungal hyphae to first penetrate the plant's outer cell layers and ultimately gain entrance into the inner cortex. Once inside the plant's inner cortex, the hyphae form arbuscules, which are highly branched structures that provide a surface for nutrient exchange from the AMF to the plant, and vesicles that are AMF structures used for storing lipids and other nutrients. The hyphal network running through the plant roots and connecting all of the exchange and storage structures is referred to as the intraradical mycelium (IRM). Simultaneously, two types of hyphae grow outside of the roots in the soil. Unbranched runner hyphae leave one root to infect other roots, while highly branched absorptive hyphae take up and transport nutrients from the soil (Friese & Allen 1991). The hyphae outside the roots are called extraradical mycelium (ERM) and are also responsible for spore formation.

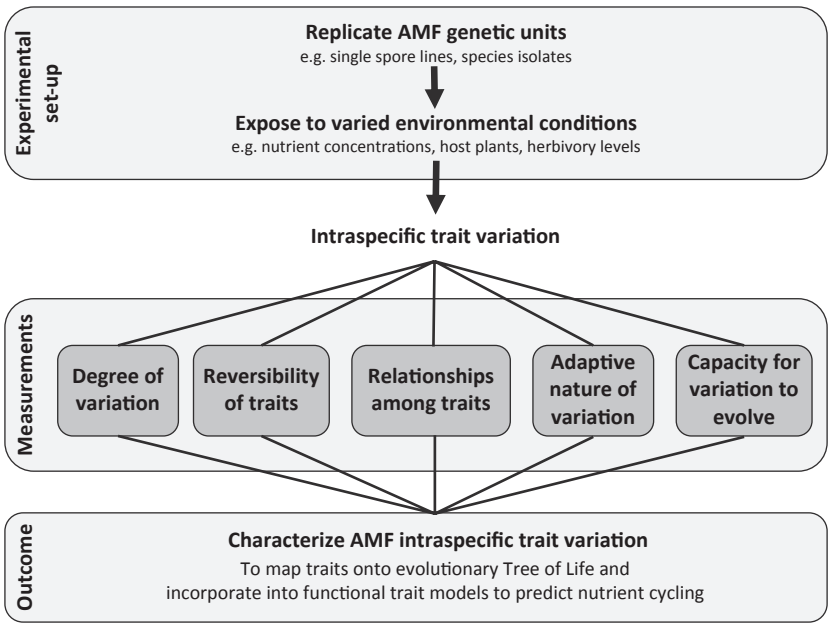

Fig. 1. Components of phenotypic plasticity framework for characterizing intraspecific trait variation in arbuscular mycorrhizal fungi. Note, the five measurements are neither sequential steps, nor mutually exclusive, and all five may be explored in the same experimental set-up.

Together, the ERM and IRM form a network specialized for the uptake and transport of nutrients.

The size or extent of many fungal traits (e.g. network size, network connectedness) can be viewed as proxies for the level of nutrient flux in the system. For example, large IRM, large ERM and numerous arbuscules are indicative of a high degree of nutrient flux (Jakobsen, Abbott & Robson 1992; Mader *et al.* 2000; Fitter 2006), while numerous vesicles and spores indicate nutrients that are held by the AMF and not passed on to the plant (Johnson 1993; Smith & Read 2008; Ijdo *et al.* 2010; Table 1). For other traits, the relationship between the traits and nutrient flux has not been determined. For example, it is probable that mycelial networks with a high number of connections (i.e. points where hyphae have intersected, fused and exchange resources) have a higher nutrient transport rate than a mycelial network with fewer connections, but this has not been tested. Traits relevant to nutrient flux and their established and/or hypothesized influences on nutrient flux are a key starting point for investigating intraspecific trait variation (Table 1).

Arbuscular mycorrhizal fungi hyphae are multinucleate, containing multiple nuclei within a common cytoplasm (Cooke, Gemma & Koske 1987). In addition, AMF are heterokaryotic, meaning neighbouring nuclei are not necessarily genetically identical. Heterokaryosis is potentially achieved by multiple mechanisms. Evidence suggests that AMF spores contain genetically different nuclei (Kuhn, Hijri & Sanders 2001). Therefore, individual AMF, defined as the collection of hyphae derived from a single spore (Kuhn, Hijri & Sanders 2001), may begin life already in a multigenomic state. It is also possible for the hyphae from two closely related individuals that encounter each other in the soil to fuse (termed 'anastomosis') and share cytoplasm (Giovannetti *et al.* 2004; Croll *et al.* 2009). This can result in either a unidirectional or bidirectional flow of nuclei and nutrients (Croll *et al.* 2009), creating another avenue for heterokaryosis. This within-individual genetic variation is cited as a potential factor driving high intraspecific trait variation in AMF (Ehinger *et al.* 2012). We address the genetic mechanisms potentially underlying intraspecific trait variation in the next section.

Phenotypic plasticity framework

FRAMEWORK OVERVIEW

Phenotypic plasticity is defined as the ability of one genotype to produce multiple phenotypes depending on environmental conditions (West-Eberhard 1989). While phenotypic plasticity is a mechanism that generates intraspecific trait variation, the presence of intraspecific trait variation in AMF is not conclusive evidence that phenotypic plasticity is the mechanism responsible for it. This means that for AMF, we need to be wary of using the classical definition of phenotypic plasticity because the multigenomic nature of AMF makes it difficult to know the genetic basis of intraspecific trait variation. Regardless of the mechanism generating intraspecific variation (i.e. whether it is true phenotypic plasticity or not), it is clear that AMF trait values can vary according to environmental conditions (Hoeksema *et al.* 2010). Classic phenotypic plasticity experiments are then very useful in revealing this variation (Fig. 1). We thus refer to our framework as a 'Phenotypic Plasticity Framework' because it uses experimental approaches from the phenotypic plasticity literature, even though phenotypic plasticity may not be the mechanism generating this variation.

To illustrate, imagine hyphae with identical collections of genes producing different phenotypes in different environments, this may be considered phenotypic plasticity in the classic sense (Fig. 2a). However, if different genomes are responsible for the different traits in the different

Table 1. Examples of arbuscular mycorrhizal fungi traits related to nutrient flux and how to measure them

Trait	Growth system*	How to measure	Correlation between trait value and nutrient flux†	Environmental factors that induce intraspecific trait variation
Intraradical colonization				
Total root colonization	Soil/ROC	Visually quantify stained roots using microscope[1]	↑[2,3,4]	Simulated herbivory[5], nutrient concentration [6,7], intraspecific competition[7]
Structure quantification	Soil/ROC	Visually quantify proportion of vesicles and arbuscules in roots[1]	↑[8,9]	Arbuscules: host plant strain[11], simulated herbivory[5], nutrient concentration[12]
			↓[10]	Vesicles: simulated herbivory[5], nutrient concentration[6], host plant[13]
Arbuscule size	Soil/ROC	Measure arbuscule dimensions in stained roots[14]	↑	None reported
Intraradical mycelial gene copy number	Soil/ROC	Use quantitative PCR to measure relative abundances of gene copy numbers[15,16]	↑	Interspecific competition[17]
Extraradical colonization				
Hyphal length	Soil/ROC	Stain and quantify hyphae that have been isolated from growth medium[6, 18, 19]	↑[20]	Nutrient concentration[6, 7, 21], intraspecific competition[7]
Extraradical mycelial biomass	Soil/ROC	Isolate hyphae from growth medium and weigh (possible in sand and ROC systems)[22]	↑[23,24,25]	Nutrient concentration and host plant[26]
Extraradical mycelial gene copy number	Soil/ROC	Use quantitative PCR to measure relative abundances of gene copy numbers[15, 16]	↑[23, 24]	Interspecific competition[17]
Glomalin production	Soil	Extract and quantify glomalin from soil[27]	↓[28]	None reported
Branched absorbing structure quantification	ROC	Visually quantify number of structures per unit area under microscope [29]	↑	Nutrient concentration[29]
Hyphal branching frequency	ROC	Calculate number of branches within a length of hyphae[30]	↑	None reported
Network connectivity	ROC	Determine fusion compatibility/incompatibility[31, 32]	↑	Host plant[32]
Nuclei density	ROC	Stain extraradical mycelium and quantify nuclei per unit area under epifluorescence microscope [32]	↑	None reported
Nutrient transfer				
Mineral nutrient uptake by arbuscular mycorrhizal fungi (AMF)	Soil/ROC	Quantify labelled nutrients in plant tissues that were directly accessible by AMF only and not plant roots using mesh bags[20] or split ROC plates	↑	Nutrient patch concentrations[20]
Carbon uptake	Soil/ROC	Measure labelled carbon in fungal biomass after labelling plants with $^{13}CO_2$ [33,34,35]; in ROC systems use scintillation counting to measure radioactivity of fungal biomass after labelling root with ^{14}C-sucrose [35]	↑	Nutrient concentration[36]
Form of P storage	Soil/ROC	Extract and compare phosphate pool distributions to determine what form (accessible or inaccessible) of phosphate fungi is storing[35]	↑[36]	Nutrient concentration[36]
Ability to control plant's direct P uptake pathway	Soil/ROC	Quantify labelled P in plant tissues that are only accessible by AMF and not plant roots[37,38,39]	↑↓	None reported
Nutrient transfer gene expression	Soil/ROC	Measure upregulation and downregulation of nutrient transporters[40]; site specificity of gene expression can be identified with laser microdissection[41,42]	↑↓	Nutrient concentration[40]
Reproduction				
Spore density	Soil/ROC	Spores can be collected from soil or growth media[43] or visually quantified in ROCs	↓[44, 45]	Simulated herbivory[5], nutrient concentration[21, 26, 29], host plant[26]

(continued)

Table 1. (continued)

Trait	Growth system*	How to measure	Correlation between trait value and nutrient flux†	Environmental factors that induce intraspecific trait variation
Spore size	Soil/ROC	Measure spore dimensions and calculate volume[46]	↓	None reported
Nuclei per spore	Soil/ROC	High *z*-resolution microscopy for three-dimensional reconstruction[46]	↑↓	None reported
Genetic variation between nuclei	Soil/ROC	Sequence individual nuclei within single spore[47]	↑↓	Host plant[47]

[1]McGonigle *et al.* (1990); [2]Mader *et al.* (2000); [3]Sanders *et al.*(1977); [4]Smith *et al.* (1994); [5]Klironomos, McCune & Moutoglis (2004); [6]Abbott, Robson & Deboer (1984); [7]Li *et al.*(2008); [8]Fitter (2006); [9]Johnson *et al.* (2010); [10]Johnson (1993); [11]De Deyn *et al.* (2009); [12]Braunberger, Miller & Peterson (1991); [13]Sisaphaithong *et al.* (2012); [14]Javot *et al.* (2007); [15]Gorzelak *et al.* (2012); [16]Thonar, Erb & Jansa (2012); [17]Engelmoer, Behm & Kiers (2013); [18]Hart & Reader (2002); [19]Miller, Jastrow & Reinhardt (1995); [20]Cavagnaro *et al.* (2005); [21]Koch, Croll & Sanders (2006); [22]Declerck, Fortin & Strullu (2005); [23]Jakobsen, Abbott & Robson (1992); [24]Jansa, Mozafar & Frossard (2003); [25]Hodge, Campbell & Fitter (2001); [26]Ehinger, Koch & Sanders (2009); [27]Wright & Upadhyaya (1996); [28]Wilson *et al.* (2009); [29]Bago *et al.* (2004); [30]Schnepf, Roose & Schweiger (2008); [31]Croll *et al.* (2009); [32]Giovannetti *et al.* (2004); [33]Manefield *et al.* (2002); [34]Vandenkoornhuyse *et al.* (2007); [35]Kiers *et al.* (2011); [36]Takanishi *et al.* (2009); [37]Smith, Smith & Jakobsen (2003); [38]Smith, Smith & Jakobsen (2004); [39]Smith & Smith (2012); [40]Fiorilli, Lanfranco & Bonfante (2013); [41]Balestrini *et al.* (2007); [42]Gomez & Harrison (2009); [43]Gerdemann & Nicolson (1963); [44]Ijdo *et al.* (2010); [45]Smith & Read (2008); [46]Marleau *et al.* (2011); [47]Angelard *et al.* (2013).

*Growth system refers to whole plants grown in soil, root organ cultures (ROC) or both.

†↑ indicates positive correlation; ↓indicates negative correlation; ↑↓ indicates both positive and negative correlations are possible. Arrows without references indicate hypothesized relationships.

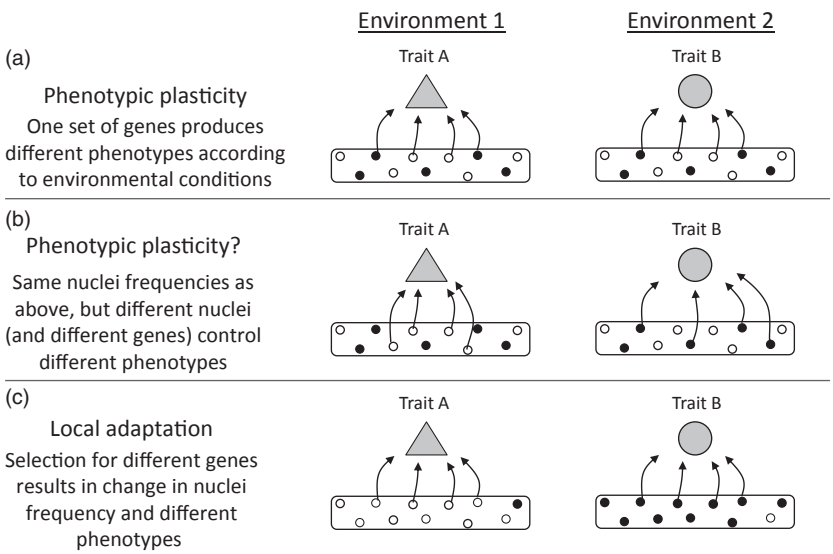

Fig. 2. Possible mechanisms for the genetic basis of intraspecific variation in traits due to the genetically non-identical nature of arbuscular mycorrhizal fungi nuclei. Arbuscular mycorrhizal fungi (AMF) are represented by the rounded-edge rectangles in each panel, and the genetic identity of nuclei within AMF is represented by either black or white circles. (a) For classic phenotypic plasticity, different traits are produced from the same genotype (both black and white nuclei). (b) Alternatively without changing the nuclei frequencies, different nuclei could be responsible for different traits, that is, one trait value is due to black nuclei and the other due to white nuclei. This may also be considered phenotypic plasticity. (c) A third option is local adaptation, where environmental conditions create selection pressure for different nuclei, which determine different trait values.

environments, it is questionable whether this can be considered true phenotypic plasticity (Fig. 2b). The confusion arises because we do not yet have a working definition for phenotypic plasticity under multigenomic conditions. Lastly, hyphae may have different collections of genes due to stochastic processes such as drift, or local adaptation may select for certain genes in different environments. Under these conditions, different traits would be expressed in different environments, but the mechanism would not be phenotypic plasticity (Fig. 2c).

Classic phenotypic plasticity experiments involve exposing replicate genotypes, which could be clones, siblings or even members of the same population, to varying environmental conditions and measuring trait values. This provides a structured way to measure intraspecific variation in functional traits. In the following two sections, we provide suggestions and considerations for selecting genetic units and set-ups for measuring traits in AMF within the experiments outlined in the framework (Fig. 1).

To adequately quantify AMF intraspecific trait variation, we advocate initially using a laboratory approach. The goal is to eventually adapt these methods to field experiments once sufficient molecular techniques have been developed. In a field situation, it is common for plant roots to be colonized by multiple AMF species and for one AMF to colonize multiple plants simultaneously (Opik *et al.* 2009). This adds additional layers of complexity to already complicated organisms. Laboratory settings permit fine control of the genetic content of the AMF under study, which is less possible to control in the field. In addition, because AMF species are morphologically indistinguishable, many traits cannot be identified to the species level when there is a mix of species present, which makes it impossible to quantify intraspecific trait variation. For example, if multiple AMF species are present, arbuscule density can be quantified in a root section (Table 1); however, it would be impossible to determine the species identity of the arbuscules in the root based on morphological characters alone.

Laboratory settings permit investigations into the biological potential of AMF. The trade-off is that ecological conditions are greatly simplified and possibly even artificial, which makes it imperative to follow these experiments with field experiments. At the conclusion of this article, we discuss possibilities for how to adapt these methods to field conditions.

GENETIC UNITS

To conduct the experiments in our phenotypic plasticity framework (Fig. 1), it is necessary to identify a replicable genetic unit of AMF. The lower the genetic variation in the genetic unit, the lower the potential for extraneous 'noise' in the experiment and the higher the potential for repeatable results. Ideal material for experiments would be AMF cultures derived from single spores. However, these can be quite time intensive to develop, and localized genetic adaptation in response to environmental conditions can still occur in these lines (Angelard *et al.* 2013). If single spore lines are not available, replicates within a species isolate are acceptable. It would be informative to investigate whether the range of trait variation within single spore lines is equivalent to that among single spore lines, or for instance among mixed spore lines.

MEASURING TRAITS

Once the genetic unit is identified, it is necessary to select a proper cultivation system for the AMF so that the traits of interest can be measured. Within a laboratory setting, two main options exist for cultivating AMF and their hosts: whole plants in soil/sand and transformed root organ cultures (ROCs) grown on Petri plates (Becard & Fortin 1988; Declerck, Fortin & Strullu 2005; Table 1). ROCs have many advantages over whole plants for measuring certain traits. For example, some traits, particularly those pertaining to ERM anastomosis, are only quantifiable using ROCs. In addition, many traits, such as spore density, can be measured repeatedly and non-destructively over time. ROCs also have many significant drawbacks. Namely, they are a highly contrived laboratory system, only a limited number of host plant species are available (~5 species) and grow in a narrow range of nutrient conditions. These factors create a risk of ecologically irrelevant AMF-host plant-nutrient combinations. It is necessary for the researcher to identify the advantages and disadvantages of each growth system for investigating a particular question. Beyond identifying which growth system is appropriate for the species and traits of interest, it is also important to determine how, when and where to measure the traits. Environmental conditions can vary in both space and time, so it may be informative to incorporate repeated sampling in a spatial and/or temporal dimension (e.g. Jakobsen, Abbott & Robson 1992).

Below, we outline five components of a plasticity framework for measuring intraspecific variation in AMF traits (Fig. 1). For each component, we describe the concept within the context of the classic phenotypic plasticity definition and how it can be adapted to measuring intraspecific trait variation in AMF. We provide experimental designs for measuring that aspect of intraspecific trait variation and suggestions for appropriate statistical analyses where applicable. In addition, we retain the term 'intraspecific' to describe trait variation within an AMF lineage with the acknowledgement that the AMF species concept is still under debate (van der Heijden, Scheublin & Brader 2004; Stockinger, Kruger & Schussler 2010). The five components to our framework are not sequential steps, nor are they mutually exclusive, and all five can possibly be investigated within a single experimental set-up.

1. Quantifying the degree of intraspecific trait variation

The degree of intraspecific trait variation can be quantified as the range of trait values expressed across a range of environmental conditions. Higher variation means larger differences in trait values in different environments. Trait variation can be quantified and visually represented by plotting trait values against different environments, such that species-specific differences in trait variation are evident (Fig. 3). The degree of trait variation can also vary across traits; just because one trait in an organism is variable does not mean others are variable.

Experimentally, trait variation in AMF is measured by exposing replicate individuals to different environments and measuring trait values in each environment. The different environments tested can be discrete, such as different host plants, or continuous such as a gradient of soil nutrient levels or changing CO_2 levels. Both abiotic and biotic variables have been shown to induce intraspecific trait variation. For

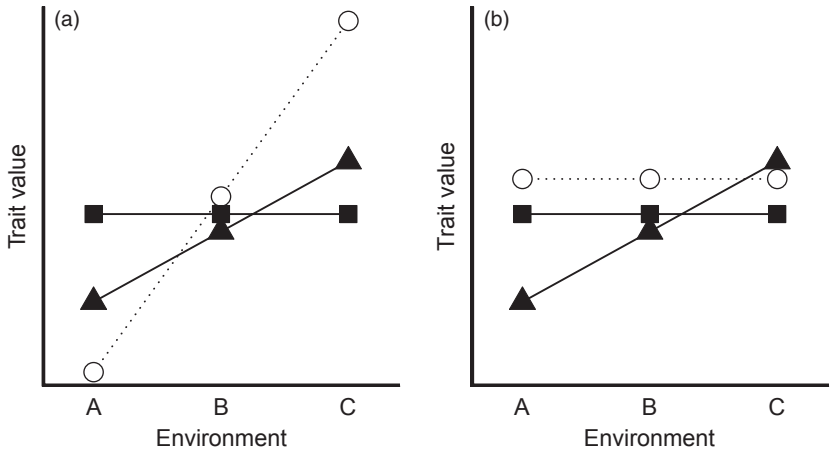

Fig. 3. Theoretical examples of trait variation and corresponding fitness with respect to environmental conditions. In both panels, the squares and triangles represent different species, while the open circles represent ideal trait values that confer the highest fitness in those environments. The degree of trait variation in each species is the same in the two panels: the triangle species has a higher degree of trait variation than the square species. (a) Trait variation in the triangle species is considered adaptive because the ideal trait values (open circles) are different in each environment and the triangle species' trait values follow this pattern. (b) Trait variation is not adaptive in this scenario because the ideal trait value does not change according to environmental conditions.

example, the percentage of vesicles in colonized roots in a whole-plant experiment was reduced by high concentrations of soil phosphorus (Abbott, Robson & Deboer 1984) and varied according to host plant with a lower percentage in sorghum, relative to wheat and barley (Sisaphaithong *et al.* 2012; Table 1). Similarly, in an ROC experimental study, spore density was not fixed and changed with respect to phosphorus concentration and host species (Ehinger, Koch & Sanders 2009).

While many studies have demonstrated environmentally induced intraspecific trait variation in AMF (Table 1), this is rarely the target of the experiment. Most experiments that document AMF trait variation are designed to determine which AMF species and/or environments best support plant growth. Shifting to an AMF-centric trait approach will provide even larger, more detailed data sets on AMF traits relevant to nutrient cycling (Alberton, Kuyper & Gorissen 2005; Chagnon *et al.* 2013).

2. Reversibility of traits

Once an organism commits to a developmental pathway, is it fixed or reversible? There is evidence to suggest that some traits are more reversible than others (Hoverman & Relyea 2007; Metlen, Aschehoug & Callaway 2009). Given the genetic nature of AMF, traits may be recalcitrant if trait values are the result of local adaptation, but more reversible if they are the result of phenotypic plasticity (Fig. 2). In addition, the type of trait can influence how reversible it is; morphological traits such as hyphal branching are likely to be less reversible than physiological traits such as nutrient uptake rate.

To test the reversibility of traits, organisms are exposed to an environmental condition to induce a response in a trait. The trait is measured, and then the environment is removed or switched to a different state, and the trait is monitored for changes. Some environments are much easier to reverse or remove than others in an experimental context. Experimentally switching between drought and non-drought conditions or from low to high nutrient conditions, for example, is relatively easy. Switching host plants is more challenging. The reversibility of traits is especially relevant to global change, because many factors such as temperature and precipitation are predicted to increase in variability, in contrast to constant gradual increases (Karl & Trenberth 2003).

3. Identifying relationships among traits

The responses of individual traits to environmental changes are rarely independent. Identifying correlations between pairs or groups of traits in their responses to similar conditions can elucidate a species' resource allocation strategies and constraints on responding to different environments. Traits may be positively or negatively correlated, and the direction of the correlation likely depends on the traits and environmental context involved. Negative correlations can indicate a trade-off in investing in one trait over the other, while positive correlations suggest that both traits respond in the same manner to the environmental conditions.

Experiments for identifying how trait correlations change with environmental conditions are identical to experiments for quantifying the degree of trait variation, except multiple traits are measured and compared. The exact comparison depends on the number of traits involved in the analysis and the question under investigation. To illustrate ways to visualize the relationships among traits, we simulated a data set with the same statistical characteristics of the experimental data from Klironomos, McCune & Moutoglis (2004). This experiment involved exposing a host plant, *Bromus inermis,* inoculated with one of three AMF isolates, *Glomus intraradices* (Schenk & Smith)*, Glomus etunicatum* (Becker & Gerdemann)*,* and *Glomus mosseae* (Nicolson & Gerdemann), to three levels of herbivory: none, low and high herbivory. At the conclusion

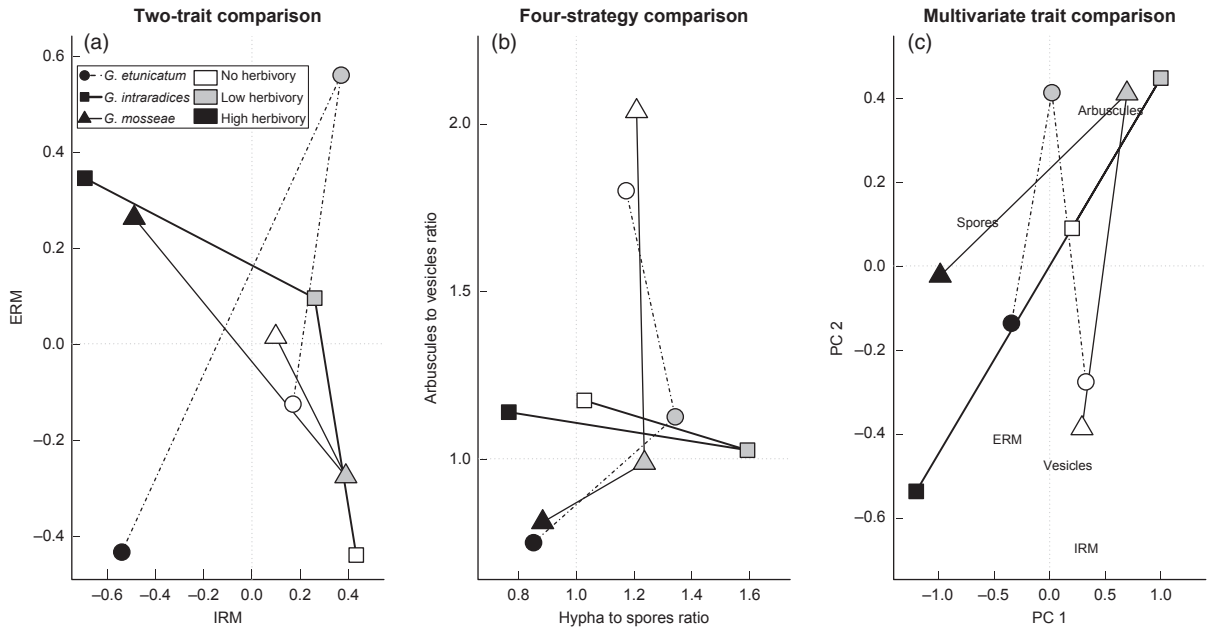

Fig. 4. Examples of methods to visualize relationships between traits and strategies. Data in figures for the three species under three herbivory conditions were simulated to have the same statistical characteristics of the data from Klironomos, McCune & Moutoglis (2004). (a) Relationship between standardized mean intraradical mycelium and extraradical mycelium size. Zero indicates the mean value across all observations within a species; values above zero are greater than the mean, and below zero are less than the mean. (b) Relative investment in four different strategies: arbuscule density (resource exchange) versus vesicle density (resource storage) relative to hyphal size (growth) versus spore density (reproduction). Values of 1 represent equal investment in the two strategies along that axis. (c) Phenotypic trajectories of the three species under the three herbivory treatments. Points represent mean principal components analysis (PCA) scores for the first two PCA axes. Position of words indicates loadings (correlations) of the five traits along the first two PCA axes.

of the experiment, they measured AMF traits, including IRM size, ERM size, arbuscule density, vesicle density and spore density.

When comparing traits that differ in scale, it can be helpful to standardize the data within each trait to have a mean of zero and standard deviation of one (i.e. subtract the mean from each observation and divide the difference by the standard deviation) so that the traits are evaluated on the same scale. We performed this standardization on the simulated data set prior to any further analyses. For simple correlations between two traits, such as IRM and ERM, plotting each trait on a separate axis is useful for visualizing how their relationship changes with different environmental conditions (Fig. 4a). This analysis suggests that under most conditions, there was a negative relationship between ERM and IRM (i.e. most points are in the upper left and lower right quadrants), indicating a possible trade-off. However, for *G. etunicatum* under both levels of herbivory, but not the control, there was a positive relationship between ERM and IRM.

For multiple traits, trait ratios can be calculated to identify strategies or trade-offs of interest. We compared the ratio between reproduction (spore density) and growth (IRM + ERM) to the ratio between resource storage (vesicle density) and exchange (arbuscule density; Fig. 4b). In this case, *G. etunicatum* and *G. mosseae* exhibit similar strategies: high growth and resource exchange under no herbivory shifting to reproduction and resource storage under high herbivory (Fig. 4b). In comparison, *G. intraradices* is relatively invariant along the storage exchange axis and only shifts investment between growth and reproduction in response to herbivory (Fig. 4b).

Lastly, multivariate ordination methods such as phenotypic trajectory analysis (Adams & Collyer 2009) provide a third option for examining trait relationships. A phenotypic trajectory is the collection of points within a species starting at the lowest treatment, in this case no herbivory, and ending at the highest treatment. First, a principle components analysis (PCA) is conducted on the matrix of standardized trait values. The mean PCA scores from the first two axes comprise the points in the phenotypic trajectory. Then, geometric statistical techniques can be used to analyse the sizes, shapes and orientations of the phenotypic trajectories in multivariate trait space (Adams & Collyer 2009). Phenotypic trajectory analysis on the simulated data shows that across species, the treatments cluster in multivariate trait space (Fig. 4c). ERM, vesicles and IRM cluster together and away from spores and arbuscules, suggesting a possible trade-off in these traits (Fig. 4c). In addition, the three species trajectories are similar in size ($P = 0.32$) and shape ($P = 0.87$), but differ in orientation ($P < 0.001$) with *G. intraradices* oriented differently than the other two (Fig. 4c). This indicates that the relationship between traits and potentially resource allocation strategies in *G. intraradices* differs from the other two species.

While many studies record multiple trait values (e.g. Klironomos, McCune & Moutoglis 2004; Pivato *et al.* 2007; Opik & Moora 2012) and have identified trait correlations

Table 2. Resource allocation strategies and trait ratios for further investigation

Strategy	Trait ratios
Growth versus reproduction	Fungal abundance relative to spore density[1]
Nutrient exchange versus nutrient storage	Arbuscule density and/or extraradical mycelium (ERM) size relative to vesicle and/or spore density[2,3,4]
Nutrient exchange versus nutrient foraging	Root fungal biomass relative to mycelial fungal biomass[5,6]
Nutrient foraging versus host colonization	Absorptive hyphae relative to runner hyphae[7]
Foraging strategy	Nutrients taken up from highly concentrated patches relative to total soil nutrients[8]
Cost benefit ratio	Nutrients provided to plant relative to carbon allocated to fungal partner[2]
Allometric ratio	Investment in structures related to nutrient transfer (e.g. ERM, arbuscules) relative to investment in all structures[9]

[1]Klironomos, McCune & Moutoglis (2004); [2]Kiers *et al.* (2011); [3]Verbruggen *et al.* (2012); [4]Braunberger, Miller & Peterson (1991); [5]Hart & Reader (2002); [6]Powell *et al.* (2009); [7]Friese & Allen (1991); [8]Cavagnaro *et al.* (2005); [9]Johnson *et al.* (2003).

under single environmental conditions (Gamper *et al.* 2008), analyses of how the relationships between traits change with changing environmental conditions are more rare (Braunberger, Miller & Peterson 1991). However, these can be extremely useful in elucidating particular resource strategies. The simulated data set illustrated just a sample of potentially useful strategies to investigate, but additional strategies might also be informative (Table 2).

4. Assessing adaptive nature of intraspecific trait variation

Intraspecific variation in a trait simply means that its value changes with environmental conditions. However, a change in a trait does not mean that the change is beneficial (i.e. increases fitness) to the organism. For example, having environmentally induced variation rather than fixed values in traits related to nutrient uptake may increase AMF fitness by allowing AMF to reach difficult to access nutrients in the soil. Alternatively, environmentally induced variation in traits related to reproduction may not be beneficial if it results in low or zero reproductive output. Understanding the conditions under which trait variation is beneficial (Fig. 3a) and not beneficial (Fig. 3b) is important. If changes in trait values result in increased fitness of the organism in that environment relative to the unchanged option, the trait variation is categorized as adaptive (Ghalambor *et al.* 2007).

While adaptive intraspecific trait variation is a straightforward concept to describe, it is difficult to measure experimentally. First, assessing fitness can be difficult in any species, especially AMF, and using traits as proxies is a common practice. For AMF, spore density, colonization level and mycelial biomass may be useful proxies for AMF fitness. Secondly, experiments for testing the adaptive nature of trait variation involve comparing fitness of individuals with an environmentally induced trait to individuals without that trait in the same environment. For certain traits and species, this is relatively easy and could take the form of, for example, exposing tadpoles to predator cues to induce an antipredator morphological response. Then, tadpoles with the predator-induced trait and tadpoles without the trait would be exposed to predators, and survival would be measured (Benard 2006). In a second well-known example, *Impatiens capensis* seedlings were grown to induce high- or low-light phenotypes. Then, seedlings were transplanted such that both high- and low-light phenotype individuals were planted in high and low light conditions, and survival over the course of the experiment was recorded (Dudley & Schmitt 1996).

In both the *Impatiens* and tadpole examples, individuals that had previous exposure to the testing environment performed better than those that had not (Dudley & Schmitt 1996; Benard 2006), indicating that the induced trait changes were adaptive. The success of these experiments was largely due to the fact that the traits were morphological and were not reversible over the timeframe of the experiment. For organisms such as plants and AMF, which are more difficult to move and 'test' in different environments, examples of adaptive trait variation from the literature are scarce. A useful avenue of research may be first measuring the reversibility (see above) of a morphological trait, then measuring the fitness of individuals with and without that trait during an experimental timeframe where the trait is not reversible.

5. Genotype × environment interaction: capacity for intraspecific trait variation to evolve

If there is variation in the degree of trait variation across genotypes within a population, this is termed a genotype × environment interaction and indicates that there is potential for trait variation to evolve in the presence of appropriate selection pressure. Thus, the degree of trait variation itself can be thought of as a trait that can evolve, potentially independent from evolution in the mean trait value (Ghalambor *et al.* 2007). This can be visualized by illustrating traits without (Fig. 5a) and with (Fig. 5b) a genotype × environment interaction. Experimental set-ups that test for genotype × environment interactions are conceptually straightforward: replicate individuals of each genotype (or clone or sibling) are tested in each environment. Then, using a standard ANOVA framework, significant genotype × environment interactions can be identified.

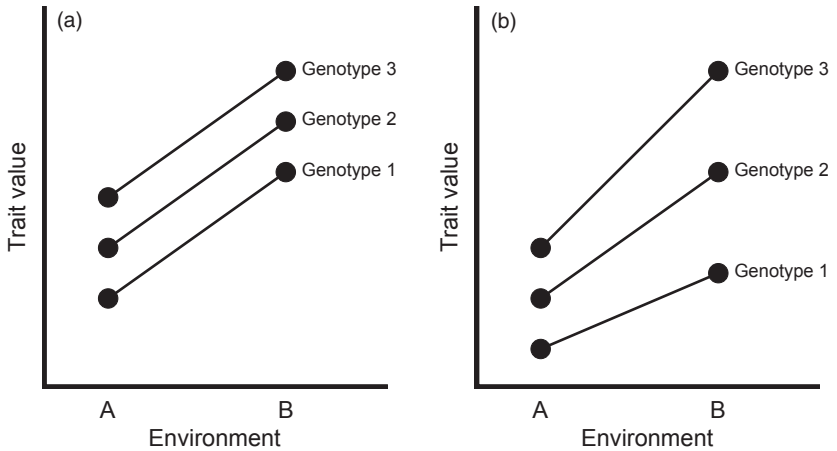

Fig. 5. Theoretical example of investigating a genotype × environment interaction. (a) No genotype × environment interaction. Although the three genotypes tested have different mean trait values in the two environments, their trait variation (slopes) is identical across environments, and thus, there is no genetic variation in trait variation. (b) A genotype × environment interaction. The three genotypes vary in their level of trait variation with genotype 3 having the highest, and thus, there is potential for mean trait variation in the population to evolve.

For AMF, the challenge in identifying genotype × environment interactions lies in selecting the genotypes. Ideally, single spore lines could be treated as genotypes and tested under different environmental conditions. Using this approach, Angelard *et al.* (2013) demonstrated a significant genotype × environment interaction for AMF growth of single spore lines propagated on different host plants. If single spore lines are not available, other ways to distinguish genotypes are necessary, such as different source populations or different generations of an experimental evolution study.

Future steps

We devised this framework to help structure much needed research on quantifying intraspecific trait variation in AMF (Fig. 1), with the ultimate goal of including AMF in functional trait statistical methods. In order to use functional trait methods to predict changes in nutrient cycles in a community in response to global change, three sets of data are needed: (i) trait values for the species in the community, (ii) relationship between trait value and nutrient cycling, (iii) response of trait to global change (Suding *et al.* 2008). Our framework provides avenues for obtaining all of these necessary data. In the most simplified example, researchers could use component 1 of the framework, quantifying the degree of intraspecific variation, to record how a trait such as arbuscule density is related to phosphorus flux under varying global change environmental scenarios, such as different rainfall regimes. This would provide basic information for AMF species from a particular community, allowing us to make crude predictions regarding how phosphorus cycling could change under altered precipitation. Incorporating more components of the framework would increase the confidence in the predictions of the trait values, their roles, and how they might fluctuate. For example, component 2 would provide an indication of how reversible arbuscule density is to changing precipitation regimes. Component 3 would show whether other traits are also important with respect to phosphorus cycling and precipitation. Components 4 and 5 would provide ways to predict whether trait values would be constant in the future or whether they might evolve and change with respect to changing precipitation conditions. At present, how trait values influence nutrient flux is still not known for many traits (Table 1), thus recording nutrient values in experiments would provide valuable information. As more information on different fungal species and isolates is collected, this could be mapped onto the Tree of Life to and incorporated into predictive models (Fig. 1).

While our framework provides the experimental designs necessary to quantify intraspecific trait variation, this is just the first step. Intraspecific trait variation in AMF is predicted to be high (Koch *et al.* 2004; Munkvold *et al.* 2004; Ehinger *et al.* 2012), which may make it difficult to incorporate AMF into the current functional trait models that assume a single trait value per species. Adapting trait-based models to include trait variation may be necessary and beneficial for other taxa as well (Ives, Midford & Garland 2007; Berg & Ellers 2010).

The simplicity and tractability of laboratory experiments is essential for the beginning stages of quantifying intraspecific trait variation. However, in nature AMF exist in much more complex environments with respect to both abiotic and biotic components. For example, a recent ROC experiment suggested that species interactions such as interspecific competition can significantly alter intraspecific trait values (Engelmoer, Behm & Kiers 2013). This underscores the importance of devising methods for conducting experiments in more natural, species-rich conditions. In the future, it will be necessary to increase the complexity of laboratory experiments and, where possible, conduct them under field conditions. With the recent development of molecular markers, it is now possible to conduct experiments involving mixed AMF species and distinguish and quantify them at the end (Gorzelak *et al.* 2012). These techniques are currently limited to quantifying only the abundance of each species, while quantifying traits to the species-level is not yet possible. The development of new laser microdissection techniques that permit very precise analysis of transcripts from a given location (Balestrini *et al.* 2007; Gomez & Harrison 2009) may overcome these limitations and allow species-specific trait quantification in mixed AMF species assemblages.

We have taken an AMF-centric view in outlining our framework, but AMF are not the only partners in this mutualism. The plant hosts, upon which AMF rely as their sole

source of carbohydrates, are incredibly plastic and respond to environmental changes. In fact, global change scenarios predict increases in both CO_2 levels and soil nutrients, and these changes can significantly alter the stability of plant-AMF mutualisms (Johnson 2010; Johnson *et al.* 2013). How AMF respond to the changing nutrient relationship with their hosts may be a function of the degree of classic phenotypic plasticity in traits related to nutrient exchange (Kiers *et al.* 2010). If, for example, plants reduce the amount of carbon allocated to AMF, then AMF with high plasticity in carbon requirements will likely be more successful than less plastic species. Quantifying intraspecific trait variation in AMF is not only necessary for understanding how AMF will affect nutrient cycling, but also for understanding how changes in nutrient cycling will affect AMF.

At present, we do not know whether intraspecific trait variation in AMF is generated by phenotypic plasticity (Fig. 2). Phenotypic plasticity is often considered a rapid mechanism for responding to changing environmental conditions, which provides organisms with the traits they need in a short amount of time. In contrast, adaptation can take multiple generations before optimal trait values spread through the population, and environmental conditions may change within that timeframe, rendering the new trait less optimal. In AMF, local adaptation may be more rapid if it is based on the change in nuclei frequencies within an individual, but slower if it must involve population-level changes in gene frequencies. Thus, determining whether the genetic basis of intraspecific trait variation in AMF is phenotypic plasticity or local adaptation is a key goal, as the ecological consequences of the two mechanisms differ greatly (Beldade, Mateus & Keller 2011). Future work should concentrate on elucidating the mechanisms driving intraspecific trait variation in AMF and determine whether classic phenotypic plasticity plays a significant role. As our understanding of AMF genetics increases in the coming years, we will be better positioned to incorporate AMF into functional trait models of global nutrient cycling.

In a broader context, understanding current intraspecific trait variation in AMF will provide insight into the evolutionary history of fungal traits. Mapping trait values onto a Tree of Life phylogeny can illustrate how traits evolved over time, and how labile they are. The degree of intraspecific variation can be thought of as a species-level trait in its own right, subject to evolution. Understanding how intraspecific trait variation fluctuates along the Tree of Life can help predict the range of future fungal trait evolutionary trajectories under changing conditions. Overall, our work highlights that evolution along the tips of the Tree of Life may be equally as important as evolution across deeper level nodes in generating the trait variation that affects ecosystem processes.

Although AMF are critical for ecosystem processes such as nutrient cycling, sufficient knowledge of their traits is lacking. This knowledge is a key to understand their evolutionary history and future trajectories, and especially important to predict their responses to global change or even natural environmental fluctuations. Our framework provides a structure of experimental designs to characterize AMF traits with the goal of increasing our understanding of AMF traits to a level where they can be included in predictive models.

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References

Abbott, L.K., Robson, A.D. & Deboer, G. (1984) The effect of phosphorus on the formation of hyphae in the soil by the vesicular arbuscular mycorrhizal fungus *Glomus fasciculatum*. *New Phytologist*, **97**, 437–446.

Adams, D.C. & Collyer, M.L. (2009) A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution*, **63**, 1143–1154.

Alberton, O., Kuyper, T.W. & Gorissen, A. (2005) Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO_2. *New Phytologist*, **167**, 859–868.

Angelard, C., Tanner, C.J., Fontanillas, P., Niculita-Hirzel, H. & Sanders, I.R. (2013) Rapid genotypic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation. *The ISME Journal*, doi: 10.1038/ismej.2013.154.

Bago, B., Cano, C., Azcon-Aguilar, C., Samson, J., Coughlan, A.P. & Piche, Y. (2004) Differential morphogenesis of the extraradical mycelium of an arbuscular mycorrhizal fungus grown monoxenically on spatially heterogeneous culture media. *Mycologia*, **96**, 452–462.

Balestrini, R., Gomez-Ariza, J., Lanfranco, L. & Bonfante, P. (2007) Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. *Molecular Plant-Microbe Interactions*, **20**, 1055–1062.

Becard, G. & Fortin, J.A. (1988) Early events of vesicular arbuscular mycorrhiza formation on RI-DNA transformed roots. *New Phytologist*, **108**, 211–218.

Beldade, P., Mateus, A.R.A. & Keller, R.A. (2011) Evolution and molecular mechanisms of adaptive developmental plasticity. *Molecular Ecology*, **20**, 1347–1363.

Benard, M.F. (2006) Survival trade-offs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). *Ecology*, **87**, 340–346.

Berg, M.P. & Ellers, J. (2010) Trait plasticity in species interactions: a driving force of community dynamics. *Evolutionary Ecology*, **24**, 617–629.

Bethlenfalvay, G.J. (1983) Parasitic and mutualistic associations between a mycorrhizal fungus and soybean- the effect on phosphorus on host plant-endophyte interactions. *Physiologia Plantarum*, **57**, 543–548.

Bissett, A., Brown, M.V., Siciliano, S.D. & Thrall, P.H. (2013) Microbial community responses to anthropogenically induced environmental change: towards a systems approach. *Ecology Letters*, **16**, 128–139.

Bonfante, P. & Genre, A. (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nature Communications*, **1**, 48.

Braunberger, P.G., Miller, M.H. & Peterson, R.L. (1991) Effect of phosphorus-nutrition on morphological-characteristics of vesticular-arbuscular mycorrhizal colonization of maize. *New Phytologist*, **119**, 107–113.

Cavagnaro, T.R., Smith, F.A., Smith, S.E. & Jakobsen, I. (2005) Functional diversity in arbuscular mycorrhizas: exploitation of soil patches with different phosphate enrichment differs among fungal species. *Plant Cell and Environment*, **28**, 642–650.

Chagnon, P.L., Bradley, R.L., Maherali, H. & Klironomos, J. (2013) A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science*, **18**, 484–491.

Cheng, L., Booker, F.L., Tu, C., Burkey, K.O., Zhou, L., Shew, H.D., Rufty, T.W. & Hu, S. (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. *Science*, **337**, 1084–1087.

Cooke, J.C., Gemma, J.N. & Koske, R.E. (1987) Observations of nuclei in vesicular-arbuscular mycorrhizal fungi. *Mycologia*, **79**, 331–333.

Croll, D., Giovannetti, M., Koch, A.M., Sbrana, C., Ehinger, M., Lammers, P.J. & Sanders, I.R. (2009) Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist*, **181**, 924–937.

De Deyn, G.B., Biere, A., van der Putten, W.H., Wagenaar, R. & Klironomos, J.N. (2009) Chemical defense, mycorrhizal colonization and growth responses in Plantago lanceolata L. *Oecologia*, **160**, 433–442.

Declerck, S., Fortin, J.A. & Strullu, D.G. (2005) *In Vitro Culture of Mycorrhizas*. Springer-Verlag, Berlin.

Dudley, S.A. & Schmitt, J. (1996) Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *The American Naturalist*, **147**, 445–465.

Ehinger, M., Koch, A.M. & Sanders, I.R. (2009) Changes in arbuscular mycorrhizal fungal phenotypes and genotypes in response to plant species identity and phosphorus concentration. *New Phytologist*, **184**, 412–423.

Ehinger, M.O., Croll, D., Koch, A.M. & Sanders, I.R. (2012) Significant genetic and phenotypic changes arising from clonal growth of a single spore of an arbuscular mycorrhizal fungus over multiple generations. *New Phytologist*, **196**, 853–861.

Engelmoer, D.J.P., Behm, J.E. & Kiers, E.T. (2013) Intense competition between arbuscular mycorrhizal mutualists in an *in vitro* root microbiome negatively affects total fungal abundance. *Molecular Ecology*, doi: 10.1111/mec.12451.

Fiorilli, V., Lanfranco, L. & Bonfante, P. (2013) The expression of GintPT, the phosphate transporter of *Rhizophagus irregularis*, depends on the symbiotic status and phosphate availability. *Planta*, **237**, 1267–1277.

Fitter, A.H. (2006) What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist*, **172**, 3–6.

Friese, C.F. & Allen, M.F. (1991) The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. *Mycologia*, **83**, 409–418.

Gamper, H.A., Young, J.P.W., Jones, D.L. & Hodge, A. (2008) Real-time PCR and microscopy: are the two methods measuring the same unit of arbuscular mycorrhizal fungal abundance? *Fungal Genetics and Biology*, **45**, 581–596.

Gerdemann, J.W. & Nicolson, T.H. (1963) Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, **46**, 235–244.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.

Giovannetti, M., Sbrana, C., Avio, L. & Strani, P. (2004) Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. *New Phytologist*, **164**, 175–181.

Gomez, S.K. & Harrison, M.J. (2009) Laser microdissection and its application to analyze gene expression in arbuscular mycorrhizal symbiosis. *Pest Management Science*, **65**, 504–511.

Gorzelak, M.A., Holland, T.C., Xing, X.K. & Hart, M.M. (2012) Molecular approaches for AM fungal community ecology: a primer. *Journal of Microbiological Methods*, **90**, 108–114.

Hart, M.M. & Reader, R.J. (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist*, **153**, 335–344.

Hart, M.M., Forsythe, J., Oshowski, B., Bucking, H., Jansa, J. & Kiers, E.T. (2013) Hiding in a crowd-does diversity facilitate persistence of a low-quality fungal partner in the mycorrhizal symbiosis? *Symbiosis*, **59**, 47–56.

van der Heijden, M.G.A. & Scheublin, T.R. (2007) Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytologist*, **174**, 244–250.

van der Heijden, M.G.A., Scheublin, T.R. & Brader, A. (2004) Taxonomic and functional diversity in arbuscular mycorrhizal fungi – is there any relationship? *New Phytologist*, **164**, 201–204.

Helgason, T. & Fitter, A.H. (2009) Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). *Journal of Experimental Botany*, **60**, 2465–2480.

Hodge, A., Campbell, C.D. & Fitter, A.H. (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, **413**, 297–299.

Hoeksema, J.D., Chaudhary, V.B., Gehring, C.A., Johnson, N.C., Karst, J., Koide, R.T. *et al.* (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters*, **13**, 394–407.

Hoverman, J.T. & Relyea, R.A. (2007) How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology*, **88**, 693–705.

Ijdo, M., Schtickzelle, N., Cranenbrouck, S. & Declerck, S. (2010) Do arbuscular mycorrhizal fungi with contrasting life-history strategies differ in their responses to repeated defoliation? *Fems Microbiology Ecology*, **72**, 114–122.

Ives, A.R., Midford, P.E. & Garland, T. (2007) Within-species variation and measurement error in phylogenetic comparative methods. *Systematic Biology*, **56**, 252–270.

Jakobsen, I., Abbott, L.K. & Robson, A.D. (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist*, **120**, 371–380.

Jansa, J., Mozafar, A. & Frossard, E. (2003) Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. *Agronomie*, **23**, 481–488.

Javot, H., Penmetsa, R.V., Terzaghi, N., Cook, D.R. & Harrison, M.J. (2007) A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 1720–1725.

Johnson, N.C. (1993) Can fertilization of soil select less mutualistic mycorrhizae. *Ecological Applications*, **3**, 749–757.

Johnson, N.C. (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist*, **185**, 631–647.

Johnson, N.C., Rowland, D.L., Corkidi, L., Egerton-Warbuton, L. & Allen, E.B. (2003) Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology*, **84**, 1895–1908.

Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A. & Miller, R.M. (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 2093–2098.

Johnson, D., Martin, F., Cairney, J.W.G. & Anderson, I.C. (2012) The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New Phytologist*, **194**, 614–628.

Johnson, N.C., Angelard, C., Sanders, I.R. & Kiers, E.T. (2013) Predicting community and ecosystem outcomes of mycorrhizal responses to global change. *Ecology Letters*, **16**, 140–153.

Karl, T.R. & Trenberth, K.E. (2003) Modern global climate change. *Science*, **302**, 1719–1723.

Kiers, E.T., Palmer, T.M., Ives, A.R., Bruno, J.F. & Bronstein, J.L. (2010) Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters*, **13**, 1459–1474.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E. *et al.* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, **333**, 880–882.

Kivlin, S.N., Hawkes, C.V. & Treseder, K.K. (2011) Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry*, **43**, 2294–2303.

Klironomos, J.N., McCune, J. & Moutoglis, P. (2004) Species of arbuscular mycorrhizal fungi affect mycorrhizal responses to simulated herbivory. *Applied Soil Ecology*, **26**, 133–141.

Koch, A.M., Croll, D. & Sanders, I.R. (2006) Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. *Ecology Letters*, **9**, 103–110.

Koch, A.M., Kuhn, G., Fontanillas, P., Fumagalli, L., Goudet, J. & Sanders, I.R. (2004) High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 2369–2374.

Kuhn, G., Hijri, M. & Sanders, I.R. (2001) Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature*, **414**, 745–748.

Li, H.Y., Smith, F.A., Dickson, S., Holloway, R.E. & Smith, S.E. (2008) Plant growth depressions in arbuscular mycorrhizal symbioses: not just caused by carbon drain? *New Phytologist*, **178**, 852–862.

Mader, P., Vierheilig, H., Streitwolf-Engel, R., Boller, T., Frey, B., Christie, P. & Wiemken, A. (2000) Transport of N-15 from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytologist*, **146**, 155–161.

Manefield, M., Whiteley, A.S., Griffiths, R.I. & Bailey, M.J. (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Applied and Environmental Microbiology*, **68**, 5367–5373.

Marleau, J., Dalpe, Y., St-Arnaud, M. & Hijri, M. (2011) Spore development and nuclear inheritance in arbuscular mycorrhizal fungi. *BMC Evolutionary Biology*, **11**, 51.

McGonigle, T., Miller, M., Evans, D., Fairchild, G. & Swan, J. (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495–501.

Metlen, K.L., Aschehoug, E.T. & Callaway, R.M. (2009) Plant behavioural ecology: dynamic plasticity in secondary metabolites. *Plant Cell and Environment*, **32**, 641–653.

Miller, R.M., Jastrow, J.D. & Reinhardt, D.R. (1995) External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia*, **103**, 17–23.

Mouillot, D., Graham, N.A.J., Villeger, S., Mason, N.W.H. & Bellwood, D.R. (2013) A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, **28**, 167–177.

Munkvold, L., Kjoller, R., Vestberg, M., Rosendahl, S. & Jakobsen, I. (2004) High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist*, **164**, 357–364.

Opik, M. & Moora, M. (2012) Missing nodes and links in mycorrhizal networks. *New Phytologist*, **194**, 304–306.

Opik, M., Metsis, M., Daniell, T.J., Zobel, M. & Moora, M. (2009) Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist*, **184**, 424–437.

Parrent, J.L., Peay, K., Arnold, A.E., Comas, L.H., Avis, P. & Tuininga, A. (2010) Moving from pattern to process in fungal symbioses: linking functional traits, community ecology and phylogenetics. *New Phytologist*, **185**, 882–886.

Pauls, S.U., Nowak, C., Balint, M. & Pfenninger, M. (2013) The impact of global climate change on genetic diversity within populations and species. *Molecular Ecology*, **22**, 925–946.

Pivato, B., Mazurier, S., Lemanceau, P., Siblot, S., Berta, G., Mougel, C. & van Tuinen, D. (2007) Medicago species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist*, **176**, 197–210.

Powell, J.R., Parrent, J.L., Hart, M.M., Klironomos, J.N., Rillig, M.C. & Maherali, H. (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 4237–4245.

Sanders, I.R. & Croll, D. (2010) Arbuscular Mycorrhiza: the challenge to understand the genetics of the fungal partner. *Annual Review of Genetics*, Vol 44 (eds A. Campbell, M. Lichten & G. Schupbach), pp. 271–292. Annual Reviews, Palo Alto, CA, USA.

Sanders, F.E., Tinker, P.B., Black, R.L.B. & Palmerley, S.M. (1977) Development of endomycorrhizal root systems 1. Spread of infection and growth-promoting effects with 4 species of vesicular-arbuscular endophyte. *New Phytologist*, **78**, 257–268.

Schnepf, A., Roose, T. & Schweiger, P. (2008) Growth model for arbuscular mycorrhizal fungi. *Journal of the Royal Society Interface*, **5**, 773–784.

Sisaphaithong, T., Kondo, D., Matsunaga, H., Kobae, Y. & Hata, S. (2012) Expression of plant genes for arbuscular mycorrhiza-inducible phosphate transporters and fungal vesicle formation in sorghum, barley, and wheat roots. *Bioscience Biotechnology and Biochemistry*, **76**, 2364–2367.

Smith, S.E. & Read, D.J. (2008) *Mycorrhizal Symbiosis*. Academic Press, New York, NY, USA.

Smith, S.E. & Smith, F.A. (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia*, **104**, 1–13.

Smith, S.E., Smith, F.A. & Jakobsen, I. (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology*, **133**, 16–20.

Smith, S.E., Smith, F.A. & Jakobsen, I. (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist*, **162**, 511–524.

Smith, S.E., Dickson, S., Morris, C. & Smith, F.A. (1994) Transfer of phosphate from fungus to plant in VA mycorrhizas- calculation of the area of symbiotic interface and fluxes of P from 2 different fungi to *Allum porrum* L. *New Phytologist*, **127**, 93–99.

Stockinger, H., Kruger, M. & Schussler, A. (2010) DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist*, **187**, 461–474.

Suding, K.N., Lavorel, S., Chapin, F.S., Cornelissen, J.H.C., Diaz, S., Garnier, E., Goldberg, D., Hooper, D.U., Jackson, S.T. & Navas, M.L. (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology*, **14**, 1125–1140.

Takanishi, I., Ohtomo, R., Hayatsu, M. & Saito, M. (2009) Short-chain polyphosphate in arbuscular mycorrhizal roots colonized by Glomus spp.: a possible phosphate pool for host plants. *Soil Biology & Biochemistry*, **41**, 1571–1573.

Thonar, C., Erb, A. & Jansa, J. (2012) Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities marker design, verification, calibration and field validation. *Molecular Ecology Resources*, **12**, 219–232.

Treseder, K.K. (2013) The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil*, **371**, 1–13.

Van Bodegom, P.M., Douma, J.C., Witte, J.P.M., Ordonez, J.C., Bartholomeus, R.P. & Aerts, R. (2012) Going beyond limitations of plant functional types when predicting global ecosystem-atmosphere fluxes: exploring the merits of traits-based approaches. *Global Ecology and Biogeography*, **21**, 625–636.

Vandenkoornhuyse, P., Mahe, S., Ineson, P., Staddon, P., Ostle, N., Cliquet, J.B., Francez, A.J., Fitter, A.H. & Young, J.P.W. (2007) Active root-inhabiting microbes identified by rapid incorporation of plant-derived carbon into RNA. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 16970–16975.

Verbruggen, E., El Mouden, C., Jansa, J., Akkermans, G., Buecking, H., West, S.A. & Kiers, E.T. (2012) Spatial structure and interspecific cooperation: theory and an empirical test using the mycorrhizal mutualism. *The American Naturalist*, **179**, E133–E146.

Verbruggen, E., Veresoglou, S.D., Anderson, I.C., Caruso, T., Hammer, E.C., Kohler, J. & Rillig, M.C. (2013) Arbuscular mycorrhizal fungi – short-term liability but long-term benefits for soil carbon storage? *New Phytologist*, **197**, 366–368.

Webb, C.T., Hoeting, J.A., Ames, G.M., Pyne, M.I. & Poff, N.L. (2010) A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecology Letters*, **13**, 267–283.

West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, **20**, 249–278.

Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A. & Hartnett, D.C. (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecology Letters*, **12**, 452–461.

Wright, S.F. & Upadhyaya, A. (1996) Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science*, **161**, 575–586.

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