Letters

Forces that structure plant communities: quantifying the importance of the mycorrhizal symbiosis

Understanding forces that structure plant communities is a key issue in plant community ecology. Traditionally, ecologists have focused on the absolute and the relative roles of competition and herbivory (Sih *et al.*, 1985) and on the relative role of dispersal limitation vs biotic interactions (Huston, 1999), as well as on neutral models (Hubbell, 2001) and on their niche-based counterparts (Cadotte *et al.*, 2008). Increasingly, mycorrhizal ecologists have argued that mycorrhizal fungi should also be considered as fundamentally important determinants of plant community composition. This conviction is motivated by a growing body of research which demonstrates that mycorrhizal fungi can have large effects on plant growth and may modify the strength of other interactions (van der Heijden, 2002), which is taken as proof that mycorrhizas should have a strong effect on plant community structure. We agree that the *potential influence* of mycorrhizal fungi on structuring plant communities has been conclusively shown. However, the realization of such potential under variable environmental conditions and complex biotic interactions, and the *relative influence* of the mycorrhizal symbiosis compared with other biotic and abiotic drivers, have only been addressed in a few studies (see Table 1) and remain uncertain for most systems. Interestingly, we find no studies where the relative influence of ectomycorrhizal or ericoid mycorrhizal fungi on plant community structure has been quantified. This may reflect the difficulty of measuring plant community outcomes (e.g. plant community diversity or evenness) in these symbioses, which tend to be formed by long-lived plants. Nonetheless, the widespread failure of ectomycorrhizal plants to establish in the absence of ectomycorrhizas suggests that the effect size of ectomycorrhizal fungi on plant community structure is likely to be large. Quantification, in this case, may only be possible through the creative utilization of individual-based models of forest community dynamics (e.g. SORTIE; Pacala *et al.*, 1996). Similar debates about the relative importance of predation and competition in structuring plant communities have been addressed through syntheses and meta-analyses of published experimental studies (Sih *et al.*, 1985; Gurevitch *et al.*, 1992). These approaches have shown that for terrestrial plants, herbivores have more frequent and stronger effects on community structure than interspecific competition. Currently there is an insufficient number of published studies on the relative contribution of the mycorrhizal symbiosis (compared with other factors) to attempt a meta-analytical approach.

Three general approaches can be used to increase our understanding of the role of mycorrhizal symbioses on plant community structure:

Approach 1: The *proportion of the variance in plant community composition that is attributed to the presence and composition of mycorrhizal fungi* is a useful measure that would allow us to evaluate the mycorrhizal contribution to plant community composition. This requires the manipulation of mycorrhizal fungal communities in the field, either with fungal-addition treatments in landscapes where the fungi have been severely disturbed (Mendes *et al.*, 2010), or with fungal-removal treatments using fungicides (Helgason *et al.*, 2007), rotated cores with nylon mesh windows (Johnson *et al.*, 2001) or the repeated growth of non-mycorrhizal plants (Stinson *et al.*, 2006). It is noted that, similarly to other field manipulations, mycorrhizal field experiments also have their limitations. However, the concern of not having the perfectly controlled experiment should not deter us from using this approach. Similar constraints exist for manipulations of other biotic groups (e.g. competitors and herbivores), yet we have a much better understanding of the influence of these other groups *only because* of the long history of experimentally manipulating them in the field. Manipulations of mycorrhizal fungi should also be conducted across environmental gradients because many abiotic factors (e.g. temperature, moisture and fertility) are known to interact strongly with mycorrhizal diversity and functioning. In addition, other creative methods may be possible, which do not necessarily add fungi to a mycorrhizal-free soil substrate or attempt to completely eliminate fungi. Rather it may be useful to manipulate their composition and diversity in the field. For example, Opik *et al.* (2010) showed that there are probably two general categories of arbuscular mycorrhizal (AM) fungi: globally distributed generalist culturable taxa; and narrowly distributed specialized unculturable taxa. Therefore, it would be possible to increase the abundance and diversity of AM fungi and to manipulate the presence of generalist/specialist AM fungi in the ecosystem by culturing generalists and directly extracting

Table 1 A summary of selected multifactor manipulation studies of plant communities, where one factor is the mycorrhizal symbiosis

References	System	Mycorrhizal manipulation	Other factors manipulated	Plant community metrics	Proportion of total variance attributed to mycorrhizal symbiosis	Proportion of total variance attributed to other factor(s)
Collins & Foster (2009)	Temperate prairie grassland; mesocosm	Presence/absence; soil inoculum	Five levels of phosphorus availability	Diversity (H)	0.28 main effect + 0.13 in interaction	0.24 + 0.13 in interaction
Gange & Brown (2002)	Temperate old field grassland	Fungicide	Insecticide	Richness		Insecticide explains 1.3× variation of fungicide
Grime *et al.* (1987)	Temperate grassland; microcosm	Presence/absence	Soil heterogeneity, grazing	Diversity (H) richness	0.26 0.44	0.11 0.18
Rowe *et al.* (2009)	Temperate steppe	Soil inoculum	Nitrogen reduction (sucrose addition)	Richness	No significant effect	No significant effect
Smith *et al.* (1999)	Temperate prairie grassland	Fungicide	Removal of two dominant grasses	Richness		Plant competition explained up to 1.7× more variation than mycorrhizal symbiosis
Stampe & Daehler (2003)	Subtropical old-field grassland; microcosm	0, three single Glomeromycota isolates, and one combination of all three	Disturbance (clipping)	Diversity (H)	0.23	0.12
Stein *et al.* (2009)	Temperate grassland	Presence/absence of two inocula or field soil inoculum	Presence of hemiparasitic plant	Evenness	0.39	0.004
van der Heijden *et al.* (2008)	Temperate sand dune grassland; microcosm	Mix of four Glomeromycota isolates	Nitrogen addition	Functional group evenness	0.57 + 0.19 in interaction	0.21 + 0.19 in interaction
Vogelsang *et al.* (2006)	Temperate prairie grassland	0, 1 and 6 Glomeromycota isolates	Soil phosphorus 0, 1 and 5 types of P	Diversity (H)	For Myc diversity: 0.18 Myc composition: 0.26 + 0.20 interaction	0.02 0.04 + 0.20 interaction
Zobel *et al.* (1999)	Subarctic forest understorey	Fungicide	Acid rain, heavy metal	Richness	Fungicide decreased richness. No impact of acid rain and heavy metal	

When not available from the paper, we captured mean and SEM from figures using ImageJ 1.42q (NIH, USA). We then calculated sums of squares using GraphPad Prism 5.02 (GraphPad Software Inc., La Jolla, CA, USA). Proportion variance attributed to mycorrhizal symbiosis vs variance attributed to other factor(s) for a given response variable was calculated as the proportion of factor sums of squares to total sums of squares. When there was a significant interaction term this respective SS was added to both factors, as indicated. When only *F*-values were reported, we used the ratios of these to indicate relative effects.
Myc, mycorrhizal fungal.

specialists from the field. For example, in glasshouse experimental systems, inocula from arable and diverse natural ecosystems can be used, which may yield different fungal diversities and different proportions of generalist/specialist AM fungi. Similarly, in the field it would be possible to add either generalist culturable fungi (and thus decrease the share of specialists in diverse natural communities) or inocula from diverse natural ecosystems.

Approach 2: To further determine the contribution of mycorrhizal symbioses relative to other interacting biotic and abiotic factors, *experimental complexity will have to increase through the use of multifactorial designs.* This will require manipulating the presence and composition of mycorrhizal symbionts along with other factors. We recommend that studies should first focus on comparing the effects of mycorrhizas with other biotic factors that have already been shown to have a major influence on plant communities (e.g. herbivory, competition and pathogens). As a follow-up, we then suggest extending such studies across environmental gradients (e.g. by conducting similar studies under different abiotic conditions, as in Approach 1). Such studies are easier to conduct in the glasshouse but need to be compared with patterns in the field. Several studies have taken this approach. For example, Grime *et al.* (1987) compared the effects of soil heterogeneity, grazing and mycorrhizal symbioses on plant diversity and found that the presence of mycorrhizal fungi explained a greater proportion of the variance in plant diversity than the other factors combined. Vogelsang *et al.* (2006) went further by manipulating the composition and diversity of mycorrhizal fungi, as well as soil phosphorus availability, and found that fungal composition and diversity explained a much greater proportion of the observed variance in plant diversity. Other studies have also taken a similar approach of studying the effect of mycorrhizal fungi in the context of other factors (see Table 1), and the proportion of variance explained by the effect of mycorrhizas ranges from 0 to > 50% (Fig. 1). One limitation of these glasshouse manipulation studies is that any observed effects may not scale to the field (Read, 2002), so we recommend that simultaneous glasshouse–field comparisons are also made. For example, Pringle & Bever (2008) set up parallel studies of plant response to mycorrhizal fungi in growth chambers and in the field and found that mycorrhizal effects were correlated between the two environments. It should also be emphasized that because mycorrhizal fungi are difficult to control in the field, they may not be consistently eliminated, either spatially or temporally. Thus, variance in field experiments may be underestimated. Parallel glasshouse–field analyses are also encouraged because some of the confounding factors can be set as treatments in the glasshouse to yield more accurate estimates of variance.

Approach 3: Finally, inferences as to *the relative importance of forces of plant community structure are derived from monitoring these potential forces, as well as plant community*
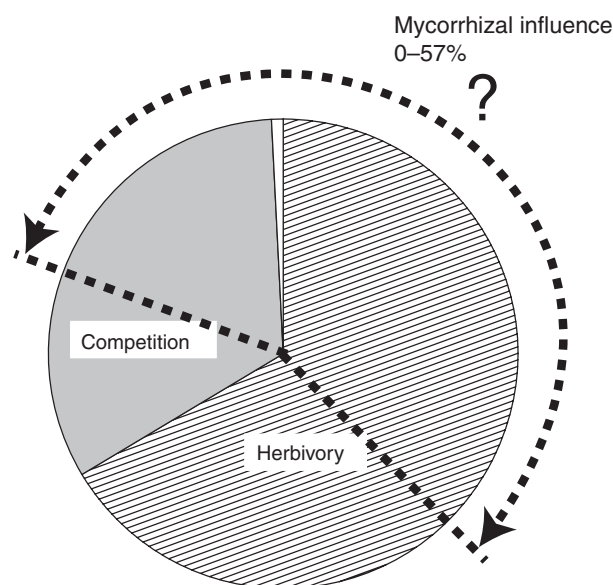

Fig. 1 Percentage variance in plant community composition that is explained by competition among plants vs herbivory (Sih *et al.*, 1985). We propose that a similar analysis using mycorrhizal fungi would be very informative (data from Table 1 indicate that mycorrhizal symbioses could explain 0–57% of the variance in plant community structure).

composition over time. This approach takes advantage of variation in plant and fungal community composition in the field, and uses measures of potential forces at time 1 as predictors of plant community composition at time 2 using the analytical framework of structural equation modelling (Shipley, 2002). This method is based on using natural or experimentally driven variation, or patterns through time, while measuring different variables that relate mycorrhizal abundance to plant community changes, via different mechanistic (direct and indirect) pathways. This approach could be better informed by analyses that explicitly compare the strength of different pathways (e.g. using pulse-label experiments and subsequent isotope analyses; or using the output of simulation community models). We recommend that the scientific community carefully choose good model field sites to focus on for long-term study, especially sites that may have fewer problems with confounding variables.

In most cases, species richness and evenness have been the main metrics of plant community responses measured following mycorrhizal manipulations (Table 1). Further consideration should be given to other robust metrics that can be used to compare community outcomes across systems. These might include total productivity, ecosystem resilience and plant functional group composition (Lavorel *et al.*, 1997).

In conclusion, we recommend a redirection of mycorrhizal studies in plant community ecology, departing from the already answered question of whether mycorrhizal symbioses *can* affect plant community structure, and move on to the questions of *how much* they affect community structure

under different conditions and in different systems. This requires an approach that not only centres on more complex experimental and survey designs, but also evaluates the proportion of variation explained by the other major factors. As in many other areas of ecology, it would help to move beyond the Fisherian approach of significance testing (noting the P-value; the effect is/is not significant) to ascertaining the variability explained by the components of more complex combinations of factors. In ANOVA terms this amounts to evaluating the Sum of Squares (e.g. Morin *et al.*, 1988), but other, more complex, techniques are available (e.g. partition of variances of dissimilarities in Permutational Multivariate Analysis of Variance (Anderson, 2001), or Structural Equation Modelling (Shipley, 2002). In addition, there should be concerted efforts to replicate studies across host plant communities, environmental gradients and in a wide variety of ecosystems and biogeographical regions. Several ecology studies show that the importance or the intensity of biotic interactions vary between ecosystems, often in a systematic way (Sih *et al.*, 1985; Bertness & Ewanchuk, 2002). For mycorrhizal studies, soil fertility may be an important determinant of the strength of mycorrhizal effects (Johnson, 2009), but studies across other environmental gradients may provide important insights on the relative contribution of mycorrhizal symbioses to plant community composition. In doing so, we would also recommend for studies to report a consistent measure of variables that are likely to affect the functioning of the symbiosis (such as soil nutrient levels and species identity). Relative importance might also be context dependent, being captured as a statistical interaction term among different factors. For example, the effect of mycorrhizal fungal community composition on plant diversity was dependent upon phosphorus addition (Vogelsang *et al.*, 2006). Although quantification of *relative influence* is a significant challenge, we argue that only by doing so will mycorrhizal effects be integrated into a comprehensive understanding of plant community ecology. There are also important applied implications: only proper quantification of impact can justify economic investment into managing mycorrhizal symbioses in restoration, agriculture or forestry. While quantification raises the spectre that the mycorrhizal symbiosis may not be a significant driver of plant community dynamics in some or many ecosystems, even this would be useful in allowing mycorrhizal ecologists to refocus efforts.

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Meetings

Plants and the Earth system – past events and future challenges

25th New Phytologist/Colston Research Society Symposium – Colonization of the terrestrial environment, Bristol, UK, September 2010

The huge diversity of land plants we see on earth today underpins our atmosphere, our environment and our society. But how and when did they get here, and what will happen to them, and to us, in the future?

The 25th New Phytologist/Colston Research Symposium 'Colonization of the terrestrial environment' (http://www.newphytologist.org/colonization/default.htm) brought together scientists from diverse backgrounds – geology, atmospheric science, palaeobotany, plant physiology, molecular, genetic and evo-devo – to discuss this key event in Earth's history.

> *'...land plants (otherwise known as "land survival devices for cyanobacteria"!) could not have evolved without interplay between organisms (bacteria and algae), rocks and the atmosphere.'*