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REVIEW AND SYNTHESIS

A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi

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Abstract

Mycorrhizal fungi influence plant growth, local biodiversity and ecosystem function. Effects of the symbiosis on plants span the continuum from mutualism to parasitism. We sought to understand this variation in symbiotic function using meta-analysis with information theory-based model selection to assess the relative importance of factors in five categories: (1) identity of the host plant and its functional characteristics, (2) identity and type of mycorrhizal fungi (arbuscular mycorrhizal vs. ectomycorrhizal), (3) soil fertility, (4) biotic complexity of the soil and (5) experimental location (laboratory vs. field). Across most subsets of the data, host plant functional group and N-fertilization were surprisingly much more important in predicting plant responses to mycorrhizal inoculation ('plant response') than other factors. Non-N-fixing forbs and woody plants and C_4 grasses responded more positively to mycorrhizal inoculation than plants with N-fixing bacterial symbionts and C_3 grasses. In laboratory studies of the arbuscular mycorrhizal symbiosis, plant response was more positive when the soil community was more complex. Univariate analyses supported the hypothesis that plant response is most positive when plants are P-limited rather than N-limited. These results emphasize that mycorrhizal function depends on both abiotic and biotic context, and have implications for plant community theory and restoration ecology.

Keywords

Arbuscular mycorrhizas, ectomycorrhizas, inoculation, meta-analysis, nitrogen, phosphorous, plant functional group, soil microorganisms, symbiosis.

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INTRODUCTION

Most plant species belong to families that typically form root symbioses with mycorrhizal fungi, often with dramatic consequences for plant growth and reproduction (Koide 2000), plant community structure (Grime *et al.* 1987; Hartnett & Wilson 2002) and ecosystem functions (Rillig 2004). Although these symbioses are cited in textbooks as clear examples of mutualism and plants often benefit from the association, the interaction might better be viewed as

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exhibiting a continuum of outcomes as the fungi can sometimes be of little net benefit to host plants or even function as a net parasitism. Different variables control whether a symbiosis between a mycorrhizal plant and fungus will develop as a mutualism or parasitism, including host plant characteristics, fungal characteristics, soil biotic and abiotic conditions, and experimental procedures (Modjo *et al.* 1987; Johnson *et al.* 1997; Klironomos 2003; Jones & Smith 2004); however, predictions regarding the importance of these different variables have typically been tested in isolation, with individual studies conducted using restricted subsets of plants and fungi. We currently lack an understanding of the relative importance and average magnitude of these different variables for mycorrhizal associations.

We used meta-analyses to synthesize available data and address these deficiencies. Meta-analysis provides a quantitative method for integrating results from many different experiments to answer broad questions, taking into account variation among studies in levels of replication and data dispersion, and providing quantitative estimates for experimental effects and relationships among variables (Hedges & Olkin 1985; Gurevitch & Hedges 1999). Meta-analysis has been used to test the importance of single factors for variability in outcomes of the ectomycorrhizal (EM) symbiosis (Karst *et al.* 2008), to examine responses of arbuscular mycorrhizal (AM) and EM symbioses to N, P and CO_2 fertilization (Treseder 2004), to test whether AM fungi affect plant–pathogen interactions (Borowicz 2001), to compare the impacts of different agricultural management practices on AM colonization and resulting growth responses of crop plants (Lekberg & Koide 2005), and to compare the relative importance for plants of mycorrhizal symbioses vs. other types of interactions (Morris *et al.* 2007). Our meta-analysis is distinct in that we used multi-factor statistical models to simultaneously estimate the relative importance and magnitude of the effects of multiple predictor variables on plant response to inoculation with mycorrhizal fungi, focusing on predictor variables in five categories: (1) the identity of the host plant and its functional characteristics, (2) the identity (genus) and type of mycorrhizal fungi (AM vs. EM), (3) soil fertility (N-fertilization and P-fertilization treatments), (4) the biotic complexity of the soil (sterilized vs. non-sterilized soil, single species vs. multispecies mycorrhizal inoculum and augmentation of non-mycorrhizal microbes) and (5) experimental location (laboratory vs. field).

MATERIALS AND METHODS

Overview of approach to analysis

We employed multi-factor statistical models to assess the relative importance of different factors to the response of plants to mycorrhizal inoculation. The methods for multi-factor meta-analysis are not well developed in the statistical literature, and widely available meta-analysis software (e.g., Metawin 2.0, Rosenberg *et al.* 2000) do not accommodate such a multi-factor approach. Datasets for multi-factor meta-analysis are inherently observational in nature because the values of the explanatory variables have not been independently manipulated. Some combinations of study characteristics used as explanatory variables are likely to be much more common than other combinations, generating autocorrelation and incomplete orthogonality among the explanatory variables. The commonly used stepwise multiple regression approach to selecting a single statistical model based on *P*-values associated with individual factors can lead to substantial errors in model selection and parameter estimation (Chatfield 1995; Burnham & Anderson 2002; Whittingham *et al.* 2006). We used information–theoretic criteria to rank candidate multiple regression models having different combinations of explanatory variables and to rank the relative importance of the variables in those models. We also estimated the parameters associated with important explanatory variables, while controlling for the effects of other explanatory variables in the best multi-factor models, rather than testing their statistical significance (Burnham & Anderson 2002). Overall, this analytical approach allows for inference from multiple models and focuses inference on the weight of evidence in the data for different models and factors and on the size and direction of effects. Furthermore, this approach avoids testing null hypotheses about individual factors that may not belong in the best model or models.

Literature search and dataset construction

We searched the ISI Web of Science database (1968–2004) using the key words *mycorrhiz** and *inocul** on 22 January 2005 to generate a set of 1852 publications. Because we did not anticipate screening all 1852 publications, we screened a random subset (*c.* 20%) of those publications for studies to be included in our meta-analysis. We defined a 'study' as any comparison of average plant performance between plants that were inoculated with mycorrhizal fungi (AM or EM) and plants that were not inoculated. We did not include studies in which different levels of mycorrhizal colonization were produced by adding fungicide to the roots of a subset of plants. Individual publications frequently yielded multiple studies, for example comparing the response of different host plants to inoculation or the response of the same host plant to inoculation with different fungi and our initial screening identified 1167 studies (from 134 publications) containing appropriate data. Because this initial compilation of studies included substantially fewer studies involving EM fungi compared with AM fungi, we added an additional 827

studies (from 49 publications) on EM fungi that were used in a previous meta-analysis and that met our criteria for inclusion (Karst *et al.* 2008). The latter studies were originally selected by screening, using similar criteria as our original search, the 3591 publications produced by a search of the ISI Web of Science database (1965–2006) using the key word *ectomycorrhiza*.

From each study, we collected data on plant performance with and without mycorrhizal inoculation, as well as the following 14 study characteristics to be used as explanatory variables in multi-factor meta-analyses:

PlantFunctionalGroup: A categorical fixed-effect variable with up to six levels corresponding to putative plant functional groups: C_4 grasses, C_3 grasses, N-fixing forbs (i.e. forbs with N-fixing bacterial symbionts), non-N-fixing forbs, N-fixing woody plants and non-N-fixing woody plants.

MycorrhizaType: A categorical fixed-effect variable with two levels, AM and EM.

FungalGenus: A categorical fixed-effect variable with seven levels, including five EM levels (*Laccaria*, *Pisolithus*, *Hebeloma*, *Scleroderma* and 'other EM genera') and two arbuscular-mycorrhizal (AM) levels (*Glomus* and 'other AM genera').

InoculumComplexity: A categorical fixed-effect variable with three levels: whole soil inoculum (presumably containing multiple fungal species as well as diverse non-mycorrhizal microbes and other biota), multiple-species inoculum (containing multiple fungal species but little or no other soil biota) and single-species inoculum (containing only a single fungal species and little or no other soil biota).

Sterility: A categorical fixed-effect variable with two levels: sterilized (background soil medium was sterilized before the experiment was conducted) and not sterilized.

MicrobeControl: A categorical fixed-effect variable with three levels: No added non-mycorrhizal microbes (non-mycorrhizal microbes were not added or supplemented in the experiment, either to all the background soil or to the non-inoculated pots), microbial wash (an aqueous filtrate of non-mycorrhizal microbes was added), or other microbial addition (non-mycorrhizal microbes were supplemented in another form, usually using rhizosphere soil from non-mycorrhizal culture plants).

Location: A categorical fixed-effect variable with two levels, laboratory (greenhouse or growth chamber) and field (e.g. agricultural field, forest).

N-fertilization, *P-fertilization* and *Fertilization*: Categorical fixed-effect variables. *N-fertilization* and *P-fertilization* had two levels (fertilized or not) and *Fertilization* had four levels (fertilized with N but not P, fertilized with P but not N, fertilized with both N and P, and fertilized with neither N nor P). *Fertilization* was not used in the same statistical models with *N-fertilization* and *P-fertilization*, but rather was used as an alternative to those two separate factors. Because actual levels of soil fertility were rarely reported, these fertilization variables are the best available approach to assess the potential importance of N and P availability for mycorrhizal function (also see *Univariate tissue nutrient analyses* below).

N-fertilization × *MycorrhizaType*: A fixed-effect variable, testing the interaction between two variables described above, *N-fertilization* and *MycorrhizaType*.

PlantSpecies and *PlantFamily*: Categorical random-effect variables. Each level of *PlantSpecies* is designated by a unique combination of plant genus and specific epithet. The largest data subset (Analysis 1) contained more than 130 plant species in 27 different plant families.

PlantSpecies × *PlantFunctionalGroup*: A random-effect variable included only in models containing *PlantFunctionalGroup* as a fixed factor.

Because most studies lacked information on one or more of these 14 variables and missing data were not compatible with our approach to multi-factor meta-analysis, we created four different subsets of the data for analysing different subsets of the 14 explanatory variables in separate meta-analyses. Each of these data subsets contained complete information on a subset of the explanatory variables, allowing analyses of the relative importance of those variables in that data subset. Table 1 lists the explanatory variables analysed for each of the four data subsets and Appendix S1 provides additional details on how candidate explanatory variables were chosen and scored and how multiple data subsets were chosen for meta-analysis. Appendices S2 and S3 contain, respectively, the data used in each of the analyses and the full bibliographic references for the publications from which those data were extracted. Chaudhary *et al.* (2010) provide a detailed description of the database and web interface tools that we developed to facilitate efficient and accurate compilation of data for complex multi-factor meta-analysis.

Calculation of effect sizes

Whole plant (root and shoot) biomass and shoot biomass were the most commonly reported measures of plant response to mycorrhizal inoculation; in our analyses, we used whole plant biomass when it was available and otherwise used shoot biomass. For each experimental comparison between inoculated treatments and non-inoculated controls, we calculated an effect size for plant biomass based on mean values in the inoculated and non-inoculated groups. Specifically, effect size of inoculation was calculated as the log response ratio of inoculated to non-inoculated plant biomass: $\ln(X_i/X_n)$, where X_i is the mean biomass in an inoculated treatment and X_n is the mean biomass in a non-inoculated control. This metric is positive for a beneficial effect of inoculation on plant biomass, and

Table 1 Summary of which candidate explanatory variables were included in analysis of each of the four data subsets. An 'X' indicates inclusion of a candidate explanatory variable in the analysis of a particular data subset

Explanatory variables	1: AM and EM fungi (n = 616 studies)	2: AM fungi only (n = 420 studies)	3: Single-species inocula only (n = 524 studies)	4: Laboratory studies of AM fungi only (n = 306 studies)
PlantFunctionalGroup	X	X	X	X
MycorrhizaType	X			
FungalGenus			X	
InoculumComplexity	X	X		X
Sterility				X
MicrobeControl				X
Location	X	X	X	
N-fertilization	X	X	X	X
P-fertilization	X	X	X	X
Fertilization	X	X	X	X
N-fertilization × MycorrhizaType	X			
PlantFamily	X	X	X	X
PlantSpecies	X	X	X	X
PlantSpecies × PlantFunctionalGroup	X	X	X	X

negative for a detrimental effect on plant biomass. We used the log response ratio (rather than other commonly used metrics for effect size such as Hedges' d) because it provides a standardized, unit-less measure of overall performance in inoculated treatments relative to non-inoculated controls, allowing valid comparisons among studies. Moreover, log response ratios have particularly favourable statistical properties for meta-analysis (Hedges *et al.* 1999). Scatter plots of effect size vs. the sample size of each study did not reveal any patterns indicative of publication bias, for example a lack of studies with both low effect size and low sample size.

Multi-factor meta-analysis

We used the MIXED procedure in SAS (SAS v. 9.1; SAS Institute, Inc., Cary, NC, USA) for all analyses, employing restricted maximum likelihood estimation of parameters. We first used a pure random-effects model to estimate the overall weighted mean effect size (i.e. the log response ratio of plant response to mycorrhizal inoculation) and random between-studies variance component (*sensu* van Houwelingen *et al.* 2002), with each effect size estimate weighted by the reciprocal of the within-study variance (which we estimated as the summed number of replicates in the inoculated treatments and non-inoculated controls) plus the maximum likelihood estimate of the residual between-studies variance component. We used this weighting method in lieu of the actual estimated effect size variance from each study, because far more studies reported levels of replication than reported actual measures of dispersion (SD, SE or confidence intervals) that could be used to calculate variance. Thus, we made the assumption that studies with higher levels of replication provided more precise estimates of effect size and those studies were given higher weight in the meta-analysis.

For each of the four separate analyses (Table 1), we explored the relative importance of different fixed factors by analysing a series of mixed-effect multiple meta-regression models, including the global model containing all of the fixed factors being considered for that dataset, as well as each of the nested subset models containing at least one fixed factor. Within each analysis, each candidate model was ranked according to an information-theoretic criterion (AIC_c, Akaike's Information Criterion corrected for small samples, which converges on AIC for large samples). An Akaike weight (w_i) was calculated for each model, which corresponds approximately to the likelihood that model is the best model among those being considered. Inference was then based on a 95% confidence set of models, based on cumulative w_i of the best models. For each predictor variable, its relative importance with regards to plant response to mycorrhizal inoculation was then determined based on the sum of w_i of the models in the 95% confidence set in which that predictor appeared. Predictor variables with a summed w_i less than 0.5 were considered relatively unimportant (Burnham & Anderson 2002). Further details on how these analyses and calculations were carried out can be found in Appendix S4.

Univariate tissue nutrient analyses

Overall, plants benefit most from mycorrhizal mutualisms in nutrient limited soils and benefit least in high fertility soils

since plants have less to gain from trading C for fungal-derived nutrients (Koide 1991; Schwartz & Hoeksema 1998; Jones & Smith 2004); however, interactions between concentration levels of different nutrients may also be important for determining symbiosis function. For example, mycorrhizal benefits to plants may be greatest when plants are P-limited but not N-limited because N limitation reduces plant photosynthetic capacity and thus C supply for the symbiosis (Johnson *et al.* 2003; Johnson 2010). Although soil and plant tissue nutrient concentration data were not reported frequently enough to be included as predictor variables in our multi-factor analyses, plant tissue nutrient concentration data – which can be useful indicators of plant nutrient status – were reported often enough (95 AM studies and 35 EM studies from 25 publications) for some separate, supportive univariate analyses, to aid interpretation of our multifactor meta-analysis results with respect to P- and N-fertilization. In particular, tissue N : P ratios < 14 or > 16 have been postulated to indicate N limitation or P limitation, respectively, with ratios between 14 and 16 likely indicating that the two elements are not limiting or are co-limiting (Koerselman & Meuleman 1996). Therefore, the tissue N : P ratios of non-mycorrhizal plants may also be a useful indicator of plant responsiveness to mycorrhizal inoculation. Because plant response to mycorrhizal inoculation depends on relative limitation of plant growth by C, N and P; and because photosynthesis (C acquisition) is often reduced in N-limited plants due to the high relative abundance of N in the photosynthetic enzymes (Chapin 1980), we predicted that mycorrhizas should be most beneficial when P is relatively more limited than N. We tested this prediction with two analyses: First, we estimated the linear relationship between plant biomass response to inoculation (log response ratio) and final tissue N : P ratio (natural log-transformed) of control (non-inoculated) plants using un-weighted maximum-likelihood parameter estimation in SAS PROC MIXED. Second, we used a *t*-test to compare plant biomass response to inoculation between N-limited (tissue N : P < 14 in non-inoculated plants) and P-limited (tissue N : P > 16 in non-inoculated plants) studies. Finally, to better understand the relationship between N-fertilization and P-fertilization (predictor variables in our meta-analyses) and the tissue N and P data used in these univariate analyses, we used *t*-tests to compare tissue N : P ratios of non-inoculated plants between studies that were N-fertilized and those that were not and between studies that were P-fertilized and those that were not.

RESULTS

Table 2 contains a summary of the results from the multi-factor meta-analyses of the four different data subsets,

Table 2 Summary of results from multi-factor meta-analyses of plant response to mycorrhizal inoculation in four different data subsets, including relative importance of candidate explanatory variables based on model selection using AIC_c (Akaike's Information Criterion corrected for small samples)

Analysis	Overall weighted mean effect size* ± SE	Relative variable importance and sum of Akaike weights (sum w_i) for each variable	Pseudo-R^2 of AIC_c-best model
1: AM and EM fungi (n = 616 studies)	0.43 ± 0.052	**PlantFunctionalGroup**† (sum w_i = 0.60) = **N-fertilization** (sum w_i = 0.60) > InoculumComplexity (sum w_i = 0.49) > MycorrhizaType (sum w_i = 0.47) > Location (sum w_i = 0.46) > P-fertilization (sum w_i = 0.20) > Fertilization (sum w_i = 0.17) > N-fertilization × MycorrhizaType (sum w_i = 0.16)	0.26
2: AM fungi only (n = 420 studies)	0.49 ± 0.063	**PlantFunctionalGroup** (sum w_i = 0.70) > **N-fertilization** (sum w_i = 0.68) > Location (sum w_i = 0.44) > Inoculum Complexity (sum w_i = 0.31) > P-fertilization (sum w_i = 0.25) > Fertilization (sum w_i = 0.23)	0.41
3: Single-species inocula only (n = 524 studies)	0.36 ± 0.051	**N-fertilization** (sum w_i = 0.67) > PlantFunctionalGroup (sum w_i = 0.42) > FungalGenus (sum w_i = 0.40) > Location (sum w_i = 0.34) > P-fertilization (sum w_i = 0.24) > Fertilization (sum w_i = 0.21)	0.23
4: Laboratory studies of AM fungi only (n = 306 studies)	0.61 ± 0.072	**PlantFunctionalGroup** (sum w_i = 0.92) = **MicrobeControl** (sum w_i = 0.92) > **InoculumComplexity** (sum w_i = 0.81) > Sterility (sum w_i = 0.48) > P-fertilization (sum w_i = 0.36) > N-fertilization (sum w_i = 0.28) > Fertilization (sum w_i = 0.11)	0.34

*Effect size was calculated as the log response ratio of inoculated to non-inoculated plant biomass: $\ln(X_i/X_n)$, where X_i is the mean biomass in an inoculated treatment and X_n is the mean biomass in a non-inoculated control treatment.
†Variables in bold type had a sum of Akaike weight (sum w_i) greater or equal to 0.5 and thus were considered relatively important.

including the overall weighted mean effect size, the relative importance of candidate explanatory variables based on model selection and the pseudo-R^2 of the AIC_c-best model. Plant response to mycorrhizal inoculation ranged widely from negative to positive (Fig. S1), but in all four subsets of data the average plant response was positive, with weighted mean effect sizes ranging from 0.36 (± 0.051 SE) to 0.61 (± 0.072). The covariance parameters for the random effects of plant taxonomy were estimated to be non-zero in the best models in all four multi-factor analyses, suggesting that plant identity explained a significant proportion of variation in outcome of the symbiosis in all four analyses. Appendix S5 includes the full model selection results for each multi-factor analysis, including information criteria for each model considered.

Analysis 1: AM and EM fungi (candidate fixed factors: PlantFunctionalGroup, MycorrhizaType, InoculumComplexity, Location, N-fertilization, P-fertilization, Fertilization, N-fertilization × MycorrhizaType)

Model selection suggested that 76 of 88 candidate models were useful for inference (Table S5-1 in Appendix S5). Among the eight fixed factors under consideration, PlantFunctionalGroup and N-fertilization were most important in explaining plant biomass response to mycorrhizal inoculation (Table 2). Among the plant functional groups considered, C_4 grasses and other non-N-fixing plants exhibited the largest responses to mycorrhizal inoculation, while C_3 grasses and N-fixing plants exhibited smaller responses (Fig. 1). In general, plant responses to mycorrhizal inoculation were substantially larger when experiments were not fertilized with N (Fig. 1). MycorrhizaType, InoculumComplexity, Location, Fertilization, P-fertilization and N-fertilization × MycorrhizaType were relatively unimportant as explanatory variables (sum $w_i < 0.5$). In the best model, the covariance parameters associated with the random effects of plant taxonomy were both estimated to be non-zero. Parameters associated with explanatory variables found to be relatively unimportant are not depicted graphically.

Analysis 2: AM fungi only (candidate fixed factors: PlantFunctionalGroup, InoculumComplexity, Location, N-fertilization, P-fertilization, Fertilization)

Model selection suggested that 28 of 40 candidate models were useful for inference (Table S5-2 in Appendix S5). PlantFunctionalGroup was most important (among the fixed factors under consideration) in explaining plant biomass response to mycorrhizal inoculation (Table 2), appearing in 12 of the 14 best models (Appendix S5), with

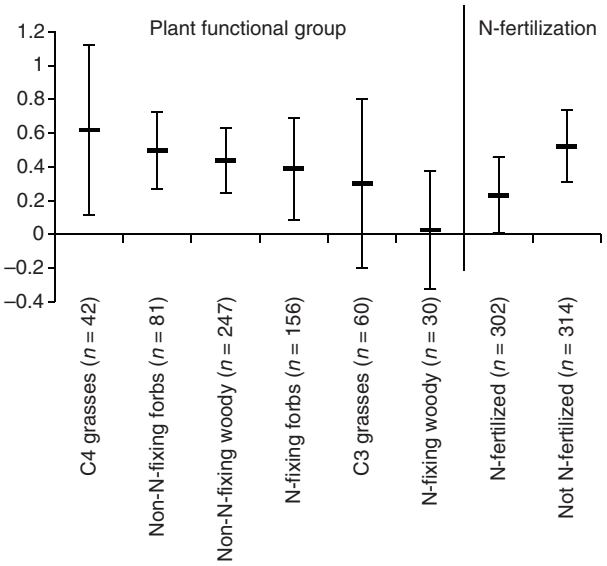

Figure 1 Parameter estimates (weighted mean ± SE) of plant response to mycorrhizal inoculation for the two important explanatory variables in Analysis 1 (AM and EM fungi): PlantFunctionalGroup and N-fertilization. The number of studies in each group is shown in parentheses.

patterns of variation among plant functional groups very similar to Analysis 1 (Fig. S2a). N-fertilization was nearly as important as PlantFunctionalGroup (Appendix S5), with plant responses to mycorrhizal inoculation substantially larger when experiments were not fertilized with N (Fig. S2b). InoculumComplexity, Location, Fertilization and P-fertilization were relatively unimportant as explanatory variables (sum $w_i < 0.5$). In the best model, the covariance parameters associated with the random effects of plant taxonomy were both estimated to be non-zero.

Analysis 3: Inocula with single AM and EM fungal species only (no mixed-species or whole-soil mycorrhizal inocula) (candidate fixed factors: PlantFunctionalGroup, FungalGenus, Location, N-fertilization, P-fertilization, Fertilization)

Model selection suggested that 31 of 40 candidate models were useful for inference (Table S5-3 in Appendix S5). N-fertilization was the only important explanatory variable among those considered (Table 2). As in Analyses 1 and 2, plant responses to mycorrhizal inoculation were substantially larger when experiments were not fertilized with N (Fig. S2c). FungalGenus, PlantFunctionalGroup, Location, Fertilization and P-fertilization were relatively unimportant as explanatory variables (sum $w_i < 0.5$). In the best model, the covariance parameters associated with the random effects of plant taxonomy were both estimated to be non-zero.

Analysis 4: Laboratory studies of AM fungi only (candidate fixed factors: PlantFunctionalGroup, InoculumComplexity, Sterility, MicrobeControl, N-fertilization, P-fertilization, Fertilization)

Model selection suggested that 30 of 80 candidate models were useful for inference (Table S5-4 in Appendix S5). PlantFunctionalGroup and MicrobeControl were the most important explanatory variables (Table 2). Parameter estimates (and their relative magnitudes) for plant functional groups were somewhat different than in Analyses 1–3, with non-N-fixing forbs benefiting the most from mycorrhizal inoculation (Fig. 2). With respect to MicrobeControl, plant response to mycorrhizal inoculation was substantially larger when non-mycorrhizal microbes were present in all treatments, compared with when they were not added to experiments (Fig. 2). InoculumComplexity was also an important explanatory variable, with plant response to inoculation substantially higher in experiments in which multiple fungal species or whole soil inoculum were used, rather than a single fungal species (Fig. 2). Sterility, Fertilization, P-fertilization and N-fertilization were relatively unimportant as explanatory variables (sum w_i < 0.5). In the best model, the covariance parameters associated with the random effects of plant taxonomy were both estimated to be non-zero.

Univariate tissue nutrient analyses

Within the largest data subset, across the 130 studies reporting final tissue N and P concentration for non-inoculated plants, higher tissue N : P ratio (natural log-transformed) of non-inoculated plants was associated with increased plant response to mycorrhizal inoculation (estimated slope = 0.178, 95% confidence interval: 0.086–0.269; $F_{1,128} = 14.74$, $P = 0.0002$; Fig. 3), although even at high N : P ratios, plant responses to inoculation varied widely from negative to positive. The mean effect size of mycorrhizal inoculation on P-limited (tissue N : P > 16) plants was approximately twice that of N-limited (tissue N : P < 14) plants ($t_{121} = 2.80$, $P = 0.006$; mean ± SE of P-limited = 0.500 ± 0.063, N-limited = 0.240 ± 0.068). Compared with plants in experiments not fertilized with N, non-inoculated plants in experiments that were fertilized with N had significantly lower tissue N : P ratios ($t_{128} = 2.34$, $P = 0.021$; mean ± SE of N-fertilized = 13.1 ± 2.14, not N-fertilized = 18.8 ± 1.17; Fig. 3). Non-inoculated plants in experiments fertilized with P did not differ in their tissue N : P ratios from those that were not fertilized with P ($t_{128} = 1.22$, $P = 0.225$).

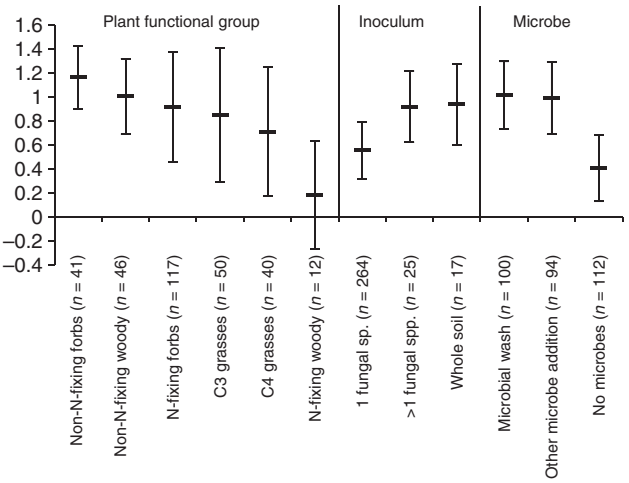

Figure 2 Parameter estimates (weighted mean ± SE) of plant response to mycorrhizal inoculation for the three important explanatory variables in Analysis 4 (laboratory studies of AM fungi only): PlantFunctionalGroup, InoculumComplexity, MicrobeControl. The number of studies in each group is shown in parentheses.

DISCUSSION

Our multi-factor meta-analyses of multiple subsets of 1,994 field and laboratory mycorrhizal inoculation studies highlight the simultaneous important influences of functional characteristics of host plants, soil fertility and complexity of

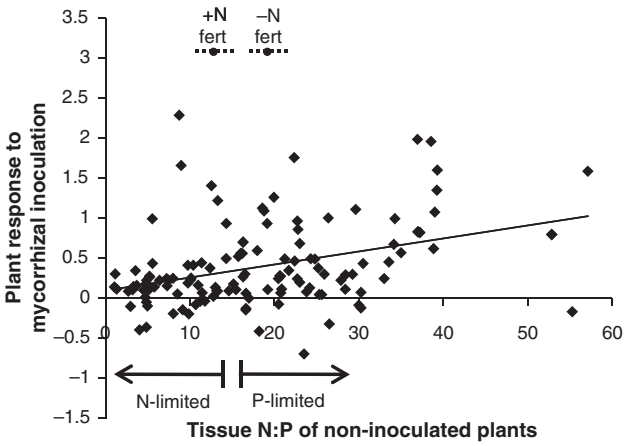

Figure 3 Parameter estimates for relationships among N-fertilization, plant response to mycorrhizal inoculation, and tissue N : P ratios of non-inoculated plants. The scatterplot shows variation in plant response to mycorrhizal inoculation (log response ratio of plant biomass) as a function of final tissue N : P ratio of non-inoculated plants ($n = 130$ studies). The fitted line shows the maximum likelihood estimated linear relationship between the two variables. The dotted lines show mean (± SE) tissue N : P ratio of non-inoculated plants in experiments that were N-fertilized ('+N fert') or not N-fertilized ('–N fert'). Putative zones of N- and P-limitation of plant growth (Koerselman & Meuleman 1996) are indicated below the horizontal axis.

the soil community, which includes both mycorrhizal fungi and non-mycorrhizal microbes, on the response of plants to mycorrhizal symbioses (Figs 1–3 and S2). Outcomes in individual studies ranged widely from positive to negative, yet the weighted average effect of inoculation on plant growth was positive (Figs 3 and S1). Our results provide insight on how mycorrhizal function depends on specific plant, soil and fungal factors, and suggest areas for further research that could increase our understanding of the complex relationship between plant and fungal symbionts and inform management practices (Kiers *et al.* 2002; Gosling *et al.* 2006; Harris 2009).

Soil fertility and nutrient limitation

For both laboratory and field studies of AM and EM fungi (Analyses 1 and 2), N-fertilization was an important predictor of plant response to mycorrhizal inoculation, and was the only important predictor in the subset of studies in which inocula consisted of single fungal species (Analysis 3). Overall, plant responses to mycorrhizal inoculation were substantially lower with N-fertilization (Figs 1 and S2b,c), regardless of mycorrhiza type (AM or EM). In contrast, P-fertilization was consistently unimportant, relative to other predictors (Table 2). This result held true whether P-fertilization was treated as a separate predictor variable (with or without P-fertilization) or was combined with N-fertilization in a four-level predictor variable (no fertilization, P-fertilization only, N-fertilization only, or both). This result also held true whether the analysis included both AM and EM fungi (Analysis 1) or focused only on AM fungi (Analyses 2 and 4).

Upon first consideration our findings for P-fertilization and N-fertilization on plant responses seem paradoxical. Our univariate analysis showed that the benefits of mycorrhizal inoculation to plant growth were greater in P-limited plants than in N-limited plants, yet our multi-factor meta-analyses show that N-fertilization was strongly associated with reduced mycorrhizal benefits (Table 2, Fig. 1). Furthermore, there is a large body of literature showing that AM symbioses are critical to the P nutrition of many plant species, and that mycorrhizal benefits are often inversely related to P availability (Stribley *et al.* 1980; Marschner & Dell 1994; Smith & Read 2008). So why do we see an association between N-fertilization and the outcome of mycorrhizal inoculation, and no such association with P-fertilization?

These apparent contradictions can be resolved if we consider relative C, N and P limitation of mycorrhizal plants and fungi, rather than focusing on each resource separately. Previous studies make the following predictions: 1) mycorrhizal benefits will be greatest when plants are P-limited but not N-limited since N limitation reduces plant photosynthetic capacity and thus C supply for the symbiosis; and 2) mycorrhizal benefits will be lowest when N, P or other belowground resources do not limit plant growth because plants will tend to reduce C allocation to roots and mycorrhizas in such an environment (Johnson *et al.* 2003; Johnson 2010). The first of these predictions is supported by our analyses showing that plant responses to mycorrhizal inoculation increased with an increase in the tissue N : P concentration of non-inoculated plants (Fig. 3) and was higher when plants were P-limited compared with N-limited (See Results). But our results for P- and N-fertilization in multi-factor meta-analyses provide mixed support for the second prediction. Why was plant response to mycorrhizal inoculation lower in N-fertilized experiments, while P-fertilization was an unimportant predictor?

Our analyses suggest a bias in the initial conditions of experiments with N-fertilization, compared with those without N-fertilization, such that the N-fertilization experiments in our data set were performed predominately in soils with relatively low inherent N availability and high P availability as indicated by the tissue N : P ratios of non-inoculated plants (Fig. 3). In other words, researchers tended to apply N fertilizer to soils with low inherent N availability and high P availability and likely reduced mycorrhizal benefits for host plants by reducing overall nutrient limitation, especially relative to soils with relatively low P availability. In contrast, relative N : P limitation, as indicated by tissue N : P ratios of non-inoculated plants, did not differ between experiments with and without P-fertilization. This last point may explain why P-fertilization was not an important predictor variable in the multifactor meta-analyses. Future analyses focusing on studies reporting actual measurements of soil fertility and densities of fungal mycelium in the soil will clearly be useful for testing this hypothesis.

Host plant functional characteristics

We found that plant functional group was an important explanatory variable in the subset of 616 laboratory and field studies of AM and EM fungi (Analysis 1), in the subset of 420 studies that focused on laboratory and field studies of AM fungi only (Analysis 2), and in the subset of 306 laboratory studies of AM fungi (Analysis 4). The results of the model selection from these analyses suggest that host plant functional characteristics may be more important than location (laboratory vs. field), inoculum complexity (single fungal species vs. multiple fungal species vs. whole soil inoculum), P-fertilization and mycorrhiza type (AM vs. EM) in determining variation in plant response to mycorrhizal inoculation. Our approach of using lower-level plant taxonomic groupings as random effects, over which the plant functional groups were estimated (Appendix S4),

makes it unlikely that resulting estimates of differences among plant functional groups are biased by over-representation of any particular plant taxa in the dataset.

Our observation (in all analyses) that covariance parameters associated with random effects of plant taxonomy were estimated to be non-zero in the best models suggests that additional host plant characteristics, beyond those captured by our plant functional group categories, are important for understanding variation in plant response to mycorrhizal inoculation. In fact, the consistent effects of plant taxonomy even after plant functional group effects are removed supports generalization of mycorrhizal responsiveness from one plant species to closely related species. This generalization has been commonplace in the literature on mycorrhizal interactions, where mycorrhizal dependence has often been inferred from plant family (e.g., Francis & Read 1995; Pringle & Bever 2008). Our results suggest that phylogenetic analyses of mycorrhizal responsiveness of plant species (Brundrett 2002) may be increasingly informative, as more data become available on mycorrhizal responsiveness of less studied plant taxa (Brundrett 2009).

Our analysis revealed that C_4 grasses exhibited greater positive average responses to mycorrhizal inoculation compared with C_3 grasses (Figs1 and S2a). This result confirms patterns observed in previous individual experiments (Wilson & Hartnett 1998; Hartnett & Wilson 1999). This pattern was not apparent in the analysis focused only on laboratory studies of AM fungi (Fig. 2), perhaps because C_4 grasses tend to thrive in high light environments and the lower light levels in greenhouse and growth chamber studies relative to field studies may affect patterns of carbon allocation to AM symbionts and hence symbiosis function. In addition, among non-grasses, plants with N-fixing bacterial symbionts consistently exhibited smaller responses to inoculation than those without N-fixing symbionts, especially for woody plants (Figs 1, 2 and S2a). This result may indicate that the P-rich soil used in many of the studies may have precluded a net benefit from the symbiosis more strongly in N-fixing host plants, which may be neither N-limited nor P-limited in such contexts. Alternatively, the higher C costs to plants of maintaining both fungal and bacterial symbionts may result in indirect antagonistic interactions between the two symbionts (Bethlenfalvay *et al.* 1985), and/or may tip the balance towards C limitation rather than nutrient limitation in legumes, especially in the greenhouse environments in which many of these studies occurred (Bethlenfalvay *et al.* 1982).

Diversity and composition of mycorrhizal fungi and non-mycorrhizal organisms

Plant responses were significantly affected by the diversity of the soil community. In an analysis that included 306 greenhouse and growth chamber studies of AM fungi (Analysis 4), plant response was substantially lower when plants were inoculated with single AM fungal species, compared with inoculations with multiple fungal species or whole soil inoculum (Fig. 2), the latter presumably containing multiple fungal species as well as non-mycorrhizal microbes, protozoa and invertebrates. The same analysis found that plant response was substantially higher when non-mycorrhizal microbes were added directly to background soil media (Fig. 2). Both factors emerged as important while controlling for whether or not the background soil medium was sterilized before the experiment was conducted (Table 2). These findings are consistent with the effects of AM fungi on plants being more positive when the symbiosis occurs in a more realistic biotic context, including multiple species of fungi and a diverse soil community. More positive response of plants to multiple AM fungal species may result from complementarity among fungal species in the benefits provided for host plants (Hart & Reader 2002; Maherali & Klironomos 2007) or from beneficial fungi being more likely to be present in the mixed inoculum (Vogelsang *et al.* 2006). Ideally, future studies will be able to elucidate whether general patterns exist for a quantitative relationship between plant response and mycorrhizal fungal diversity, such as the curvilinear pattern observed by van der Heijden *et al.* (1998). In light of the growing body of literature showing functional diversity among mycorrhizal fungal taxa (Jones & Smith 2004; Maherali & Klironomos 2007; Smith & Read 2008; Hobbie & Agerer 2010), it is perhaps surprising that fungal genus did not emerge as a relatively important predictor variable in Analysis 3 (Table 2); however, our power to test fungal genus effects was somewhat limited by the relative underrepresentation of most fungal genera (besides *Glomus*, *Pisolithus* and *Laccaria*) across published mycorrhizal inoculation experiments (Appendix S2).

Non-mycorrhizal microbes in the soil have been shown in individual studies to have significant effects on the formation and outcome of the mycorrhizal symbiosis (Linderman 1988; Frey-Klett *et al.* 2007), and the direction of such effects has ranged from positive to negative depending on the details of particular experiments (e.g. Garbaye & Bowen 1987; Piculell *et al.* 2008). One possible explanation for more positive responses of plants to mycorrhizal fungi in the presence of a more complex soil community is that non-mycorrhizal microbes include plant pathogens from which plants are protected by mycorrhizal fungi (Fitter & Garbaye 1994; Newsham *et al.* 1995). An alternative possibility is that non-mycorrhizal microbes found in mycorrhizal inoculum commonly have direct negative effects on plant growth, offsetting growth benefits of mycorrhizal fungi, so growth differences between inoculated and control plants are less than when these

same detrimental microbes are also found in the controls. Additionally, it has been well documented that predators in soil food webs can stimulate plant productivity by enhancing nutrient (particularly N) availability to plants (reviewed by Moore *et al.* 2003), and mycorrhizal fungi may facilitate plant responses to this enrichment. On the other hand, soil fungivores may consume mycorrhizal fungi, sometimes having the opposite effect on plant productivity (e.g. Warnock *et al.* 1982; Finlay 1985). Under all of these scenarios, tests with non-mycorrhizal microbes added to controls, and in the presence of a diverse soil food web, would appear to give more precise representations of the net effect of mycorrhizal fungal inoculations. However, tests without such microbial controls may be preferred in some contexts (e.g. when rhizobia are factorially manipulated with mycorrhizal fungi) and our results suggest that these studies will provide conservative estimates of the benefits of mycorrhizal inoculation. Although we still have much to learn about the mechanisms of complex interactions among plants, mycorrhizal fungi and other soil biota (Bonkowski *et al.* 2009; Kobayashi & Crouch 2009), our results – averaged across a diverse array of experimental contexts and approaches – are consistent with more positive net responses of plants to AM fungi in the presence of higher diversity in the surrounding soil biota.

Across the largest data subset, which contained both AM and EM fungal studies, mycorrhiza type (AM vs. EM) was not an important explanatory variable (Table 2). Experiments on EM symbiosis are nearly always conducted on the seedlings of woody plants, focusing on plant performance during a small part of the life cycle of those plants, whereas experiments on the AM symbiosis more often utilize herbaceous plants, and more often measure lifetime plant performance. Thus, it may be that important differences between experiments with AM and EM fungi were captured with other explanatory variables included in our analyses, especially plant functional groups and plant taxonomy, or that the two groups of symbionts have similar average effects on their hosts.

Experimental location

Individual studies have shown that effects of mycorrhizal fungi on plants differ between field vs. greenhouse or growth chamber experiments (McGonigle 1988; Newsham *et al.* 1995; Pringle & Bever 2008), and a previous meta-analysis of nearly 300 studies found specifically that beneficial effects of AM fungi on plants were smaller in field experiments compared with greenhouse or growth chamber experiments (Lekberg & Koide 2005). In our analyses, experimental location (laboratory vs. field) *per se* was a relatively unimportant factor for the outcome of the symbiosis when controlling for other more specific explanatory factors such as plant functional group and N-fertilization (Table 2). It may be that, as we suggest above for mycorrhiza type, these other more specific explanatory factors captured most of the important variation in results between laboratory and field experiments in our dataset. Moreover, in Analyses 1–3, the location variable did appear in some of the best models (Appendix S5), and parameter estimates in those analyses (data not shown) suggest a trend in the same direction as that observed by Lekberg & Koide (2005).

Composition of the database

An important consideration is that variables not included in our models could also be important for variation in the outcome of the mycorrhizal symbiosis. Indeed, despite including at least eight fixed and random factors as explanatory variables in each analysis, it is striking how much variability in plant response to inoculation that we were still unable to explain (59–77%; see Table 2). This result not only emphasizes the strong conditionality of the mycorrhizal symbiosis, but also should serve as a caution that the results of our analyses are limited in their scope of inference to the explanatory factors included in each analysis. In some cases, values for potentially important variables were simply not reported. For example, very few studies reported available soil N and P concentrations, or values for other potentially important abiotic factors, such as ambient light or soil water availability. Other factors, such as host and fungal genotypes (e.g. Munkvold *et al.* 2004; Piculell *et al.* 2008; Hoeksema *et al.* 2009), may have substantial effects on how plants respond to mycorrhizal inoculation, and yet are almost never manipulated explicitly in experiments or reported in publications. Other trophic and non-trophic interactors with plants, such as pathogens, herbivores, fungal endophytes and pollinators, may also influence plant response to mycorrhizal inoculation (Morris *et al.* 2007; Mack & Rudgers 2008; Gehring & Bennett 2009; Scervino *et al.* 2009), but their presence or abundance are not typically reported in inoculation studies. Moreover, factors for which we accounted at a coarse level, such as the addition of non-mycorrhizal microbes to controls, could in theory be parsed more finely in a way that would explain variance in plant response to mycorrhizal inoculation, for example by accounting for the presence or abundance of key functional groups among the soil microbes that could mediate different types of plant–soil feedbacks (Klironomos 2003). We focused our analysis on examining the relative importance of, and parameter estimates for, a variety of biotic factors such as host plant characteristics, N-fertilization and P-fertilization, fungal composition and diversity, and other soil biotic factors that are frequently reported in the literature, but a clear goal for future synthetic analyses

should be to continue to broaden explanatory power by accounting for the numerous ways that plant responses to mycorrhizal fungi are contingent on their environment.

A second consideration when interpreting these results is that they are influenced by the types of studies represented in the database and in each data subset analysed. For example, our results would likely differ if we had selected data from only unmanaged systems, instead of including a substantial number of laboratory and agricultural studies. However, the relative scarcity of studies from unmanaged field systems in our sample of the literature prevented us from conducting an analysis of whether the outcomes of mycorrhizal inoculations tend to differ among major natural ecosystem types or climates, or between managed and unmanaged field systems.

A final factor to consider is that the mycorrhizal symbioses in natural field systems are often established on a target plant through mycelial growth from an existing mycelial network (Newman 1988; Simard & Durall 2004). In contrast, in a typical laboratory or field inoculation experiment colonization usually takes place from spores or fragmented mycelium (but see van der Heijden & Horton 2009). As a result, the C costs of establishing the symbiosis in unmanipulated field systems may less often be fully paid by the target host plant or colonization may be more rapid from an existing mycelial network (e.g. Puschel *et al.* 2007), increasing the net benefits provided by mycorrhizal fungi to host plants in unmanipulated field systems relative to the inoculation experiments analysed here. Moreover, plant and fungal taxa may vary in their abilities to develop a functional symbiosis from whole mycelial growth vs. spores or fragmented mycelium, and the relative importance of different inoculum sources may depend on disturbance history of a field site (Requena *et al.* 1996), contributing to variation in outcomes among different studies.

CONCLUSIONS

This intensive meta-analysis of mycorrhizal inoculation experiments significantly advances our understanding of the relative importance of the identity and functional characteristics of host plant species, nutrient availability, the identity and diversity of mycorrhizal fungi, and other biotic factors in the soil for the function of mycorrhizal associations. Recently, ecologists have begun to propose specific hypotheses on how simultaneous positive and negative interactions influence plant community dynamics (Lortie *et al.* 2004; Brooker *et al.* 2008; Maestre *et al.* 2009). That plant functional groups and plant taxonomy emerged as important predictors in our analyses, relative to other factors, supports the hypothesis that plant species differences in mycorrhizal response are central for how plant communities are structured by mycorrhizal fungi (van der Heijden 2002). The counterintuitive relationship we observed across studies between N-fertilization and plant response to mycorrhizal inoculation points to the increasing importance of further empirical studies that explicitly test how the mycorrhizal symbiosis functions across environmental gradients (McGill *et al.* 2006; Brooker *et al.* 2008), especially factorial gradients of N and P availability that influence plant and fungal resource stoichiometry. Finally, our observation that plant responses to mycorrhizal inoculation are more positive in the context of a more diverse soil microbial community demonstrates what may be a widespread link between biodiversity and ecosystem function, and highlights the potential for utilizing soil microbial communities and positive species interactions to establish plant communities for ecosystem restoration (Halpern *et al.* 2007; Harris 2009).

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REFERENCES

Bethlenfalvay, G.J., Pacovsky, R.S., Bayne, H.G. & Stafford, A.E. (1982). Interactions between nitrogen-fixation, mycorrhizal colonization, and host-plant growth in the *Phaseolus–Rhizobium–Glomus* symbiosis. *Plant Physiol.*, 70, 446–450.

Bethlenfalvay, G.J., Brown, M.S. & Stafford, A.E. (1985). *Glycine–Glomus–Rhizobium* symbiosis II. Antagonistic effects between

mycorrhizal colonization and nodulation. *Plant Physiol.*, 79, 1054–1058.

Bonkowski, M., Villenave, C. & Griffiths, B. (2009). Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant Soil*, 321, 213–233.

Borowicz, V.A. (2001). Do arbuscular mycorrhizal fungi alter plant–pathogen relations? *Ecology*, 82, 3057–3068.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G. *et al.* (2008). Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, 96, 18–34.

Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytol.*, 154, 275–304.

Brundrett, M.C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil*, 320, 37–77.

Burnham, K.P. & Anderson, D.R. (2002). *Model Selection and Multimodel Inference: A Practical Information–Theoretic Approach*. Springer Science/Business Media/LLC, New York, NY.

Chapin, F.S. (1980). The mineral nutrition of wild plants. *Annu. Rev. Ecol. Syst.*, 11, 233–260.

Chatfield, C. (1995). Model uncertainty, data mining and statistical inference. *J. R. Stat. Soc. A*, 158, 419–466.

Chaudhary, V.B., Walters, L., Bever, J.D., Hoeksema, J.D. & Wilson, G.W.T. (2010). Advancing synthetic ecology: a database system to facilitate complex ecological meta-analyses. *Bull. Ecol. Soc. Am.*, in press.

Finlay, R.D. (1985). Interactions between soil microarthro-pods and endomycorrhizal associations of higher plants. In: *Ecological Interactions in Soils: Plants, Microbes, and Animals* (eds Fitter, A.H., Atkinson, D., Read, D.J. & Usher, M.B.). Blackwell Scientific, Oxford, pp. 319–333.

Fitter, A.H. & Garbaye, J. (1994). Interactions between mycorrhizal fungi and other soil organsisms. *Plant Soil*, 159, 123–132.

Francis, R. & Read, D.J. (1995). Mutualism and atagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. *Can. J. Bot.*, 73(Suppl. 1), S1301–S1309.

Frey-Klett, P., Garbaye, J. & Tarkka, M. (2007). The mycorrhiza helper bacteria revisited. *New Phytol.*, 176, 22–36.

Garbaye, J. & Bowen, G.D. (1987). Effect of different microflora on the success of ectomycorrhizal inoculation of *Pinus radiata*. *Can. J. For. Res.*, 17, 941–943.

Gehring, C. & Bennett, A. (2009). Mycorrhizal fungal–plant–insect interactions: the importance of a community approach. *Environ. Entomol.*, 38, 93–102.

Gosling, P., Hodge, A., Goodlass, G. & Bending, G.D. (2006). Arbuscular mycorrhizal fungi and organic farming. *Agric. Ecosyst. Environ.*, 113, 17–35.

Grime, J.P., Mackey, J.M.L., Hillier, S.H. & Read, D.J. (1987). Floristic diversity in a model system using experimental microcosms. *Nature*, 328, 420–422.

Gurevitch, J. & Hedges, L.V. (1999). Statistical issues in ecological meta-analysis. *Ecology*, 80, 1142–1149.

Halpern, B.S., Silliman, B.R., Olden, J.D., Bruno, J.P. & Bertness, M.D. (2007). Incorporating positive interactions in aquatic restoration and conservation. *Front. Ecol. Environ.*, 5, 153–160.

Harris, J. (2009). Soil microbial communities and restoration ecology: facilitators or followers? *Science*, 325, 573–574.

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

Hartnett, D.C. & Wilson, G.W.T. (1999). Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, 80, 1187–1195.

Hartnett, D.C. & Wilson, G.W.T. (2002). The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant Soil*, 244, 319–331.

Hedges, L.V. & Olkin, I. (1985). *Statistical Methods for Meta-analysis*. Academic Press, Orlando.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150–1156.

van der Heijden, M.G.A. (2002). Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search for underlying mechanisms and general principles. In: *Mycorrhizal Ecology* (eds van der Heijden, M.G.A. & Sanders, I.R.). Springer-Verlag, Berlin, pp. 243–266.

van der Heijden, M.G.A. & Horton, T.R. (2009). Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *J. Ecol.*, 97, 1139–1150.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T. *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72.

Hobbie, E.A. & Agerer, R. (2010). Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant Soil*, in press; DOI: 10.1007/s11104-009-0032-z.

Hoeksema, J.D., Piculell, B. & Thompson, J.N. (2009). Within-population genetic variability in mycorrhizal interactions. *Commun. Integr. Biol.*, 2, 110–112.

van Houwelingen, H.C., Arends, L.R. & Stijnen, T. (2002). Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat. Med.*, 21, 589–624.

Johnson, N.C. (2010). Tansley review: resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytol.*, in press; DOI: 10.1111/j.1469-8137.2009.03110.x.

Johnson, N.C., Graham, J.H. & Smith, F.A. (1997). Functioning of mycorrhizas along the mutualism–parasitism continuum. *New Phytol.*, 135, 1–12.

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

Jones, M.D. & Smith, S.E. (2004). Exploring functional definitions of mycorrhizas: are mycorrhizas always mutualisms? *Can. J. Bot.*, 82, 1089–1109.

Karst, J., Marczak, L., Jones, M.D. & Turkington, R. (2008). The mutualism-parasitism continuum in ectomycorrhizas: a quantitative assessment using meta-analysis. *Ecology*, 89, 1032–1042.

Kiers, E.T., West, S.A. & Denison, R.F. (2002). Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation. *J. Appl. Ecol.*, 39, 745–754.

Klironomos, J.N. (2003). Variation in plant response to native and exotic mycorrhizal fungi. *Ecology*, 84, 2292–2301.

Kobayashi, D.Y. & Crouch, J.A. (2009). Bacterial/Fungal Interactions: from Pathogens to Mutualistic Endosymbionts. *Annu. Rev. Phytopathol.*, 47, 63–82.

Koerselman, W. & Meuleman, A.F.M. (1996). The vegetation N : P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, 33, 1441–1450.

Koide, R.T. (1991). Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol.*, 117, 365–386.

Koide, R.T. (2000). Mycorrhizal symbiosis and plant reproduction. In: *Arbuscular Mycorrhizas: Physiology and Function* (eds Kapulnik, Y. & Douds, D.D.). Kluwer Academic Publishers, Norwell, MA, USA, pp. 19–46.

Lekberg, Y. & Koide, R.T. (2005). Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytol.*, 168, 189–204.

Linderman, R.G. (1988). Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology*, 78, 366–371.

Lortie, C.J., Brooker, R.W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F.I. *et al.* (2004). Rethinking plant community theory. *Oikos*, 107, 433–438.

Mack, K.M.L. & Rudgers, J.A. (2008). Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. *Oikos*, 117, 310–320.

Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J. Ecol.*, 97, 199–205.

Maherali, H. & Klironomos, J.N. (2007). Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, 316, 1746–1748.

Marschner, H. & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant Soil*, 159, 89–102.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends Ecol. Evol.*, 21, 178–185.

McGonigle, T.P. (1988). A numerical analysis of published field trials with vesicular–arbuscular mycorrhizal fungi. *Funct. Ecol.*, 2, 473–478.

Modjo, H.S., Hendrix, J.W. & Nesmith, W.C. (1987). Mycorrhizal fungi in relation to control of tobacco stunt disease with soil fumigants. *Soil Biol. Biochem.*, 19, 289–295.

Moore, J.C., McCann, K., Setala, H. & De Ruiter, P.C. (2003). Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics? *Ecology*, 84, 846–857.

Morris, W.F., Hufbauer, R.A., Agrawal, A.A., Bever, J.D., Borowicz, V.A., Gilbert, G.S. *et al.* (2007). Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. *Ecology*, 88, 1021–1029.

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

Newman, E.I. (1988). Mycorrhizal links between plants – their functioning and ecological significance. *Adv.Ecol. Res.*, 18, 243–270.

Newsham, K.K., Fitter, A.H. & Watkinson, A.R. (1995). Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J. Ecol.*, 83, 991–1000.

Piculell, B.J., Hoeksema, J.D. & Thompson, J.N. (2008). Interactions of biotic and abiotic environmental factors in an ectomycorrhizal symbiosis, and the potential for selection mosaics. *BMC Biol.*, 6, 23 (11 pages).

Pringle, A. & Bever, J.D. (2008). Analogous effects of arbuscular mycorrhizal fungi in the laboratory and a North Carolina field. *New Phytol.*, 180, 162–175.

Puschel, D., Rydlova, J. & Vosatka, M. (2007). The development of arbuscular mycorrhiza in two simulated stages of spoil-bank succession. *Appl. Soil Ecol.*, 35, 363–369.

Requena, N., Jeffries, P. & Barea, J.M. (1996). Assessment of natural mycorrhizal potential in a desertified semiarid ecosystem. *Appl. Environ. Microbiol.*, 62, 842–847.

Rillig, M.C. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecol. Lett.*, 7, 740–754.

Rosenberg, M.S., Adams, D.C. & Gurevitch, J. (2000). *Metawin Version 2.0: Statistical Software for Meta-analysis*. Sinauer Associates, Inc., Sunderland, MA.

Scervino, J.M., Gottlieb, A., Silvani, V.A., Pergola, M., Fernandez, L. & Godeas, A.M. (2009). Exudates of dark septate endophyte (DSE) modulate the development of the arbuscular mycorrhizal fungus (AMF) *Gigaspora rosea*. *Soil Biol. Biochem.*, 41, 1753–1756.

Schwartz, M.W. & Hoeksema, J.D. (1998). Specialization and resource trade: biological markets as a model of mutualisms. *Ecology (Washington DC)*, 79, 1029–1038.

Simard, S.W. & Durall, D.M. (2004). Mycorrhizal networks: a review of their extent, function, and importance. *Can. J. Bot.*, 82, 1140–1165.

Smith, S.E. & Read, D.J. (2008). *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, Amsterdam.

Stribley, D.P., Tinker, P.B. & Rayner, J.H. (1980). Relation of internal phosphorus concentrations and plant weight in plants infected by vesicular–arbuscular mycorrhizas. *New Phytol.*, 86, 261–266.

Treseder, K.K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytol.* 164: 347–355. *New Phytol.*, 164, 347–355.

Vogelsang, K.M., Reynolds, H.L. & Bever, J.D. (2006). Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytol.*, 172, 554–562.

Warnock, A.J., Fitter, A.H. & Usher, M.B. (1982). The influence of a springtail *Folsomia candida* (Insecta: Collembola) on the mycorrhizal association of Leek, *Allium porum* and the vesicular–arbusuclar endophyte *Glomus fasiculatus*. *New Phytol.*, 90, 285–292.

Whittingham, M.J., Stephens, P.A., Bradbury, R.B. & Freckleton, R.P. (2006). Why do we still use stepwise modelling in ecology and behaviour? *J. Anim. Ecol.*, 75, 1182–1189.

Wilson, G.W.T. & Hartnett, D.C. (1998). Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *Am. J. Bot.*, 85, 1732–1738.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Variation in effect size among the 616 studies included in the largest data subset (Analysis 1), ranked from largest to smallest.

Figure S2 Parameter estimates (weighted mean ± SE) for the important explanatory variables in Analyses 2 (AM fungi only) and 3 (single-species inocula only).
Appendix S1 Further details on generation of hypotheses, data extraction, construction of data subsets and creation of candidate statistical models.
Appendix S2 File containing the data used in the multi-factor meta-analyses and the univariate tissue nutrient analyses.
Appendix S3 Full bibliographic references for publications from which analysed data were extracted.
Appendix S4 Detailed description of how multi-factor mixed-model meta-analysis was conducted for Analyses 1–4.
Appendix S5 Model selection results from multi-factor meta-analyses of the four data subsets.

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