A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies

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Summary

• Numerous field studies have measured mycorrhizal dynamics under additions of nitrogen (N), phosphorus (P), or atmospheric CO_2 to test the hypothesis that plants should invest in mycorrhizal fungi when soil nutrients are limiting.

• Here meta-analyses were used to integrate nutrient responses across independent field-based studies. Responses were compared between ecto- and arbuscular mycorrhizal fungi, and among fertilizer types, methods of measurement, biomes, and lead investigators. Relationships between degree of response and study length, fertilization rates, total amounts of nutrients applied, and numbers of replicates were also tested.

• Across studies, mycorrhizal abundance decreased 15% under N fertilization and 32% under P fertilization. Elevated CO_2 elicited a 47% increase. Nitrogen effects varied significantly among studies, and P effects varied significantly among lead investigators. Most other factors did not affect mycorrhizal responses.

• These results support the plant investment hypothesis, and suggest that global standing stocks of mycorrhizal fungi may increase substantially under elevated CO_2 but decline moderately under P additions. Effects of N deposition may be difficult to predict for individual ecosystems, with a slightly negative influence overall.

Key words: carbon dioxide enrichment, global change, meta-analysis, mycorrhizal fungi, nitrogen fertilization, nutrient limitation, plant investment, phosphorus fertilization.

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Introduction

Since nitrogen (N), phosphorus (P), and carbon (C) are each required by mycorrhizal fungi, the availability of each nutrient could control mycorrhizal abundance. Plants provide C by transferring carbohydrates via roots; soils supply N and P. One of the more widely tested hypotheses within the field of mycorrhizal ecology is that plants should invest more C in mycorrhizal fungi where N or P are limiting to plant growth, since mycorrhizal fungi contribute to nutrient uptake by plants (Mosse & Phillips, 1971). Conversely, if N or P availability rises, a decline in mycorrhizal abundance is expected as plants allocate carbohydrates elsewhere and mycorrhizal fungi become C-limited (Read, 1991). An alternate possibility is that mycorrhizal fungi are directly limited by soil nutrient availability and should proliferate following additions of N or P (Treseder & Allen, 2002). These mechanisms apply to both arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi.

Controls over mycorrhizal dynamics by C, N, and P are germane to global change studies. Enrichment of atmospheric CO_2 typically augments photosynthesis (Bazzaz, 1990; Poorter, 1993) and increases nutrient limitation in plants (Oren *et al.*, 2001; Schlesinger & Lichter, 2001; Finzi *et al.*, 2002), while fertilization with N and P (as land is converted to agriculture) and anthropogenic N deposition enhance soil fertility (Vitousek, 1994). Humans may be altering global and regional distributions of this ecologically and economically important microbial group.

To what extent do large-scale field experiments support the hypothesis that mycorrhizal fungi will increase under elevated CO_2 but decrease under additions of N and P? By contrast to glasshouse studies, field-based manipulations of CO_2, N and P can capture complex conditions that could influence

mycorrhizal abundance, including natural climatic variability, intact soil fauna and microbial communities, and established soil structure. As such, results from field experiments are particularly useful in predicting mycorrhizal feedbacks in ecosystems under global change. Previous reviews have reported high variation among studies in AM colonization of roots under elevated CO_2 (Staddon & Fitter, 1998) and in external hyphal lengths of AM and ECM fungi under N enrichment (Treseder & Allen, 2000), so that delineations of general responses are difficult. Meta-analysis provides a quantitative, statistical means of integrating independent results, and of identifying aspects of experimental design that might contribute to variation among studies (Gurevitch *et al.*, 1992; Gurevitch & Hedges, 1993, 1999; Arnqvist & Wooster, 1995). This study applied this approach to a dataset compiled from 31 published N fertilization studies, 20 P fertilization studies, and 14 elevated CO_2 studies. It focused on below-ground changes in standing crops of the fungi. Separate meta-analyses were conducted for N, P, and CO_2.

Materials and Methods

Sources of data

Selection criteria Meta-analyses were performed on data acquired from published sources that met specific criteria (Table 1). In particular, the present study focused on field studies in which mycorrhizal abundance was measured in response to long-term (> 2-months), large-scale (> 1-m^2) manipulations of N, P, or CO_2 availability, in comparison with an unmanipulated control. Short-term or smaller-scale studies were not included, because it is possible that mycorrhizal fungi could temporarily proliferate to exploit small 'hot spots' of nutrients (Jackson *et al.*, 1990; Hagerberg *et al.*, 2003). If so, short-term responses would not necessarily reflect long-term effects. In CO_2 experiments, this study included free-air CO_2 enrichment (FACE), open-top chamber, and closed-chamber designs if they were established on pre-existing soil. Planted vegetation was accepted in the case of agricultural systems only, because my objective was to include studies that represented natural systems as closely as possible in order to best approximate widespread effects of global change. In addition, I limited my data collection to results in which means, standard deviations, and replicate numbers were reported or could be determined. This latter specification unavoidably excluded six N-fertilization studies and eight P-fertilization studies that were otherwise qualified. In all cases, the unit of replication was the plot. Correlations between pre-existing levels of soil N or P and mycorrhizal biomass were not considered.

Because one assumption of meta-analysis is that studies are independent from one another (Gurevitch & Hedges, 1999), I used only one set of data from a given system. For instance, mycorrhizal abundance was often measured several times within a given study. In these cases, I restricted my analyses to the latest sampling date, since global change is often long-term. (Mycorrhizal responses did not vary significantly with study length in the ensuing meta-analyses.) If more than one publication presented results from the same field plots, I relied upon data from the most recent paper. In addition, several studies applied nutrients at a range of levels; in these cases, I only included data associated with the highest application rates. Conversely, if a particular publication reported results from more than one study system that could reasonably be considered independent (e.g. different geographical location, fertilizer type, ecosystem, or dominant vegetation), each system was designated as a different study. Effects of N, P, and CO_2 were examined in individual meta-analyses in order to avoid redundancy of control groups within studies that simultaneously tested more than one effect (Gurevitch & Hedges, 1993).

Data acquisition

For each study, meta-analysis requires the mean, standard deviation (SD), and replicate number (n) for the control as well as the nutrient-addition treatment. When means and errors were presented in a graph, the image was digitized and Grab-It! software was used to estimate values (Preble, 1998). If standard errors (SE) were reported, these were transformed according to the equation: $SE = SD \cdot (n^{-\frac{1}{2}})$. Unidentified error bars were assumed to represent standard error.

Indices of mycorrhizal abundance

The most common measures of mycorrhizal abundance were percentage root length colonized (for AM fungi) or percentage root tips colonized (for ECM fungi), both of which are hereafter referred to as '% colonization'. Other approaches included spore counts per gram soil (AM) and hyphal length per gram soil (AM and ECM). When more than one index of mycorrhizal abundance was reported within a given study, percentage colonization data were selected in order to facilitate comparisons with other studies that measured colonization only. Data regarding production of ECM sporocarps was not included, as the analysis focused on below-ground dynamics.

Statistics

Meta-analyses were used to determine the significance of mycorrhizal responses to nutrient enrichment. For each study and each type of nutrient addition (N, P, or CO_2), the effect size was calculated as the natural log of the response ratio ('R'), which is the mean of the treatment divided by the mean of the control (Hedges *et al.*, 1999). An R of 1 indicates that the nutrient addition had no effect. The estimate of variance within each study was represented as $v_{\ln R}$, which is a function

Table 1 Characteristics of studies included in meta-analyses, including response ratios (R) and variation within studies ($v_{\ln R}$)

Study	Identifier	Mycorrhizal type[a]	Additions[b]	Study length (yr)	Replicates[c]	Unit of measure	Biome	*R*	ln *R*	$v_{\ln R}$
Nitrogen fertilization										
Anderson & Liberta (1992)		AM	56	1.25	5	% colonization	Temperate grassland	1.18	0.16	0.01
Bentivenga & Hetrick (1992)		AM	100	6.00	4	% colonization	Temperate grassland	0.86	−0.15	0.00
Cornwell *et al*. (2001)		AM	60	0.33	5	% colonization	Woodland/shrubland	0.98	−0.02	0.08
Egerton-Warburton & Allen (2000)		AM	60	2.67	10	spore count	Woodland/shrubland	0.32	−1.14	0.01
Ellis *et al*. (1992)		AM	90	8.00	4	% colonization	Agricultural	0.87	−0.14	0.02
Grogan & Chapin (2000)		AM	200	0.17	3	% colonization	Temperate grassland	0.12	−2.16	0.24
Hutchinson *et al*. (1998)	Dorset	AM	1000	2.00	8	% colonization	Temperate forest	1.11	0.11	0.04
Hutchinson *et al*. (1998)	Loring	AM	1000	3.00	8	% colonization	Temperate forest	0.30	−1.20	0.03
Johnson *et al*. (2003)	Kellogg	AM	120	9.00	5	hyphal length	Agricultural	0.57	−0.56	0.07
Johnson *et al*. (2003)	Cedar Creek	AM	170	10.00	5	hyphal length	Agricultural	0.51	−0.68	0.13
Johnson *et al*. (2003)	Sevilleta	AM	100	3.00	10	hyphal length	Desert	0.73	−0.32	0.10
Lansing (2003)	Juniper	AM	100	4.00	3	% colonization	Temperate forest	1.14	0.13	0.01
Lansing (2003)	Sugar maple	AM	100	4.00	3	% colonization	Temperate forest	0.88	−0.13	0.01
Lansing (2003)	Poplar	AM	100	4.00	3	% colonization	Temperate forest	1.13	0.12	0.01
Treseder & Vitousek (2001)	N-limited site	AM	100	12.00	4	% colonization	Tropical forest	1.02	0.02	0.15
Treseder & Vitousek (2001)	fertile site	AM	100	4.00	3	% colonization	Tropical forest	0.72	−0.32	0.11
Treseder & Vitousek (2001)	P-limited site	AM	100	6.00	3	% colonization	Tropical forest	0.75	−0.29	0.08
Baum & Makeschin (2000)		ECM	100	11.00	9	% colonization	Agricultural	0.87	−0.14	0.03
Baum *et al*. (2002)	Abbachhof	ECM	100	9.00	9	% colonization	Agricultural	0.35	−1.05	0.06
Baum *et al*. (2002)	Wildeshausen	ECM	100	4.00	9	% colonization	Agricultural	1.73	0.55	0.05
Fransson *et al*. (2001)		ECM	80	14.00	3	% colonization	Boreal forest	0.95	−0.05	0.00
Karen and Nylund (1997)		ECM	100	4.00	4	% colonization	Temperate forest	1.33	0.29	0.17
Lansing (2003)	Balsam poplar	ECM	100	4.00	3	% colonization	Boreal forest	0.97	−0.03	0.00
Lansing (2003)	Oak	ECM	100	4.00	3	% colonization	Temperate forest	0.92	−0.08	0.00
Lansing (2003)	Pinyon pine	ECM	100	4.00	3	% colonization	Temperate forest	1.00	0.00	0.00
Lansing (2003)	Red pine	ECM	100	4.00	3	% colonization	Temperate forest	0.98	−0.02	0.00
Lansing (2003)	White spruce	ECM	100	4.00	3	% colonization	Boreal forest	1.00	0.00	0.00
Termorshuizen (1993)	Dwingeloo NH_4	ECM	60	3.00	3	% colonization	Temperate forest	0.57	−0.56	0.10
Termorshuizen (1993)	Dwingeloo NO_3	ECM	60	3.00	3	% colonization	Temperate forest	0.99	−0.01	0.30
Termorshuizen (1993)	Liessel NH_4	ECM	60	3.00	3	% colonization	Temperate forest	1.00	0.00	0.02
Termorshuizen (1993)	Liessel NO_3	ECM	60	3.00	3	% colonization	Temperate forest	0.99	−0.01	0.02
Phosphorus fertilization										
Anderson & Liberta (1992)		AM	56	1.25	5	% colonization	Temperate grassland	0.88	−0.13	0.03
Bentivenga & Hetrick (1992)		AM	10	6.00	4	% colonization	Temperate grassland	0.69	−0.37	0.01
Cornwell *et al*. (2001)		AM	20	0.33	5	% colonization	Woodland/shrubland	0.48	−0.73	0.13
Gavito & Miller (1998)		AM	30	0.17	16	% colonization	Agricultural	0.92	−0.08	0.02
Grogan & Chapin (2000)		AM	200	0.17	3	% colonization	Temperate grassland	0.88	−0.12	0.04
Hicks & Loynachan (1987)		AM	112	1.00	19	% colonization	Agricultural	0.20	−1.61	0.06
Kahiluoto *et al*. (2001)	Maaninka	AM	45	20.00	6	% colonization	Agricultural	0.66	−0.42	0.24
Kahiluoto *et al*. (2001)	Mietoinen	AM	45	20.00	4	% colonization	Agricultural	0.64	−0.45	0.20
Martensson & Carlgren (1994)	Ultuna	AM	45	28.00	4	spore count	Agricultural	0.01	−4.21	69.48

Table 1 *continued*

Study	Identifier	Mycorrhizal type[a]	Additions[b]	Study length (yr)	Replicates[c]	Unit of measure	Biome	*R*	ln *R*	$v_{\ln R}$
Martensson & Carlgren (1994)	Offer	AM	45	28.00	4	spore count	Agricultural	0.14	−1.97	0.77
Pellet & El-Sharkawy (1993)		AM	100	2.00	12	% colonization	Agricultural	0.70	−0.36	0.02
Sanginga *et al*. (1996)	Degraded	AM	7	0.27	8	% colonization	Agricultural	1.33	0.29	0.10
Sanginga *et al*. (1996)	Compound	AM	7	0.27	8	% colonization	Agricultural	1.62	0.48	0.09
Thomson *et al*. (1992)		AM	352	2.00	3	% colonization	Agricultural	0.66	−0.42	0.04
Treseder & Vitousek (2001)	N-limited site	AM	100	12.00	4	% colonization	Tropical forest	0.23	−1.46	0.13
Treseder & Vitousek (2001)	Fertile site	AM	100	4.00	4	% colonization	Tropical forest	0.50	−0.69	0.18
Treseder & Vitousek (2001)	P-limited site	AM	100	6.00	3	% colonization	Tropical forest	0.41	−0.90	0.48
Vanlauwe *et al*. (2000)		AM	7	0.31	6	% colonization	Agricultural	1.63	0.49	0.02
Baum & Makeschin (2000)		ECM	50	11.00	9	% colonization	Agricultural	0.69	−0.38	0.07
Pampolina *et al*. (2002)		ECM	1000	2.00	4	hyphal length	Agricultural	0.52	−0.66	0.20
Elevated CO_2										
Allen, MF (unpublished data)		AM	550	1.50	3	% colonization	Woodland/shrubland	2.26	0.82	0.04
Rillig *et al*. (1999a)	Serpentine	AM	700	4.00	10	% colonization	Temperate grassland	1.56	0.44	0.01
Rillig *et al*. (1999a)	Sandstone	AM	700	4.00	10	% colonization	Temperate grassland	1.73	0.55	0.03
Rillig *et al*. (2000)		AM	569	20.00	4	% colonization	Temperate grassland	3.45	1.24	0.12
Rillig *et al*. (2001)		AM	566	0.50	4	hyphal length	Agricultural	3.50	1.25	0.01
Rogers *et al*. (1992)		AM	550	0.12	3	% colonization	Agricultural	1.18	0.17	0.03
Runion *et al*. (1994)		AM	550	0.33	8	% colonization	Agricultural	1.03	0.03	0.00
Fransson *et al*. (2001)		ECM	700	3.00	3	% colonization	Boreal forest	0.93	−0.07	0.00
Kasurinen *et al*. (1999)		ECM	595	3.00	4	% colonization	Temperate forest	0.75	−0.28	0.06
Langley *et al*. (2003)		ECM	696	3.00	8	#colonized tips/ cm^{-1} root	Woodland/shrubland	1.21	0.19	0.01
Lukac *et al*. (2003)	*Populus alba*	ECM	550	3.00	3	% colonization	Agricultural	1.56	0.45	0.00
Lukac *et al*. (2003)	*Populus nigra*	ECM	550	3.00	3	% colonization	Agricultural	1.25	0.22	0.03
Lukac *et al*. (2003)	*Populus x euramericana*	ECM	550	3.00	3	% colonization	Agricultural	1.00	0.00	0.10
Rey *et al*. (1997)		ECM	700	4.50	6	% colonization	Temperate forest	1.72	0.54	0.05

[a]AM, arbuscular mycorrhizal; ECM, ectomycorrhizal.
[b]For N or P fertilization: kg ha^{-1} yr^{-1}. For elevated CO_2: ppm CO_2 in enriched treatment. Ambient was typically 350–370 ppm.
[c]Where replicate number was uneven between control and treatment, lower replicate number is reported.

of means, standard deviations and replicate numbers for controls and treatments (Hedges *et al.*, 1999). To determine if R deviated significantly from 1 across studies (i.e. nutrient additions had a significant general effect), a random effects model using MetaWin software was applied (Rosenberg *et al.*, 2000). Random effects models allow comparisons among groups in a framework similar to analysis of variance (ANOVA). In addition, significant variation in R among studies can be assessed. Responses between AM and ECM fungi were sequentially compared, among types of N or P fertilization applied (e.g. ammonium nitrate vs. ammonium sulphate), among methods of measurement, among biomes, and among lead investigators (i.e. first authors). Continuous model meta-analyses was also used to test for relationships between R and study length, levels of nutrient addition, total amounts of nutrients applied (in the case of N or P fertilization), the product of study length and CO_2 concentration in the enriched treatment (in the case of elevated CO_2), or numbers of replicate plots. Statistical results reported include R; 95% confidence intervals for R (CI); degrees of freedom (d.f.); total heterogeneity in R among studies (Q_T); and in the case of comparisons among groups, the difference among group cumulative effect sizes (Q_M), and the residual error (Q_E) (Rosenberg *et al.*, 2000).

Results

Nitrogen fertilization

Across studies, N fertilization reduced mycorrhizal abundance by an average of 15% (Fig. 1), but with significant variation among studies (Q_T = 100, d.f. = 30; $P < 0.00001$). Moreover, a meta-analysis restricted to percentage colonization data indicated a smaller, but still significant, decrease of 5.8% (Table 2), again with significant heterogeneity among studies (Q_T = 84.8, d.f. = 26, $P < 0.00001$). Aspects of experimental design influenced how mycorrhizal fungi responded to N. In particular, declines in mycorrhizal abundance were slightly more pronounced under higher rates of N application ($R = -2.54 \times 10^{-4} * [\text{rate}] + 0.905$, $P = 0.020$), although two studies with application rates of 1000 kg N ha^{-1} h^{-1} (Hutchinson *et al.*, 1998) had large leverage. When these two studies were omitted, no significant effects of application rate were apparent. Replicate number was weakly negatively related to R ($R = -0.0372 * [\text{replicate number}] + 0.964$, $P = 0.007$), potentially because studies with more replicate plots also had higher rates of N additions (Table 1). Neither the total amount of nitrogen added nor the duration of fertilization was a significant factor. Likewise, we found no significant effects of mycorrhizal type, fertilization type, measurement index, biome, or lead investigator (Table 2).

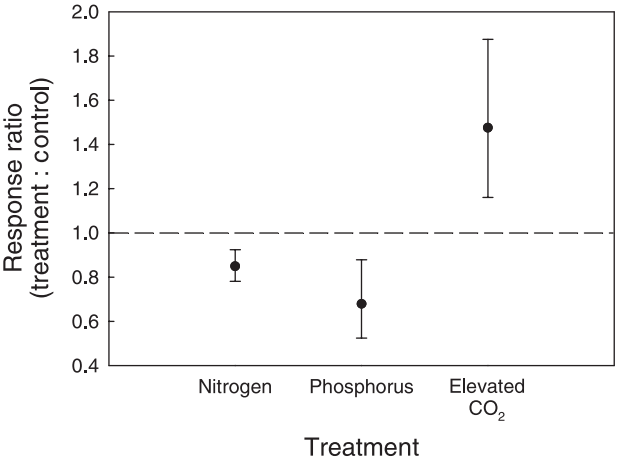

Fig. 1 Responses of mycorrhizal fungi to nitrogen fertilization, phosphorus fertilization, and elevated CO_2 in field studies. A response ratio > 1 indicates an increase in abundance relative to the control, and < 1 indicates a decrease. Symbols are means ± 95% confidence intervals. Responses were significant in each case, as confidence intervals did not overlap with 1. Thirty-one studies were represented for N, 20 for P, and 14 for elevated CO_2.

Phosphorus fertilization

Mycorrhizal fungi declined moderately under P fertilization, with an average reduction of 32% (Fig. 1). Moreover, variation among studies was nonsignificant (Q_T = 22.5, d.f. = 19, P = 0.259), indicating consistency among systems in mycorrhizal responses to P. Response ratios did not differ between AM and ECM fungi, among type of fertilizer applied, among biomes, among measurement types, or as a function of fertilization rate, fertilization duration, total amount of P added, or replicate number. However, R varied significantly among lead investigators (Table 2). When analysis was restricted to studies that reported percentage colonization, P effects were still significant (Table 2).

Elevated CO_2

By contrast to N and P fertilization, CO_2 enrichment consistently and strongly increased mycorrhizal growth, by an average of 47% across all studies (Fig. 1), and by 36% within studies that measured percentage colonization (R = 1.36, CI of 1.11–1.68, number of studies = 12). Among the study characteristics examined, none contributed significantly to differences among studies (Table 2), and there was no significant variation among studies in general (Q_T = 14.5, d.f. = 13, P = 0.342). We could not test for differences among measurement types, since percentage colonization was the only metric used by more than one study.

Discussion

For each nutrient examined, results from the meta-analyses supported the hypothesis that mycorrhizal fungi are more

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Table 2 Statistical results of comparisons among groups

Comparison	Group[a]	*R*	95% CI	Number of studies	Q_M	Q_E	*P*-value
Nitrogen fertilization							
Mycorrhizal type	AM fungi	0.761	0.675–0.858	17	8.03	99.1	0.081
	ECM fungi	0.947	0.845–1.06	14			
Fertilizer type	$NaNO_3$	1.08	0.598–1.94	3	5.18	83.5	0.492
	NH_4NO_3	0.858	0.779–0.944	18			
	$(NH_4)_2SO_3$	0.711	0.506–0.999	5			
	NH_4NO_3 + urea	0.808	0.325–2.01	3			
Measurement	% colonization	0.942	0.890–0.997	27	6.56	85.8	0.091
	Hyphal length	0.577	0.256–1.30	3			
Biome	Temperate grassland	0.897	0.562–1.43	3	37.3	84.2	0.094
	Woodland/shrubland	0.402	0.071–2.27	2			
	Agricultural	0.777	0.593–1.02	6			
	Temperate forest	0.932	0.834–1.04	13			
	Tropical forest	0.807	0.333–1.96	3			
	Boreal forest	0.972	0.694–1.36	3			
Lead authors	Hutchinson	0.540	0.082–3.54	2	24.4	50.8	0.096
	Johnson	0.577	0.259–1.29	3			
	Lansing	0.984	0.916–1.06	8			
	Treseder	0.805	0.348–1.87	3			
	Baum	0.849	0.482–1.50	3			
	Termorshuizen	0.938	0.674–1.31	4			
Phosphorus fertilization							
Mycorrhizal type	AM fungi	0.687	0.523–0.902	18	0.078	21.9	0.789
	ECM fungi	0.611	0.004–94.4	2			
Fertilizer type	Superphosphate	0.694	0.520–0.926	16	2.78	17.6	0.185
	$Ca(H_2PO_4)_2$	0.134	0.000–32 180	2			
Measurement	% colonization	0.707	0.544–0.920	17	2.92	19.7	0.151
	Spore count	0.135	0.000–27 677	2			
Biome	Temperate grassland	0.819	0.252–2.67	3	3.94	17.0	0.236
	Agricultural	0.735	0.521–1.04	13			
	Tropical forest	0.347	0.070–1.73	3			
Lead investigator	Kahiluoto	0.647	0.009–47.1	2	23.9	2.13	0.017
	Martensson	0.135	0.000–8238	2			
	Sanginga	1.45	0.094–22.3	2			
	Treseder	0.331	0.112–0.977	3			
Elevated CO_2							
Mycorrhizal type	AM fungi	1.84	1.22–2.77	7	3.39	9.29	0.108
	ECM fungi	1.19	0.785–1.79	7			
Biome	Woodland/shrubland	1.62	0.025–104	2	1.58	8.66	0.701
	Temperate grassland	1.98	0.592–6.60	3			
	Agricultural	1.48	0.907–2.40	6			
	Temperate forest	1.15	0.014–96.4	2			
Lead investigator	Rillig	2.31	1.19–4.48	4	3.03	3.57	0.178
	Lukac	1.32	0.464–3.75	3			

[a]Groups are included only when represented by two or more studies.

abundant where plants are more limited by soil nutrients. However, responses to N were less consistent than were responses to P and elevated CO_2, given the heterogeneity in N effects among studies. Replicate numbers within N studies influenced response ratios, but not substantially. What other characteristics of the studies might be responsible for the remaining variation in N effects? It is possible that mycorrhizal fungi may not be as effective in facilitating plant uptake of inorganic N compared with inorganic P (Mosse & Phillips, 1971; Smith & Read, 1997). In particular, nitrate is more mobile in the soil than is phosphate, so diffusion or mass flow may supply N at adequate rates in nitrate-rich systems. Under these circumstances, plant investment in mycorrhizal fungi may be minimal even in control plots. Alternately, mycorrhizal growth may be N-limited in some ecosystems (Treseder & Allen, 2002) so that N fertilization increases mycorrhizal abundance. Nitrogen effects were positive in 23% of studies (Table 1). Regardless of the mechanism, the significant variation

in N responses among studies indicates that predictability of N deposition effects on mycorrhizal biomass for any given ecosystem is relatively low. The smaller confidence intervals for N effects vs P or CO_2 effects (Fig. 1) reflect the larger number of N studies included in the meta-analyses.

Although most study variables did not significantly influence mycorrhizal responses to P fertilization, in many cases the number of studies represented within groups was low (Table 2). For example, ECM responses to P were determined in two studies only. Likewise, all but two studies applied superphosphate as the source of P. Hyphal length was used as an index of abundance in two P studies, compared with 17 P studies reporting percentage colonization. Tropical forests and temperate grasslands were represented by three P studies each. These small sample sizes limit my ability to determine whether these variables are important factors in mycorrhizal responses to P.

Seven biomes were included in the meta-analyses, albeit unequally. Agricultural systems were the most common, comprising 25 of 65 cases (Table 1). Deserts were the least common, with one study represented. Moreover, all data from natural tropical forests were collected in Hawaii. A more diverse sampling of nutrient effects within tropical forests, deserts, boreal forests, and woodlands/shrublands would improve the possibility of establishing general patterns among and within biomes.

To include studies that encompassed as broad a range of regions and biomes as possible, data on hyphal lengths and spore counts was incorporated, in addition to % colonization. However, % colonization is not necessarily comparable with the others, since this parameter is a function of standing root length as well as mycorrhizal biomass (Allen, 2001). Colonization levels can be interpreted as an assessment of relative allocation toward mycorrhizal fungi by plants. For this reason, additional meta-analyses were conducted on % colonization data only. Effects of N, P, and CO_2 remained significant – but smaller – despite the reduction in sample size. Standing stocks of fine roots tend to increase under elevated CO_2 (Rogers *et al.*, 1994). Thus, total mycorrhizal biomass may be more strongly affected by CO_2 enrichment than would be indicted by % colonization alone. Root responses to N and P are more variable (Ostertag, 2001), so it is difficult to relate % colonization to mycorrhizal biomass in fertilization studies without specific data from each study. Even though percentage colonization tended to be associated with smaller response ratios, there was no evidence for significant differences among types of measurements used.

In summary, mycorrhizal abundance generally increases under elevated CO_2 and declines in response to N and P fertilization across studies. Plants may adjust allocation of C to mycorrhizal fungi according to the degree to which plant growth is N or P limited, as hypothesized (Mosse & Phillips, 1971; Read, 1991). Direct limitation of mycorrhizal fungi by soil nutrients appears to be at most a secondary control, evident in a subset of studies. In respect of environmental change, global standing stocks of mycorrhizal fungi may be substantially augmented by atmospheric CO_2 enrichment and moderately reduced by P fertilization. Anthropogenic N deposition effects might vary among ecosystems, with a slightly negative influence overall. These shifts in mycorrhizal dynamics may elicit corresponding shifts in ecosystem dynamics, including nutrient uptake by plants (Smith & Read, 1997), trace gas emissions (Redeker *et al.*, 2004), carbon sequestration in glomalin (Treseder & Allen, 2000), and aggregate formation in the soil (Rillig *et al.*, 1999b).

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