

A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO₂ in field studies

Kathleen K. Treseder

Department of Ecology and Evolutionary Biology and Department of Earth System Science, University of California, Irvine, CA 92697, USA

Summary

Author for correspondence:

Kathleen K. Treseder

Tel: +949-824-7634

Fax: +949-824-2181

Email: Treseder@uci.edu

Received: 28 February 2004

Accepted: 8 May 2004

- Numerous field studies have measured mycorrhizal dynamics under additions of nitrogen (N), phosphorus (P), or atmospheric CO₂ 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 CO₂ 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 CO₂ 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.

© *New Phytologist* (2004) doi: 10.1111/j.1469-8137.2004.01159.x

New Phytologist (2004)

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 CO₂ 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 CO₂ but decrease under additions of N and P? By contrast to glasshouse studies, field-based manipulations of CO₂, 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 CO₂ (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 CO₂ studies. It focused on below-ground changes in standing crops of the fungi. Separate meta-analyses were conducted for N, P, and CO₂.

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-m²) manipulations of N, P, or CO₂ 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 CO₂ experiments, this study included free-air CO₂ 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 CO₂ 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 CO₂), 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 ($V_{In,R}$)

Study	Identifier	Mycorrhizal type ^a	Additions ^b	Study length (yr)	Repli-cates ^c	Unit of measure	Biome	R	In R	$V_{In,R}$
<i>Nitrogen fertilization</i>										
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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (1998)	Dorset	AM	1000	2.00	8	% colonization	Temperate forest	1.11	0.11	0.04
Hutchinson <i>et al.</i> (1998)	Loring	AM	1000	3.00	8	% colonization	Temperate forest	0.30	-1.20	0.03
Johnson <i>et al.</i> (2003)	Kellogg	AM	120	9.00	5	hyphal length	Agricultural	0.57	-0.56	0.07
Johnson <i>et al.</i> (2003)	Cedar Creek	AM	170	10.00	5	hyphal length	Agricultural	0.51	-0.68	0.13
Johnson <i>et al.</i> (2003)	Sevilleita	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 <i>et al.</i> (2002)	Abbachhof	ECM	100	9.00	9	% colonization	Agricultural	0.35	-1.05	0.06
Baum <i>et al.</i> (2002)	Wildeshausen	ECM	100	4.00	9	% colonization	Agricultural	1.73	0.55	0.05
Fransson <i>et al.</i> (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)	Liesel NH ₄	ECM	60	3.00	3	% colonization	Temperate forest	1.00	0.00	0.02
Termorshuizen (1993)	Liesel NO ₃	ECM	60	3.00	3	% colonization	Temperate forest	0.99	-0.01	0.02
<i>Phosphorus fertilization</i>										
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 <i>et al.</i> (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 <i>et al.</i> (2001)	Maaninka	AM	45	20.00	6	% colonization	Agricultural	0.66	-0.42	0.24
Kahiluoto <i>et al.</i> (2001)	Mietoinen	AM	45	20.00	4	% colonization	Agricultural	0.64	-0.45	0.20
Martensson & Carligen (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)	Repli- cates ^c	Unit of measure	Biome	R	In R	V _{in,R}
Martensson & Carligen (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
Sangana <i>et al.</i> (1996)	Degraded	AM	7	0.27	8	% colonization	Agricultural	1.33	0.29	0.10
Sangana <i>et al.</i> (1996)	Compound	AM	7	0.27	8	% colonization	Agricultural	1.62	0.48	0.09
Thomson <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (2002)		ECM	1000	2.00	4	hyphal length	Agricultural	0.52	-0.66	0.20
<i>Elevated CO₂</i>										
Allen, MF (unpublished data)		AM	550	1.50	3	% colonization	Woodland/shrubland	2.26	0.82	0.04
Rillig <i>et al.</i> (1999a)	Serpentine	AM	700	4.00	10	% colonization	Temperate grassland	1.56	0.44	0.01
Rillig <i>et al.</i> (1999a)	Sandstone	AM	700	4.00	10	% colonization	Temperate grassland	1.73	0.55	0.03
Rillig <i>et al.</i> (2000)		AM	569	20.00	4	% colonization	Temperate grassland	3.45	1.24	0.12
Rillig <i>et al.</i> (2001)		AM	566	0.50	4	hyphal length	Agricultural	3.50	1.25	0.01
Rogers <i>et al.</i> (1992)		AM	550	0.12	3	% colonization	Agricultural	1.18	0.17	0.03
Runion <i>et al.</i> (1994)		AM	550	0.33	8	% colonization	Agricultural	1.03	0.03	0.00
Fransson <i>et al.</i> (2001)		ECM	700	3.00	3	% colonization	Boreal forest	0.93	-0.07	0.00
Kasurinen <i>et al.</i> (1999)		ECM	595	3.00	4	% colonization	Temperate forest	0.75	-0.28	0.06
Langley <i>et al.</i> (2003)		ECM	696	3.00	8	#colonized tips/ cm ⁻¹ root	Woodland/shrubland	1.21	0.19	0.01
Lukac <i>et al.</i> (2003)	<i>Populus alba</i>	ECM	550	3.00	3	% colonization	Agricultural	1.56	0.45	0.00
Lukac <i>et al.</i> (2003)	<i>Populus nigra</i>	ECM	550	3.00	3	% colonization	Agricultural	1.25	0.22	0.03
Lukac <i>et al.</i> (2003)	<i>Populus x</i> <i>euramericana</i>	ECM	550	3.00	3	% colonization	Agricultural	1.00	0.00	0.10
Rey <i>et al.</i> (1997)		ECM	700	4.50	6	% colonization	Temperate forest	1.72	0.54	0.05

^aAM, arbuscular mycorrhizal; ECM, ectomycorrhizal.^bFor N or P fertilization: kg ha⁻¹ yr⁻¹. For elevated CO₂: ppm CO₂ in enriched treatment. Ambient was typically 350–370 ppm.^cWhere 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

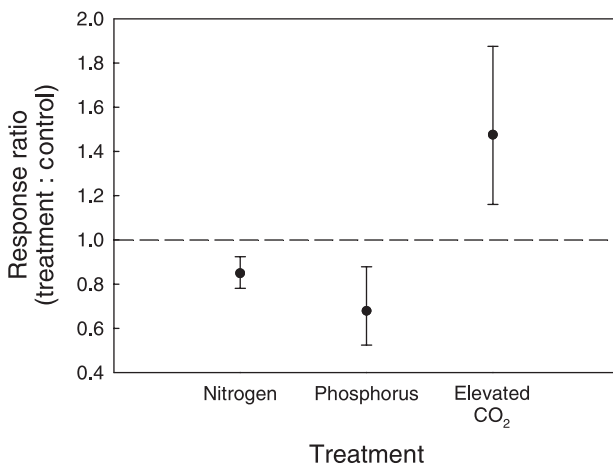


Fig. 1 Responses of mycorrhizal fungi to nitrogen fertilization, phosphorus fertilization, and elevated CO_2 in field studies. A response ratio > 1 indicates an increase in abundance relative to the control, and < 1 indicates a decrease. Symbols are means \pm 95% confidence intervals. Responses were 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 .

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 $1000 \text{ kg N ha}^{-1} \text{ h}^{-1}$ (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).

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

Table 2 Statistical results of comparisons among groups

Comparison	Group ^a	R	95% CI	Number of studies	Q _M	Q _E	P-value
<i>Nitrogen fertilization</i>							
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 ₄ NO ₃	0.858	0.779–0.944	18			
	(NH ₄) ₂ SO ₃	0.711	0.506–0.999	5			
	NH ₄ NO 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			
<i>Phosphorus fertilization</i>							
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 ₂ PO 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			
<i>Elevated CO₂</i>							
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			

^aGroups 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 CO₂, 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 CO₂ 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 CO₂ remained significant – but smaller – despite the reduction in sample size. Standing stocks of fine roots tend to increase under elevated CO₂ (Rogers *et al.*, 1994). Thus, total mycorrhizal biomass may be more strongly affected by CO₂ enrichment than would be indicated 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 CO₂ 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 CO₂ 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).

Acknowledgements

Thanks to A Cross, L Methratta, and K Turner for technical assistance and discussion, D Pellet, J Lansing, M Allen, and M Gavito for providing access to data, and all other authors whose studies were included in the analyses. This work was funded by grants from the Mellon Foundation and NSF (DEB-0107776 and DEB-0122445).

References

- Allen MF. 2001. Modeling arbuscular mycorrhizal infection: is % infection an appropriate variable? *Mycorrhiza* 10: 255–258.
- Anderson RC, Liberta AE. 1992. Influence of supplemental inorganic nutrients on growth, survivorship, and mycorrhizal relationships of *Shizachyrium scoparium* (Poaceae) grown in fumigated and unfumigated soil. *American Journal of Botany* 79: 406–414.
- Arnqvist G, Wooster D. 1995. Meta-analysis: synthesizing research findings in ecology and evolution. *Trends in Ecology and Evolution* 10: 236–240.
- Baum C, Makeschin F. 2000. Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* × *tremuloides*). *Journal of Plant Nutrition and Soil Science* 163: 491–497.
- Baum C, Weih M, Verwijst T, Makeschin F. 2002. The effects of nitrogen fertilization and soil properties on mycorrhizal formation of *Salix viminalis*. *Forest Ecology and Management* 160: 35–43.
- Bazzaz FA. 1990. The response of natural ecosystems to the rising global CO₂ levels. *Annual Review of Ecology and Systematics* 21: 167–196.
- Bentivenga SP, Hetrick BAD. 1992. The effect of prairie management practices on mycorrhizal symbiosis. *Mycologia* 84: 522–527.
- Cornwell WK, Bedford BL, Chapin CT. 2001. Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. *American Journal of Botany* 88: 1824–1829.
- Egerton-Warburton LM, Allen EB. 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications* 10: 484–496.
- Ellis JR, Roder W, Mason SC. 1992. Grain sorghum soybean rotation and fertilization influence on vesicular-arbuscular mycorrhizal fungi. *Soil Science Society of America Journal* 56: 789–794.
- Finzi AC, DeLucia EH, Hamilton JG, Richter DD, Schlesinger WH. 2002. The nitrogen budget of a pine forest under free air CO₂ enrichment. *Oecologia* 132: 567–578.
- Fransson PMA, Taylor AFS, Finlay RD. 2001. Elevated atmospheric CO₂ alters root symbiont community structure in forest trees. *New Phytologist* 152: 431–442.

- Gavito ME, Miller MH. 1998. Changes in mycorrhiza development in maize induced by crop management practices. *Plant and Soil* 198: 185–192.
- Grogan P, Chapin FS. 2000. Nitrogen limitation of production in a Californian annual grassland: The contribution of arbuscular mycorrhizae. *Biogeochemistry* 49: 37–51.
- Gurevitch J, Hedges LV. 1999. Statistical issues in ecological meta-analyses. *Ecology* 80: 1142–1149.
- Gurevitch J, Hedges. 1993. Meta-analysis: Combining the results of independent experiments. In: Scheiner SM, Gurevitch J, eds. *Design and analysis of ecological experiments*. New York, NY, USA: Chapman & Hall.
- Gurevitch J, Morrow LL, Wallace A, Walsh JS. 1992. A meta-analysis of competition in field experiments. *American Naturalist* 140: 539–572.
- Hagerberg D, Thelin G, Wallander H. 2003. The production of ectomycorrhizal mycelium in forests: Relation between forest nutrient status and local mineral sources. *Plant and Soil* 252: 279–290.
- Hedges IV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80: 1150–1156.
- Hicks PM, Loynachan TE. 1987. Phosphorus fertilization reduces vesicular arbuscular mycorrhizal infection and changes nodule occupancy of field grown soybean. *Agronomy Journal* 79: 841–844.
- Hutchinson TC, Watmough SA, Sager EPS, Karagatzides JD. 1998. Effects of excess nitrogen deposition and soil acidification on sugar maple (*Acer saccharum*) in Ontario, Canada: an experimental study. *Canadian Journal of Forest Research* 28: 299–310.
- Jackson RB, Manwaring JH, Caldwell MM. 1990. Rapid physiological adjustment of roots to localized soil enrichment. *Nature* 344: 58–60.
- Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton LM, Allen EB. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology* 84: 1895–1908.
- Kahiluoto H, Ketoja E, Vestberg M, Saarela I. 2001. Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant and Soil* 231: 65–79.
- Karen O, Nylund JE. 1997. Effects of ammonium sulfate on the community structure and biomass of ectomycorrhizal fungi in a Norway spruce stand in southwestern Sweden. *Canadian Journal of Botany* 75: 1628–1642.
- Kasurinen A, Helmisaari HS, Holopainen T. 1999. The influence of elevated CO₂ and O₃ on fine roots and mycorrhizas of naturally growing young Scots pine trees during three exposure years. *Global Change Biology* 5: 771–780.
- Langley JA, Dijkstra P, Drake BG, Hungate BA. 2003. Ectomycorrhizal colonization, biomass, and production in a regenerating scrub oak forest in response to elevated CO₂. *Ecosystems* 6: 424–430.
- Lansing JL. 2003. *Comparing arbuscular and ectomycorrhizal fungal communities in seven North American forests and their response to nitrogen fertilization*. PhD Thesis. University of California, Davis, CA, USA.
- Lukac M, Calfapietra C, Godbold DL. 2003. Production, turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated CO₂ (POPFACE). *Global Change Biology* 9: 838–848.
- Martensson AM, Carlgren K. 1994. Impact of phosphorus fertilization on VAM diaspores in two Swedish long-term field experiments. *Agriculture Ecosystems and Environment* 47: 327–334.
- Mosse B, Phillips JM. 1971. The influence of phosphate and other nutrients on the development of vesicular-arbuscular mycorrhiza in culture. *Journal of General Microbiology* 1971: 157–166.
- Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schafer KVR, McCarthy H, Hendrey G, McNulty SG, Katul GG. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature* 411: 469–472.
- Ostertag R. 2001. Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. *Ecology* 82: 485–499.
- Pampolina NM, Dell B, Malajczuk N. 2002. Dynamics of ectomycorrhizal fungi in an *Eucalyptus globulus* plantation: effect of phosphorus fertilization. *Forest Ecology and Management* 158: 291–304.
- Pellet D, El-Sharkawy MA. 1993. Cassava varietal response to phosphorus fertilization. 2. Phosphorus uptake and use efficiency. *Field Crops Research* 35: 13–20.
- Poorter H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetatio* 104: 77–97.
- Preble E. 1998. *Grab It!* In. Raleigh, NC, USA: DataTrend Software, Inc.
- Read DJ. 1991. Mycorrhizas in ecosystems – Nature's response to the 'Law of the minimum' In: Hawksworth DL, eds. *Frontiers in mycology*. Regensburg, Germany: CAB International, 101–130.
- Redeker KR, Treseder KK, Allen MF. 2004. Ectomycorrhizal fungi: A new source of atmospheric methyl halides? *Global Change Biology* (In press.)
- Rey A, Barton CVM, Jarvis PG. 1997. Belowground responses to increased atmospheric CO₂ concentrations in birch (*Betula pendula* Roth.). In: Mohren GMJ, Kramer K, Sabate S, eds. *Impacts of global change on tree physiology and forest ecosystems. Proceedings of the international conference on impacts of global change on tree physiology and forest ecosystems, held 26–29 November 1996, Wageningen, The Netherlands*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 207–212.
- Rillig MC, Field CB, Allen MF. 1999a. Soil biota responses to long-term atmospheric CO₂ enrichment in two California annual grasslands. *Oecologia (Berlin)* 119: 572–577.
- Rillig MC, Hernandez GY, Newton PCD. 2000. Arbuscular mycorrhizae respond to elevated atmospheric CO₂ after long-term exposure: evidence from a CO₂ spring in New Zealand supports the resource balance model. *Ecology Letters* 3: 475–478.
- Rillig MC, Wright SF, Allen MF, Field CB. 1999b. Long-term CO₂ elevation affects soil structure of natural ecosystems. *Nature* 400: 628.
- Rillig MC, Wright SF, Kimball BA, Pinter PJ, Wall GW, Ottman MJ, Leavitt SW. 2001. Elevated carbon dioxide and irrigation effects on water stable aggregates in a Sorghum field: a possible role for arbuscular mycorrhizal fungi. *Global Change Biology* 7: 333–337.
- Rogers HH, Prior SA, O'Neill EG. 1992. Cotton roots and rhizosphere responses to free-air CO₂ enrichment. *Critical Reviews of Plant Science* 11: 251–263.
- Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* 83: 155–189.
- Rosenberg MS, Adams DC, Gurevitch J. 2000. MetaWin: Statistical software for meta-analysis. Sunderland, MA, USA: Sinauer Associates.
- Runion GB, Curl EA, Rogers HH, Backman PA, Rodriguezkabana R, Helms BE. 1994. Effects of free-air CO₂ enrichment on microbial populations in the rhizosphere and phyllosphere of cotton. *Agricultural and Forest Meteorology* 70: 117–130.
- Sanginga N, Okogun JA, Akobundu IO, Kang BT. 1996. Phosphorus requirement and nodulation of herbaceous and shrub legumes in low P soils of a Guinean savanna in Nigeria. *Applied Soil Ecology* 3: 247–255.
- Schlesinger WH, Lichten J. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature* 411: 466–469.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*, 2nd edn. San Diego, CA, USA: Academic Press.
- Staddon PL, Fitter AH. 1998. Does elevated atmospheric carbon dioxide affect arbuscular mycorrhizas? *Trends in Ecology and Evolution* 13: 455–458.
- Termorshuizen AJ. 1993. The influence of nitrogen fertilizers on ectomycorrhizas and their fungal carpophores in young stands of *Pinus sylvestris*. *Forest Ecology and Management* 57: 179–189.
- Thomson BD, Robson AD, Abbott LK. 1992. The effect of long term applications of phosphorus fertilizer on populations of vesicular arbuscular

- mycorrhizal fungi in pastures. *Australian Journal of Agricultural Research* **43**: 1131–1142.
- Treseder KK, Allen MF. 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO₂ and nitrogen deposition. *New Phytologist* **147**: 189–200.
- Treseder KK, Allen MF. 2002. Direct N and P limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist* **155**: 507–515.
- Treseder KK, Vitousek PM. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* **82**: 946–954.
- Vanlauwe B, Nwoke OC, Diels J, Sanginga N, Carsky RJ, Deckers J, Merckx R. 2000. Utilization of rock phosphate by crops on a representative toposequence in the Northern Guinea savanna zone of Nigeria: response by *Mucuna pruriens*, *Lablab purpureus* and maize. *Soil Biology and Biochemistry* **32**: 2063–2077.
- Vitousek PM. 1994. Beyond global warming: Ecology and global change. *Ecology* **75**: 1861–1876.