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Research

Mycorrhizal phenotypes and the Law of the Minimum

Nancy Collins Johnson[1], Gail W. T. Wilson[2], Jacqueline A. Wilson[2], R. Michael Miller[3] and Matthew A. Bowker[4]

[1]School of Earth Sciences and Environmental Sustainability and Department of Biological Sciences, Northern Arizona University Flagstaff, Flagstaff, AZ 86011, USA; [2]Natural Resource Ecology & Management, Oklahoma State University, Stillwater, OK 74077, USA; [3]Biosciences Division, Argonne National Laboratory, Argonne, IL 60439, USA; [4]School of Forestry, Northern Arizona University, Flagstaff, AZ 86011, USA

Author for correspondence:
Nancy Collins Johnson
Tel: +1 928 523 6473
Email: nancy.johnson@nau.edu

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Summary

• Mycorrhizal phenotypes arise from interactions among plant and fungal genotypes and the environment. Differences in the stoichiometry and uptake capacity of fungi and plants make arbuscular mycorrhizal (AM) fungi inherently more nitrogen (N) limited and less phosphorus (P) limited than their host plants. Mutualistic phenotypes are most likely in P-limited systems and commensal or parasitic phenotypes in N-limited systems. Carbon (C) limitation is expected to cause phenotypes to shift from mutualism to commensalism and even parasitism.

• Two experiments compared the influence of fertilizer and shade on mycorrhizas in *Andropogon gerardii* across three naturally N-limited or P-limited grasslands. A third experiment examined the interactive effects of N and P enrichment and shade on *A. gerardii* mycorrhizas.

• Our experiments generated the full spectrum of mycorrhizal phenotypes. These findings support the hypothesis that mutualism is likely in P-limited systems and commensalism or parasitism is likely in N-limited systems. Furthermore, shade decreased C-assimilation and generated less mutualistic mycorrhizal phenotypes with reduced plant and fungal biomass.

• Soil fertility is a key controller of mycorrhizal costs and benefits and the Law of the Minimum is a useful predictor of mycorrhizal phenotype. In our experimental grasslands arbuscular mycorrhizas can ameliorate P-limitation but not N-limitation.

Introduction

Although mycorrhizas are generally mutually beneficial for both plants and fungi, many factors influence the symbiotic outcome of arbuscular mycorrhizal (AM) associations (Hoeksema *et al.*, 2010). Under certain conditions AM fungi have been shown to depress plant growth (Modjo & Hendrix, 1986; Peng *et al.*, 1993); consequently their function forms a continuum from mutualism to parasitism. The location of a particular AM symbiosis on this continuum can be considered its phenotype: an emergent property of interactions among plant and fungal genotypes and the environment (Johnson *et al.*, 1997). The term mycorrhizal phenotype refers to the fitness outcome for both partners in the symbiosis. In AMs, Glomeromycota are obligate biotrophs so there is never a negative response by the fungus; consequently, in the context of AM symbioses, the terms mutualism (+, +), commensalism (+, 0) and parasitism (+, −) refer to a positive fitness outcome for fungi and a positive, neutral or negative outcome for the host plants. Genotypic traits related to resource trade and control of the symbiosis in the context of local environmental conditions are important determinants of mycorrhizal phenotypes (Johnson *et al.*, 1997, 2010).

The availability of soil-derived nutrients has long been recognized as one of the more important environmental factors controlling mycorrhizal phenotype (Mosse, 1973). The extensive mycelium of AM fungi can better access immobile soil phosphorus (P) than plant roots; and AM symbioses often facilitate plant growth in P-limited soils (Koide, 1991). Despite unequivocal evidence that AM fungi can acquire and transfer substantial amounts of nitrogen (N) to their host plants (e.g. George *et al.*, 1995; Leigh *et al.*, 2008; Fellbaum *et al.*, 2012), reports are contradictory regarding the net effects of AM symbioses on plant growth in N-deficient soil. Some studies show that AM fungi increase N uptake and biomass gain of their plant hosts (e.g. Tu *et al.*, 2006) whereas others show that AM symbioses have no benefit for ameliorating N limitation (e.g. Reynolds *et al.*, 2005). These studies represent an enigma, if AM fungi can increase plant N uptake, then why is there not consistent evidence that AM fungi improve the net production of plants grown in N-deficient soil? We suggest that the answer is that the relative availability of soil N and P determines whether or not mycorrhizal benefits outweigh their costs. This environmental variable (i.e. soil nutrient supply) combined with the genetically determined capabilities of plants and fungi to trade carbon (C) for P are the key determinants of mycorrhizal phenotype.

The economics of trading partnerships provides a useful framework for understanding resource exchange among mycorrhizal fungi and their hosts (Koide & Elliott, 1989; de Mazancourt & Schwartz, 2010). Photosynthate and minerals are the commodities exchanged in mycorrhizal symbioses and the trade

value of these resources is set by their availability in the environment. The 'Law of the Minimum' states that plant production may be controlled by a single essential resource that is in limiting supply (von Liebig, 1843; van der Ploeg *et al.*, 1999). Identifying which resources are in limiting supply in the environment and determining whether mycorrhizal symbioses can enhance access to these resources provides an ecologically and evolutionarily sound approach to predicting mycorrhizal function in ecosystems (Read, 1991).

Photosynthesis provides the C currency for symbiotic exchange. As such, any condition that limits the photosynthetic capacity of a host, such as low light levels as a result of shading, may limit the amount of C available to provision fungal partners (Graham *et al.*, 1982; Pearson *et al.*, 1991). Mycorrhizal acquisition and trade of C, N and P are interconnected because the capacity of AM fungi to supply P and N to their host plants depends on the synthesis of extensive networks of external hyphae. The mycelium of AM fungi may constitute sizable C and N sinks; once sequestered into the hyphal wall matrix these resources become unavailable for plant use (Robinson & Fitter, 1999). The concentration of N in the external hyphae of AM fungi is 4–7 times greater than that of plant shoots and at least 10 times greater than that of roots (Hodge & Fitter, 2010). Consequently, AM fungi must acquire large amounts of N from the soil for their own growth, and in N-limited systems they are unlikely to transport biologically meaningful amounts of N to their host plants (*ibid*). Furthermore, the disparity in C : N ratios of plants and fungi suggest that N limitation is likely to inhibit fungal growth more severely than plant growth (Kaye & Hart, 1997; Johnson, 2010). In addition, mass flow and diffusion processes are usually enough to bring mineral-N to the root surface. This is especially so for soils where NO_3-N is the dominant form of mineral-N. These same processes are not enough to prevent the formation of a depletion zone around roots for phosphate ions primarily related to the low solubility of PO_4-P in soil solution. The growth of external AM hyphae into this depletion zone allows for mycorrhizal plants to have access to soil-P that otherwise would not be available (Miller, 1987).

There is evidence that N and P availability play different roles in the determination of mycorrhizal phenotype. Exchange of plant-derived C for fungal-derived P has been shown to enforce mycorrhizal cooperation (Hammer *et al.*, 2011; Kiers *et al.*, 2011) and mycorrhizal trade is clearly linked to biomass gain of plants and AM fungi growing in P-limited soils (Mosse, 1973). Recent evidence from root organ cultures shows that C flux from host plant to fungus may regulate N transport (Fellbaum *et al.*, 2012); however, the outcome of this for plants and fungal biomass gain has not been demonstrated. Studies using whole plants indicate that tightly linked C-for-N exchange between plants and fungi in N-limited systems does not generate bilateral control of mycorrhizal cooperation in the same manner that C-for-P exchange enforces cooperation and controls plant and fungal biomass gain in P-limited systems (Smith & Smith, 2011; Corrêa *et al.*, 2014). Instead, availability of N may influence mycorrhizas indirectly through its effects on both C supply and C demand. Ribulose bisphosphate carboxylase, the rate-limiting enzyme in photosynthesis, accounts for as much as 75% of leaf N (Chapin *et al.*, 1987); consequently, N nutrition controls the photosynthetic capacity of plants and, in turn, the amount of C that can be traded with mycorrhizal fungi. Also, the demand for photosynthate by AM fungi is influenced by N availability because fungal tissues are rich in amino acids and polymeric N including chitin and associated glycoproteins (Blackwell, 1988). In other words, soil N availability influences both the C source strength of the plant (increased at high N) and the C sink strength of the fungus (decreased at low N).

Mutually beneficial mycorrhizal phenotypes are likely to be found in P-limited systems because AM fungi can effectively trade surplus P for plant photosynthate. By contrast, mutually beneficial mycorrhizal phenotypes are not expected in N-limited systems because AM fungi are unlikely to have surplus N for trade because they have higher N requirements than their host plants. Both fertilization and shade change the C : N : P stoichiometry, and consequently, the relative costs and benefits of mycorrhizal symbioses. If the mechanism controlling mycorrhizal function is simply the balance between costs and benefits of the symbioses, then C limitation caused by insufficient light is expected to change the threshold at which C costs exceed P benefits and generate commensalism or even parasitism.

We tested these hypotheses with three experiments that examine mycorrhizal responses to fertilization and shade using big bluestem (*Andropogon gerardii*), a highly mycotrophic C_4 grass from Cedar Creek, Fermi and Konza, three North American grasslands with very different soil properties. Previous studies indicate that big bluestem genotypes and their indigenous communities of AM fungi are co-adapted to each other and their local soil environments (Johnson *et al.*, 2010; Ji *et al.*, 2013). Consequently, all three experiments maintained co-adapted systems of plant genotypes, soils,- and fungi by growing big bluestem in its local soil and naturally co-occurring AM fungi. Expt I examined mycorrhizal responses to N and P enrichment at the three sites, Expt II examined mycorrhizal response to shading at the three sites, and Expt III examined the interactive effects of nutrient enrichment and shading at a single site (Konza). Soils from Konza and Fermi are relatively more P-limited than N-limited, whereas soil from Cedar Creek is P-rich and N-limited (Johnson *et al.*, 2010). Thus, the Law of the Minimum predicts mutualistic mycorrhizas at Konza and Fermi and commensal mycorrhizas at Cedar Creek. If these predictions are supported, then the Law of the Minimum may be a useful guide to manage mycorrhizas to increase crop yield in agriculture, restore ecosystem functions, and manipulate C-sequestration in mycorrhizal hyphae.

Materials and Methods

Design of Expt I: cross-site nutrient enrichment experiment

Rhizomes of local ecotypes of big bluestem (*Andropogon gerardii* Vitman) were collected from Konza Prairie Biological Station (Konza), Fermi Natural Environmental Research Park (Fermi), and Cedar Creek Ecosystem Science Reserve (Cedar Creek) in February 2005 when the grasses were dormant. Rhizomes were

gently washed to remove soil, roots were trimmed to *c.* 5 cm and rhizomes were stored in cold, moist sand. In the spring, all roots were removed from each rhizome. Rhizome lengths were measured and 48 rhizomes of similar size from each site were selected and planted in their corresponding soil. Rhizome size varied among sites, rhizomes from Konza (mean size 16.6 mm × 1.4 mm) were significantly smaller than those from Cedar Creek (mean size 26.3 mm × 1.6 mm) and Fermi (mean size 31.9 mm × 1.6 mm). Soil was collected from each site, steam-pasteurized at 80°C for 2 h, and used to fill 48 plastic pots (11 cm wide × 14 cm tall) with 1 kg soil in each pot. Half of the pots from each site were maintained as nonmycorrhizal (NM) treatments (pasteurized soil) and the remaining half were inoculated with communities of AM fungi and other soil organisms by placing 20 g of living soil from their corresponding site directly below each rhizome during planting. All pots were amended with 50 ml of nonsterile soil sievate from the corresponding site prepared by blending soil : water in a 1 : 2 ratio and passing the slurry through a 25-μm sieve (Johnson *et al.*, 2010).

Four fertilizer treatments were established in each of the three experimental systems and each treatment was replicated six times with or without AM fungi for a total of 144 pots. At Konza and Fermi the treatments were: N enrichment, P enrichment, both N and P, and no fertilizer. Phosphorus (200 ml of 50 $\mu g\, g^{-1}$ P_2O_5: 5 $\mu g\, g^{-1}$ P) was applied once at the beginning of the experiment. Nitrogen (200 ml of 6.3 $\mu g\, g^{-1}$ NH_4NO_3: 2.3 $\mu g\, g^{-1}$ N) was applied at the beginning of the experiment and once every week for 14 wk. Previous experiments have shown that P enrichment does not influence AM structure or function in P-rich Cedar Creek soil (Johnson, 1993), so in that system, we studied an N gradient instead of an N and P factorial design. Cedar Creek soil was supplemented with 200 ml of one of four NH_4NO_3 treatments: 0N, nonamended control; 1N, 2.3 $\mu g\, g^{-1}$ N; 2N, 4.6 $\mu g\, g^{-1}$ N; 3N, 6.9 $\mu g\, g^{-1}$ N at the beginning of the experiment and every week thereafter for 14 wk. The 1N treatment was equivalent to a field application rate of 2.5 g N m^{-2} over a growing season (i.e. the 14 wk of this study). At this application rate the plant is experiencing an N supply rate at levels typically encountered in tallgrass prairie ecosystems over a growing season and includes N mineralization as well as N from dry deposition sources (Seastedt *et al.*, 1991). The 2N and 3N application levels represent N addition rates of 5 and 7.5 g N m^{-2}, and doubling and tripling of 'typical' supply rates, respectively.

Design of Expt II: cross-site shade experiment

Seeds from local big bluestem ecotypes from Cedar Creek, Fermi and Konza were germinated and 14-d-old seedlings were transplanted into plastic pots filled with 1 kg of pasteurized soils from the three prairies. For Expts II and III, we used seedlings germinated from locally collected seed rather than rhizomes to expedite the experimental set-up. AM and NM pots from the three sites were prepared as in Expt I and soil sievate was added as described above. Three shade treatments (0%, 33% and 66% light reduction) were established for each of the three sites, each treatment was replicated six times with or without AM fungi for a total of 108 pots. Shade treatments were established by adhering black polyethylene shade cloth (shadeclothstore.com) to wooden frames (1.2 m wide × 0.6 m deep × 1.2 m tall). Frames with no shade cloth were included as ambient controls.

Design of Expt III: Konza nutrient × shade experiment

Seeds from local big bluestem ecotypes from Konza were germinated and 14-d-old seedlings were transplanted into pots and filled with 1 kg of pasteurized soil from Konza. AM and NM pots from Konza were prepared and soil sievate was added as described above. Four fertilizer treatments (N enrichment, P enrichment, both N and P, and no fertilizer) were established as described in Expt I. Two shade treatments (0% and 66% light reduction) were established as described in Expt II. Each treatment was replicated six times with or without AM fungi for a total of 96 pots.

Glasshouse conditions and mycorrhizal analysis

All experiments were conducted in glasshouses at Kansas State University with temperature ranging from 20 to 25°C, and the photosynthetically active radiation in ambient light ranging from 618 to 1047 $\mu mol\, m^{-2}\, s^{-1}$. Plants were harvested after 14 wk as shoots were becoming senescent. There was no indication that plants were pot-bound at the termination of these experiments. Pots were not densely filled with root systems and root growth did not appear to be restricted by lack of available space in any treatment. Roots were washed free of soil, shoots and roots were oven dried for 72 h at 60°C and then weighed. Subsamples of roots were stained in trypan blue (Koske & Gemma, 1989) and AM colonization was measured (McGonigle *et al.*, 1990). External AM hyphae was extracted from 5 g of soil and quantified by the gridline intercept method (Miller *et al.*, 1995).

Carbon assimilation: Expt II

Photosynthetic rates of intact leaves still attached to the plant were measured using a LI-6200 portable photosynthesis system (LI-COR, Lincoln, NE, USA). One fully expanded leaf was measured for all plants at 6 wk when plants were actively growing. All measurements were carried out during midday (10:00 h to 13:00 h central daylight time (CDT)) under full sun conditions. During the measurements the air relative humidity was *c.* 70%, the temperature inside the leaf chamber was maintained between 25 and 28°C, and the ambient CO_2 concentration was 320–380 $\mu mol\, mol^{-1}$. At measurement, the portion of the leaf enclosed in the gas exchange cuvette was sampled for total leaf area (leaf length × leaf width).

Soil and nutrient analyses

Freshly collected soil samples from each site were analyzed for pH, organic matter, extractable inorganic N (NH_4^+-N and NO_3^- N) and available P (Bray test 1); inorganic N and available P were also measured from the pots at the end of the experiments. Soil organic matter was determined by direct combustion, soil

pH was measured from a 1 : 1 (based on weight) soil/water paste. Inorganic N was extracted using 2 M l^{-1} KCl and analyzed using cadmium reduction/colorimetry. Kansas State University Soil Testing Laboratory (Manhattan, KS, USA) conducted all of the soil analyses.

We used Koerselman & Meuleman (1996) thresholds of plant tissue N : P ratios as an indicator of relative N and P availability at the three sites and assumed that N-limited plants have tissue N : P < 14, P-limited plants have tissue N : P > 16, and plants with N : P ratios between 14 and 16 are either not limited by either resource or are co-limited by N and P. This N : P indicator of nutrient limitation was analyzed for Expts II and III but not for Expt I because it was initiated with rhizomes of slightly different sizes which influenced the final N : P ratios of the plants at harvest. Plant shoots were dried, ground through a 20-μm mesh, and digested with sulfuric acid and hydrogen peroxide. Phosphorus concentration of the digest was determined using the molybdate-blue method (Murphy & Riley, 1962), and N concentration was determined by a Kjeldahl method with a rapid-flow auto-analyzer.

Statistical analysis

Mycorrhizal growth response was calculated as a simple response ratio as described in Johnson (2010): $MGR = \log_e(AM/NM)$, where AM was the total dry weight of mycorrhizal plants and NM was mean DW of a nonmycorrhizal control treatment under the same nutrient conditions ($n = 6$). Data were analyzed with JMP (SAS, 2004) using ANOVA with different factors for the different experimental systems. In Expt I, the Cedar Creek system used two-way ANOVA with mycorrhizas and N as factors and the Konza and Fermi systems used three-way ANOVA with mycorrhizas, N and P as factors. Expt II used two-way ANOVA with mycorrhizas and shade as factors in all three systems. Expt III used four-way ANOVA with mycorrhizas, shade, N and P as factors in the Konza system. Following ANOVA, *post hoc* comparisons of the means were calculated using Tukey's HSD test ($P \leq 0.05$).

Results

Assessment of soil fertility and nutrient limitation

Soil texture varies across the three sites with the coarsest (sandy) soils at Cedar Creek and the finest (clayey) soils at Konza (Table 1). Cedar Creek soil contained significantly more available PO_4, less mineral N ($NH_4 + NO_3$), less organic matter, and lower pH compared to soils from the other sites (Table 1). During the course of Expt II, the mineral N concentration of Fermi and Cedar Creek soil was reduced by > 94% whereas mineral N in Konza soil decreased by only 45%. By contrast, the P concentration of Konza soil was reduced by 57% whereas in Fermi and Cedar Creek soils it was reduced by < 18% (Table 1).

Tissue N : P ratios from Expt II indicate that regardless of light treatment, NM plants from Fermi and Konza were P-limited (>> 16) whereas NM Cedar Creek plants were N-limited (<< 14). Furthermore, mycorrhizas eliminated P limitation of Fermi and Konza plants but they did not eliminate N limitation in Cedar Creek plants (Fig. 1a). Tissue N : P ratios from Expt III also indicate that at Konza, unfertilized NM plants were strongly P-limited and adding mycorrhizas eliminated this limitation (Fig. 1b). In Expt III, fertilization with P or with both P and N generated N-limited Konza plants with N : P ratios as low as Cedar Creek plants in Expt II (Fig. 1a,b). This occurred in both AM and NM plants and resulted from higher tissue P not because of lower tissue N (Supporting Information Fig. S1).

Expt I: cross-site nutrient enrichment experiment

In unfertilized Cedar Creek soil, AM and NM plants were similar in size but N enrichment caused NM plants to be larger than AM plants (Fig. 2a, Table 2). In Fermi and Konza soils,

Table 1 Soil properties of the three study systems at the onset of Expt II and from the pots after completion of the experiment

		Soil before Expt II				Soil from pots after Expt II			
Site location	Soil texture	pH	Organic matter (%)	Initial available PO_4-P (mg kg^{-1})	Initial available $NH_4 + NO_3$ (mg kg^{-1})	Final available PO_4-P (mg kg^{-1})	Final available $NH_4 + NO_3$ (mg kg^{-1})	Δ %P	Δ %N
Cedar Creek Ecosystem Science Reserve, Minnesota	Sandy loam	5.0 (0.06) a	1.4 (0.2) a	46.0 (2.1) b	8.7 (1.3) a	41.0 (2.5) b	0.5 (0.02) a	10.8	94.2
Fermi National Environmental Research Park, Illinois	Silt loam	6.9 (0.05) c	3.3 (0.3) b	12.0 (2.5) a	19.0 (4.0) b	9.9 (0.4) a	0.6 (0.04) a	17.5	96.8
Konza Prairie Biological Station, Kansas	Silty clay loam	6.6 (0.08) b	5.7 (0.1) c	17.0 (1.1) a	21.6 (2.4) b	7.2 (0.3) a	11.7 (0.9) b	57.6	45.8

Δ %P and %N indicate the change from the beginning to the end of the experiment. Data are means with 1 SE in parentheses. Values within the same column with different letters differ significantly from each other according to Tukey HSD tests.

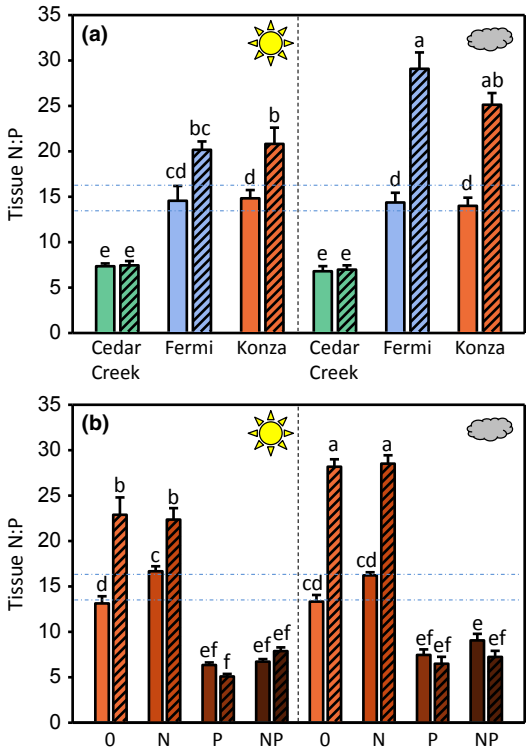

Fig. 1 Tissue nitrogen : phosphorus (N : P) ratios for mycorrhizal (open bars) and nonmycorrhizal (hatched bars) plants in Expt II (a) and Expt III (b). Light treatments are indicated as: sun symbol, full sunlight; cloud symbol, a 66% reduction in light. Fertilizer treatments in Expt III are indicated as: 0, no fertilizer; N, nitrogen alone; P, phosphorus alone; NP, enrichment with both. Horizonal dotted lines indicate threshold N : P ratios for N limitation (< 14) and P limitation (> 16). Bars represent means, + SE ($n = 6$). Bars with different letters differ significantly from each other ($P < 0.05$).

AM plants were always significantly larger than NM plants except for plants grown in P-fertilized Konza soil (Fig. 2a). In Fermi and Konza soils, N increased the biomass of AM plants but not NM plants whereas P enrichment increased the biomass of NM plants but not AM plants (Fig. 2a). Unfertilized Cedar Creek plants had significantly less AM root colonization and less external AM hyphae compared to unfertilized Fermi and Konza plants (Fig. 2b,c). Fertilization of Fermi and Konza plants with P reduced internal root colonization and external hyphae, whereas fertilization with N increased these fungal structures (Fig. 2b,c). By contrast, N fertilization of Cedar Creek soil reduced internal root colonization and had little influence on external AM hyphae. The MGR of Cedar Creek plants was significantly lower than the MGR of plants at the other sites (Fig. 2d). Nitrogen fertilization reduced MGR of Cedar Creek plants and P fertilization strongly reduced MGR of Fermi and Konza plants (Fig. 2d). Across all treatments, the amount of AM fungi inside and outside plant roots was positively correlated with MGR of plants (Fig. 3a,b).

Expt II: cross-site shade experiment

Shade significantly reduced CO_2 assimilation at all three sites, and inoculation with AM fungi increased CO_2 assimilation at

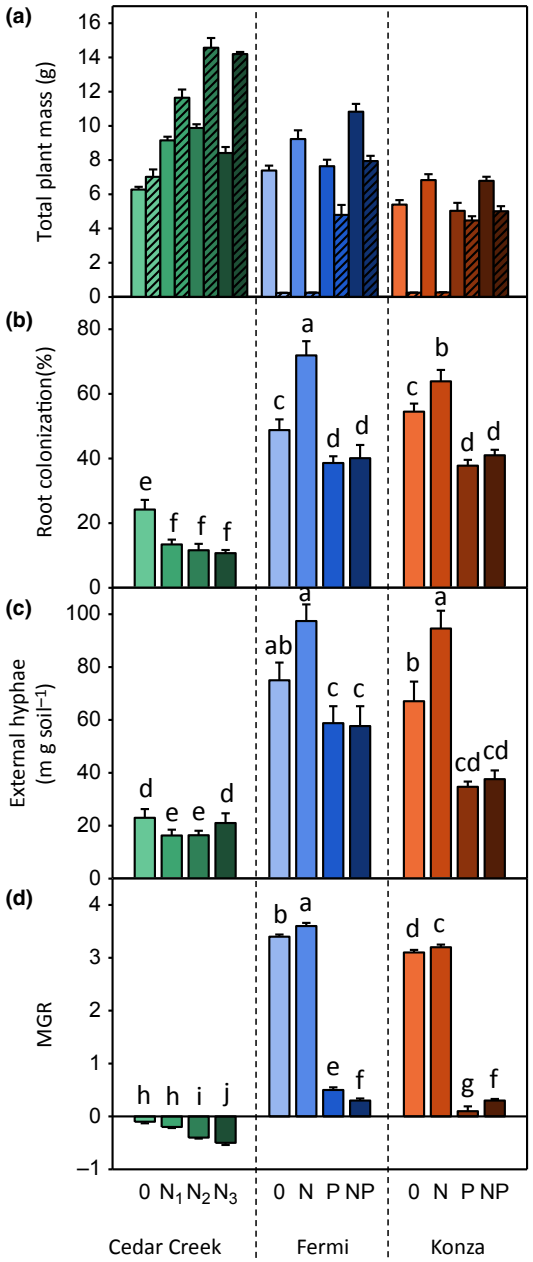

Fig. 2 Plant and fungal responses to nutrient enrichment in Expt I: total dry weight of mycorrhizal (open bars) and nonmycorrhizal (hatched bars) plants (a); root colonization by arbuscular mycorrhizal (AM) fungi (b); density of AM fungal hyphae in the soil (c); and mycorrhizal growth response (MGR) of plants (d). Nitrogen treatments in the Cedar Creek system (green bars) are indicated as: 0, no supplementary fertilizer; N_1, N_2 and N_3 for low, medium and high levels of N, respectively. Treatments in the Fermi (blue bars) and Konza (orange bars) systems are indicated as: 0, no fertilizer; N, nitrogen alone; P, phosphorus alone; NP, enrichment with both. Bars represent means, + SE ($n = 6$). Bars with different letters differ significantly from each other ($P < 0.05$).

Fermi and Konza but not at Cedar Creek (Fig. 4, Table 3). Plant biomass was significantly reduced in the 66% shade treatment at all sites (Fig. 5a). As in the previous experiment, Cedar Creek plants had significantly less mycorrhizal colonization and formed fewer external hyphae compared to Fermi and Konza plants (Fig. 5b,c). Shade significantly reduced root colonization at all

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Table 2 Expt I analysis of variance

Site	*N*	Response	Nitrogen	Phosphorus	Mycorrhizas	N × P	N × M	P × M
Cedar	48	Total plant dry mass	96.3***	–	189.0***	–	20.4***	–
	24	AM root colonization	8.3***	–	–	–	–	–
	24	External AM hyphae	1.0	–	–	–	–	–
	24	MGR	12.0***	–	–	–	–	–
Fermi	48	Total plant dry mass	53.0***	157.9***	379.1***	15.9***	2.8	85.7***
	24	AM root colonization	11.6**	32.8***	–	9.1**	–	–
	24	External AM hyphae	1.5	16.3**	–	2.2	–	–
	24	MGR	1.5	256.5***	–	1.9	–	–
Konza	48	Total plant dry mass	20.5***	107.6***	289.9***	1.04	10.1**	128.9***
	24	AM root colonization	6.5**	61.8***	–	1.6	–	–
	24	External AM hyphae	6.7**	87.7***	–	3.0	–	–
	24	MGR	2.6	201.9***	–	2.2	–	–

F-ratios from two-way (Cedar Creek) and three-way (Fermi and Konza) ANOVA for total plant mass, arbuscular mycorrhizal (AM) root colonization, external hyphae, and mycorrhizal growth response (MGR). Noninoculated plants were not included in the analysis of AM fungal responses.
Significant main effects and interactions are indicated: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

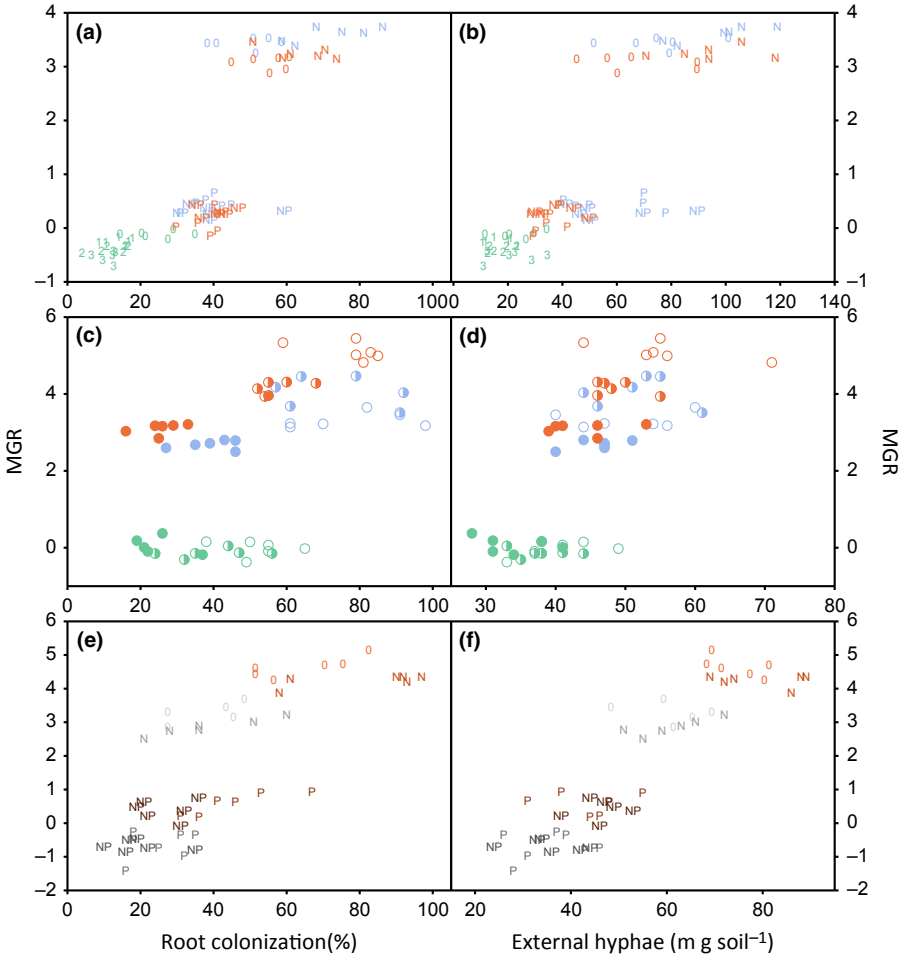

Fig. 3 Relationships between mycorrhizal growth response (MGR) and arbuscular mycorrhizal (AM) root colonization and external hyphae for Expt I (a, b), Expt II (c, d) and Expt III (e, f). Colors indicate the different sites: Cedar Creek (green), Fermi (blue) and Konza (orange) in Expts I and II. Letters indicate fertilizer treatments in Expt I and III: 0, no fertilizer; N, nitrogen alone; P, phosphorus alone; NP, enrichment with both. Solid circles, half solid, and open circles represent 66% shade, 33% shade, and full sunlight, respectively, in Expt II (c, d). Orange letters, plants grown in the full sun; gray letters, plants grown in the shade Expt III (e, f).

three sites; and it tended to reduce external hyphae, but this was only significant at Konza. Plant MGR differed significantly among sites; it was highest at Konza and lowest at Cedar Creek (Fig. 5d). The 66% shade treatment significantly reduced MGR of Konza and Fermi plants but it had no influence on MGR of Cedar Creek plants (Fig. 5d). The amount of AM fungi inside and outside plant roots was positively correlated with MGR of plants in the Fermi and Konza systems but not in the Cedar

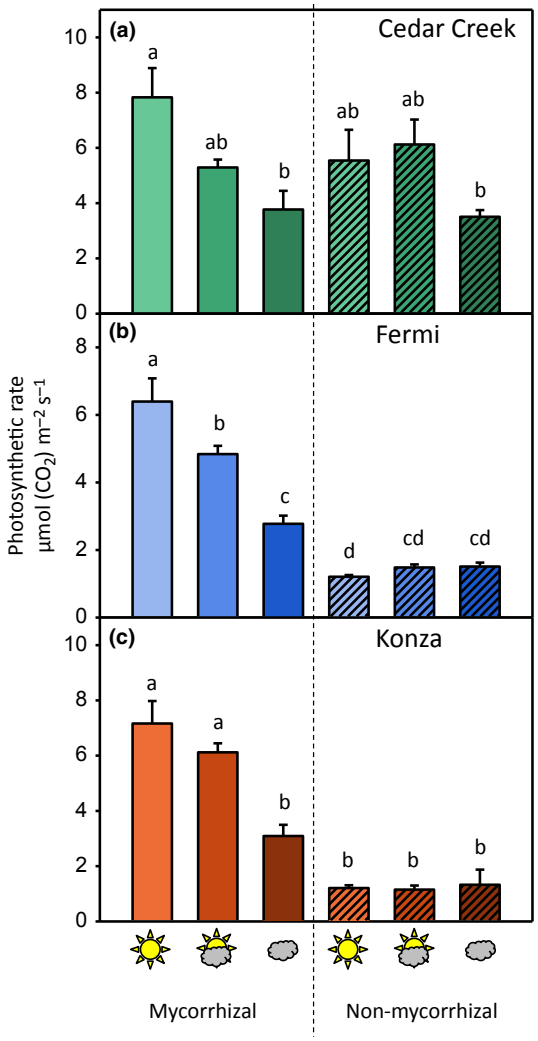

Fig. 4 Carbon assimilation rate measured in Expt II in 6-wk-old plants growing in (a) Cedar Creek (a), Fermi (b) and Konza (c) soils with and without mycorrhizas. Light treatments are indicated as: sun symbol, full sunlight; split sun/cloud, a 33% reduction in light; cloud symbol, a 66% reduction in light. Bars represent means, + SE ($n = 6$). Bars with different letters differ significantly from each other ($P < 0.05$).

Creek system (Fig. 3c,d). Plants with tissue N : P < 14 had a MGR near zero, whereas those with N : P > 16 had a MGR > 2.5 (Fig. 6a).

Expt III: Konza nutrient × light experiment

As in the previous experiments, Konza NM plants barely survived without P enrichment (Figs 2a, 5a, 7a). Shade significantly reduced plant biomass across all the nutrient treatments (Fig. 7a, Table 4). In full sun, N enrichment increased the biomass of AM plants and P enrichment and dual N and P enrichment increased the biomass of NM plants (Fig. 7a). In full sun, N enrichment alone increased root colonization, whereas in combination with shade, N fertilization did not influence internal AM colonization or external hyphae (Fig. 7b,c). Phosphorus fertilizer reduced internal colonization and external hyphae under both light

Table 3 Expt II analysis of variance

Site	*N*	Response	Light	Mycorrhizas	L × M
Cedar	36	Total plant dry mass	64.8***	0.5	1.0
	18	CO_2 Assimilation rate	7.7**	0.8	2.0
	17	AM root colonization	11.0**	–	–
	18	External AM hyphae	2.7	–	–
	18	MGR	2.2	–	–
Fermi	36	Total plant dry mass	17.2***	156.7***	17.6***
	18	CO_2 Assimilation rate	13.7**	157.0***	18.9**
	18	AM root colonization	14.7**	–	–
	18	External AM hyphae	1.4	–	–
	18	MGR	40.9***	–	–
Konza	36	Total plant dry mass	28.7***	348.6***	29.8***
	18	CO_2 Assimilation rate	9.9**	127.1***	11.4**
	18	AM root colonization	80.8***	–	–
	18	External AM hyphae	5.1*	–	–
	18	MGR	177.5***	–	–

F-ratios from two-way ANOVA for total plant mass, arbuscular mycorrhizal (AM) root colonization, external hyphae, and mycorrhizal growth response (MGR). Noninoculated plants were not included in the analysis of AM fungal responses.
Significant main effects and interactions are indicated: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

regimes (Fig. 7b,c). Both shade and fertilizer reduced MGR, and shade in combination with P enrichment generated mycorrhizal induced plant growth depression (Fig. 7d). The amount of AM fungi inside and outside plant roots was positively correlated with MGR of plants (Fig. 3c). Plants with tissue N : P < 14 (i.e. N-limited) had a MGR < 1, whereas those with N : P > 16 (i.e. P-limited) had a MGR > 2.5 (Fig. 6b).

Discussion

According to the Law of the Minimum, plant growth is controlled by the resource that is most limited in supply (von Liebig, 1843). This law is clearly an oversimplification of reality because plants are generally co-limited by multiple resources (Harpole *et al.*, 2011); a single resource may be most limiting at any one moment but the identity of that resource is likely to change through the growing season or even through a single day (Farrior *et al.*, 2013). Nevertheless, the Law of the Minimum is a central tenet of ecological stoichiometry, which is a useful framework for connecting resource supply and demand to predictions about mycorrhizal functioning (Sterner & Elser, 2002; Johnson, 2010). Mycorrhizas have been considered 'nature's response to the law of the minimum' because they enhance plant access to limiting belowground resources in a predictable way (Read, 1991). Arbuscular mycorrhizas have long been recognized for their importance to plant P nutrition, but their role in plant N nutrition has been controversial (Smith & Smith, 2011). We tested the abilities of intact mycorrhizal systems to remedy nutrient limitation in three natural grasslands and showed that AM symbiosis can completely eliminate P limitation but they cannot ameliorate N limitation (Fig. 1).

Mycorrhizal phenotypes are emergent properties of the genotypes of partnering plants and fungi and their environment. Our

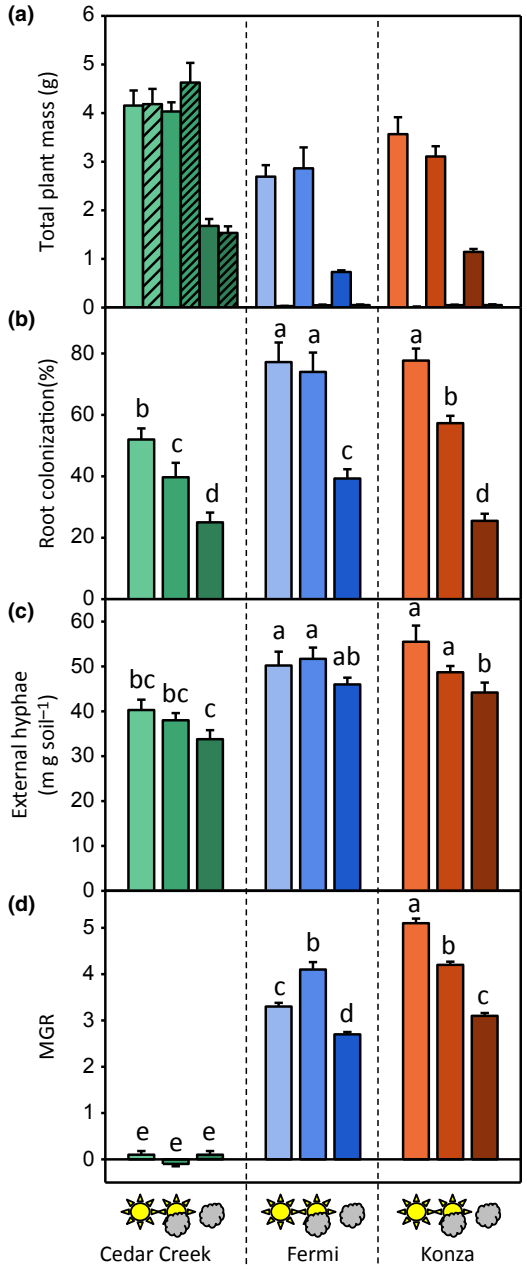

Fig. 5 Plant and fungal responses to shade in Expt II: total DW of mycorrhizal (open bars) and nonmycorrhizal (hatched bars) plants (a); root colonization by arbuscular mycorrhizal (AM) fungi (b); density of AM fungal hyphae in the soil (c); and mycorrhizal growth response (MGR) of plants (d). Light treatments are indicated as: sun symbol, full sunlight; split sun/cloud, a 33% reduction in light; cloud symbol, a 66% reduction in light. Bars represent means, + SE ($n = 6$). Bars with different letters differ significantly from each other ($P < 0.05$).

findings strongly support the hypothesis that soil fertility is an important determinant of mycorrhizal phenotype and that mutualism is most likely in P-limited soils and commensalism and parasitism are most likely in P-rich soil, especially when light is limited. As predicted, big bluestem mycorrhizas were mutualistic at Fermi and Konza, the two P-limited sites, and commensal at Cedar Creek, the P-rich site (Figs 2d, 5d). Furthermore, P fertilization of Fermi and Konza soil dramatically reduced MGR of

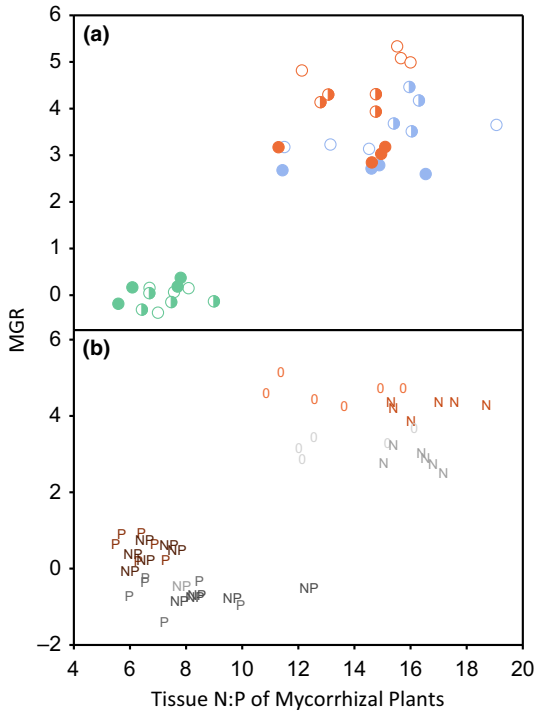

Fig. 6 Relationships between mycorrhizal growth response (MGR) and tissue nitrogen: phosphorus (N : P) ratio of mycorrhizal plants for Expt II (a), and Expt III (b). Solid circles, half solid and open circles represent 66% shade, 33% shade and full sunlight, respectively (a). Fertilizer treatments: 0, no fertilizer; N, nitrogen alone; P, phosphorus alone; NP, enrichment with both (b).

big bluestem (Fig. 2d). These patterns in MGR correspond to resource limitation as determined by mean tissue N : P ratios (Fig. 1). Mutualism was observed in plants that were P-limited or co-limited by N and P whereas commensalism was observed in plants that were strongly N-limited (Fig. 6a). Phosphorus fertilization generated commensalism or parasitism of Konza plants and reduced tissue N : P below the threshold for N limitation and to the range observed in plants grown in Cedar Creek soil (Fig. 6a,b).

Results of our studies suggest that tissue N : P is a useful predictor of mycorrhizal phenotype. In every case that MGR was high (mutualism), tissue N : P was > 10 suggesting co-limitation or P limitation, and when MGR was near zero or negative (commensal or parasitic), tissue N : P was < 14, below the threshold for N limitation (Koerselman & Meuleman, 1996; Fig. 6). Although tissue N : P ratio was a useful index of nutrient limitation in our studies of 3-month-old plants, caution is necessary when using tissue N : P ratios for inferences about plant N and P limitation. The index is likely to be most accurate when studying relatively young plants of uniform age. Studies suggest that N : P ratios may not be accurate indicators of plant nutrient status in water-limited systems or in older plants that have sequestered nutrients in their tissues (Güsewell, 2004). In such cases, it is preferable to detect plant nutrient limitation using fertilization experiments to reveal plant biomass responses to enrichment of different nutrients.

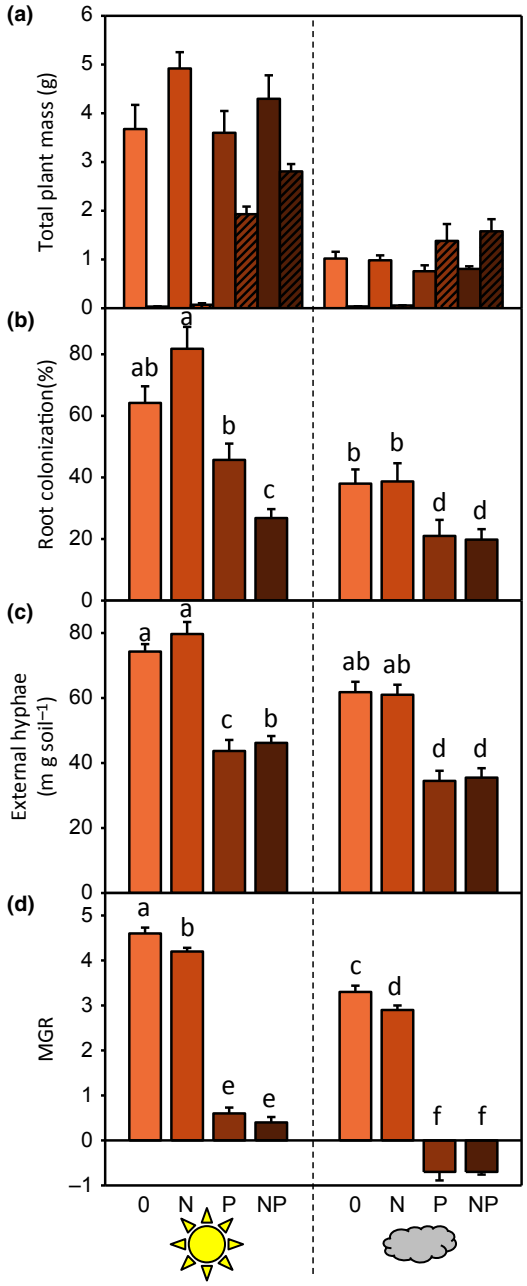

Fig. 7 Plant and fungal responses to simultaneous fertilization and shade in Expt III: total DW of mycorrhizal (open bars) and nonmycorrhizal (hatched bars) plants (a); root colonization by arbuscular mycorrhizal (AM) fungi (b); density of AM fungal hyphae in the soil (c); and mycorrhizal growth response (MGR) of plants (d). Light treatments are indicated as: sun symbol, full sunlight; cloud symbol, a 66% reduction in light. Fertilizer treatments: 0, no fertilizer; N, nitrogen alone; P, phosphorus alone; NP, enrichment with both. Bars represent means, + SE ($n = 6$). Bars with different letters differ significantly from each other ($P < 0.05$).

Our findings show that light availability was an important determinant of mycorrhizal phenotype in Fermi and Konza soils, but not in P-rich Cedar Creek soil (Fig. 5d). Simultaneous shade and P fertilizer in the Konza system (Expt III) generated a remarkable range of mycorrhizal phenotypes, from mutualism to parasitism (Figs 6b, 7d). Shade reduced the net CO_2 assimilation rate (Fig. 4) and both shade and P enrichment reduced fungal biomass, presumably because of reduced C-for-P trade. Both shade and P fertilizer reduced MGR by shifting the relative balance of commodities: shade increased the relative cost of C and fertilizer decreased the relative benefit of mycorrhizal P uptake. These results are parsimonious with the discovery that reciprocal exchange of C and P control mycorrhizal cooperation (Hammer *et al.*, 2011; Kiers *et al.*, 2011). In contrast to P, we observed little evidence to suggest that C-for-N trade directly enforces mycorrhizal cooperation by linking resource trade to biomass gain by both plant and fungal partners. Other studies have reached the same conclusion (Corrêa *et al.*, 2014). When N is in limited supply, AM fungi are unlikely to release it to their host plants until their own needs have been met (Hodge & Storer, 2014). In this regard, it is likely that plants, AM fungi and other soil microorganisms all compete for N in soils with very low N availability.

In Expt II, a 66% reduction of light reduced MGR, but a 33% reduction only reduced MGR in Konza soil; it actually increased MGR in Fermi soil. These findings suggest that the photosynthetic capacity of big bluestem must be substantially reduced to induce a limitation of C for symbiotic trade with AM fungi in P limited soils. This agrees with a study of C budgets in legume mycorrhizas (Kaschuk *et al.*, 2009), and suggests that the C cost of AM fungi is substantially lower than the photosynthetic capacity of their host plants. Cool-season grasses with a C_3 photosynthetic pathway are expected to be inherently more C-limited than C_4 grasses, such as big bluestem in our experiment, and this physiological constraint helps explain why C_3 grasses generally have a much lower dependency on mycorrhizas (Wilson & Hartnett, 1998). With ample light, big bluestem has been shown to increase its photosynthetic capacity to satisfy the C sink strength of AM fungal symbionts (Miller *et al.*, 2002). But, without sufficient light, we have shown that mycorrhizal trading partnerships become C limiting and both fungal biomass and MGR decline. Others have found evidence that shade reduces C supply to AM fungi and reduces AM fungal biomass and MGR (Graham *et al.*, 1982; Olsson *et al.*, 2010; Shi *et al.*, 2014).

Fertilization with N can either decrease or increase the abundance of AM fungi and plant MGR as seen in Expt I (Fig. 2) and in field studies (Johnson *et al.*, 2003). Nitrogen enrichment of P-rich Cedar Creek soil reduced AM colonization and MGR of plants. By contrast, N enrichment increased the abundance of AM fungi and plant MGR in Fermi and Konza soils. The Functional Equilibrium model helps explain this result (Johnson *et al.*, 2003). Nitrogen enrichment of P-rich soils may be expected to induce plants to adjust their biomass allocation in favor of shoots and away from roots and mycorrhizas. On the other hand, N enrichment of P-limited soils will exacerbate P limitation, increase the value of AM symbioses, and induce plants to increase allocation belowground to roots and AM fungi. This perspective may help reconcile inconsistencies in the literature. Reynolds *et al.* (2005) examined the effects of four different AM fungal species on five different plant species but did not observe a single case in which the fungus increased the N nutrition and biomass of their host plant. By contrast, Tu *et al.* (2006) found that AM fungi significantly increased the N uptake and biomass gain of *Avena fatua*. Soil N : P stoichiometry is the key to these

Table 4 Expt III analysis of variance

		Source of variation									
N	Response	Nitrogen	Phosphorus	Light	Mycorrhizas	N × P	N × L	P × L	N × M	P × M	L × M
95	Total plant dry mass	8.7**	37.3***	199.4***	136.3***	0.3	6.3*	2.1	0.6	68.7***	114.2***
47	AM colonization	0.1	47.2***	40.6***	–	5.2*	0.001	4.3	–	–	–
47	External AM hyphae	0.7	151.1***	28.5***	–	0.05	0.4	0.1	–	–	–
47	MGR	8.9**	1949***	211***	–	3.5	2.1	1.4	–	–	–

F-ratios from four-way ANOVA for total plant mass, arbuscular mycorrhizal (AM) root colonization, external hyphae, and mycorrhizal growth response (MGR) from plants grown in the Konza experimental system. Noninoculated plants were not included in the analysis of AM fungal responses. Significant main effects and interactions are indicated: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

contradictory findings. Reynolds *et al.* (2005) purposely used P-rich soil; consequently, similar to Cedar Creek, N enrichment generated commensal or even parasitic mycorrhizal phenotypes. By contrast, the soil used by Tu *et al.* (2006) was co-limited by both N and P (C. Tu, pers. comm.); and accordingly, N enrichment increased the relative benefit of mycorrhizal P uptake and increased MGR, just as we observed at Fermi and Konza.

The usefulness of the Law of the Minimum for predicting mycorrhizal function doesn't only pertain to N and P. It is well known that AM fungi can ameliorate plant limitation of other resources such as water and zinc (Thompson, 1994; Ryan & Angus, 2003; Augé *et al.*, 2014). Improved water relations could well be the most important function of mycorrhizas in the sandy drought-prone soil at Cedar Creek, but our experiments would never detect this because our experimental plants were never exposed to drought. Mycorrhizas are known to increase plant uptake of immobile micronutrients such as zinc, copper and manganese, and the extent to which they improve plant nutrition is influenced by the relative abundance of P and and other micronutrients (Liu *et al.*, 2000). Researchers studying fertilization trials should be aware of the potential for hidden micronutrients to influence their results (Hejcman *et al.*, 2009). For example, molybdenum, a critical co-factor in nitrogenase, is often a limiting factor for N-fixing symbioses and it is a common contaminant of P fertilizer (Barron *et al.*, 2009).

Resource limitation can be a powerful driver of community composition and genetic adaptation within populations. Natural communities of organisms are likely to shift in composition towards stoichiometric balance with their resource supply (Schade *et al.*, 2005; Danger *et al.*, 2008). When soil resources are in limited supply, plant species that form nutritional symbioses are expected to dominate the community, but fertilization will eliminate the benefits of these symbioses, and over time, plant species that invest less in useless nutritional symbioses will have a competitive advantage and come to dominate fertilized communities. We believe that this phenomenon may be driving the shifts in the species composition of grassland plants and associated AM fungi that occur in long-term fertilization studies (Johnson, 1993; Egerton-Warburton *et al.*, 2007; Johnson *et al.*, 2008; Liu *et al.*, 2012). We have found strong evidence that nutrient limitation may also be an evolutionary force that generates site-specific co-adaptation among plant genotypes and AM fungi. A reciprocal inoculation experiment clearly showed that local ecotypes of big bluestem are adapted to their local soil and indigenous AM fungal communities such that mycorrhizal exchange is maximized for the most limiting resource (N at Cedar Creek and P at Fermi and Konza) (Johnson *et al.*, 2010; Ji *et al.*, 2013).

Mycorrhizal phenotypes are shaped by complex interactions among many biotic and abiotic factors (Newsham *et al.*, 1995; Johnson *et al.*, 1997). The important insight from this work is that the Law of the Minimum provides a hypothesis-generating framework that helps identify the critical factors controlling mycorrhizal function in different systems. Arbuscular mycorrhizas are found across a remarkable diversity of ecosystems. Certainly, different factors should be expected to limit their function in systems as different as deserts and tropical rain forests. Accurate identification of these critical (limiting) factors is a first step towards developing effective management strategies to maximize AM benefits in agriculture, horticulture forestry, and restoration. For example, knowing the threshold tissue N : P ratio for mutualism will help identify the appropriate fertilization schemes to maximize mycorrhizal benefits and minimize the use of expensive fertilizers. Also, this knowledge could help manage AM fungi for their role in sequestering C in the soil. Addition of N to P-limited soils could increase AM fungal biomass and its potential to increase belowground C sinks, whereas N enrichment of P-rich soils will have the opposite effect and reduce AM fungal biomass.

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References

Augé RM, Toler HD, Saxton AM. 2014. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply

watered conditions: a meta-analysis. *Mycorrhia*. doi: 10.1007/s00572-014-0585-4.

Barron AR, Wurzburger N, Bellenger JP, Wright SJ, Kraepiel AML, Hedin LO. 2009. Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soil. *Nature Geoscience* **2**: 42–45.

Blackwell J. 1988. Physical methods for determination of chitin structure and conformation. In: Wood WA, Kellogg TS, eds. *Methods in enzymology*. New York, NY, USA: Academic Press, 436–442.

Chapin FSI, Bloom AJ, Field CB, Waring RH. 1987. Plant responses to multiple environmental factors. *BioScience* **37**: 49–57.

Corrêa A, Cruz C, Perez-Tienda J, Ferrol N. 2014. Shedding light onto nutrient responses of arbuscular mycorhial plants: nutrient interactions may lead to unpredicted outcomes of the symbiosis. *Plant Science* **221–222**: 29–41.

Danger M, Daufresne T, Lucas F, Pissard S, Lacroix G. 2008. Does Liebig's law of the minimum scale up from species to communities? *Oikos* **117**: 1741–1751.

Egerton-Warburton L, Johnson NC, Allen EB. 2007. Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. *Ecological Monographs* **77**: 527–544.

Farrior CE, Tilman D, Dybzinski R, Reich PB, Levin SA, Pacala SW. 2013. Resource limitation in a competitive context determines complex plant responses to experimental resource additions. *Ecology* **94**: 2505–2517.

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bucking H. 2012. Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **109**: 2666–2671.

George E, Marschner H, Jakobsen I. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology* **15**: 257–270.

Graham JH, Leonard RT, Menge JA. 1982. Interaction of light intensity and soil temperature with phosphorus inhibition of vesicular–arbuscular mycorrhiza formation. *New Phytologist* **91**: 683–690.

Güsewell S. 2004. N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist* **164**: 243–266.

Hammer EC, Pallon J, Hakan W, Olsson PA. 2011. Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiology Ecology* **76**: 236–244.

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB *et al.* 2011. Nutrient co-limitation of primary producer communities. *Ecology Letters* **14**: 852–862.

Hejcman M, Szakova J, Schellberg J, Srek P, Tlustos P. 2009. The Rengen Grassland Experiment: soil contamination by trace elements after 65 years of Ca, N, P, and K fertiliser application. *Nutrient Cycling in Agroecosystems* **83**: 39–50.

Hodge A, Fitter AH. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proceedings of the National Academy of Sciences, USA* **107**: 13 754–13 759.

Hodge A, Storer K. 2014. Arbuscular mycorrhizs and nitrogen: implications for individual plants through ecosystems. *Plant and Soil*. doi: 10.1007/s11104-014-2162-1.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide R, Pringle A, Zabinski C, Bever JD, Moore JN *et al.* 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* **13**: 394–407.

Ji B, Gehring CA, Wilson GWT, Miller RM, Johnson NC. 2013. Patterns of diversity and adaptation in fungal root symbionts from three prairie grasslands. *Molecular Ecology* **22**: 2573–2587.

Johnson NC. 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* **3**: 749–757.

Johnson NC. 2010. Tansley Review: resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist* **185**: 631–647.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* **135**: 575–585.

Johnson NC, Rowland DL, Corkidi L, Allen EB. 2008. Plant winners and losers during grassland eutrophication differ in biomass allocation and mycorrhizas. *Ecology* **89**: 2868–2878.

Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton L, Allen EB. 2003. Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. *Ecology* **84**: 1895–1908.

Johnson NC, Wilson GWT, Bowker MA, Wilson J, Miller RM. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences, USA* **107**: 2093–2098.

Kaschuk G, Kuyper TW, Leffelaar PA, Hungria M, Giller KE. 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biology and Biochemistry* **41**: 1233–1244.

Kaye JP, Hart SC. 1997. Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology and Evolution* **12**: 139–143.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A *et al.* 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880–882.

Koerselman W, Meuleman AFM. 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, Elliott G. 1989. Cost, benefit and efficiency of the vesicular-arbuscular mycorrhizal symbiosis. *Functional Ecology* **3**: 252–255.

Koide RT. 1991. Tansley Review No. 29 Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist* **117**: 365–386.

Koske RE, Gemma JN. 1989. A modified procedure for staining roots to detect mycorrhizas. *Mycological Research* **92**: 486–488.

Leigh J, Hodge A, Fitter AH. 2008. Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist* **181**: 199–207.

von Liebig JF. 1843. *Chemistry in its application to agriculture and physiology*. London, UK: Taylor and Walton.

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL. 2000. Acquisition of Cu, Zn, Mn, and Fe by mycorhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* **9**: 331–336.

Liu Y, Shi GX, Mao L, Cheng G, Jiang S, Ma X, An L, Du G, Johnson NC, Feng H. 2012. Direct and indirect influences of eight years of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. *New Phytologist* **194**: 523–535.

de Mazancourt C, Schwartz MW. 2010. A resource ratio theory of cooperation. *Ecology Letters* **13**: 349–359.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495–501.

Miller RM. 1987. The ecology of vesicular–arbuscular mycorrhizae in grass- and shrublands. In: Safir GR, ed. *Ecophysiology of VA mycorrhizal plants*. Boca Raton, FL, USA: CRC Press, 135–170.

Miller RM, Miller SP, Jastrow JD, Rivetta CB. 2002. Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. *New Phytologist* **155**: 149–162.

Miller RM, Reinhardt DR, Jastrow JD. 1995. External hyphal production of vesicular–arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* **103**: 17–23.

Modjo HS, Hendrix JW. 1986. The mycorrhizal fungus *Glomus macrocarpum* as a cause of tobacco stunt disease. *Phytopathology* **76**: 688–691.

Mosse B. 1973. Advances in the study of vesicular–arbuscular mycorrhizas. *Annual Review of Phytopathology* **11**: 171–196.

Murphy J, Riley JP. 1962. A modified single solution method of the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**: 31–36.

Newsham K, Fitter A, Watkinson A. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution* **10**: 407–411.

Olsson PA, Rahm J, Aliasgharzad N. 2010. Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiology Ecology* **72**: 123–131.

Pearson JN, Smith SE, Smith FA. 1991. Effect of photon irradiance on the development and activity of VA mycorrhizal infection in *Allium porrum*. *Mycological Research* **95**: 741–746.

Peng S, Eissenstat DM, Graham JH, Williams K, Hodge NC. 1993. Growth depression in mycorrhizal citrus at high-phosphorus supply. *Plant Physiology* **101**: 1063–1071.

van der Ploeg RR, Bohm W, Kirkham MB. 1999. On the origin of the theory of mineral nutrition of plants and the law of the minimum. *Soil Science Society of America Journal* **63**: 1055–1062.

Read DJ. 1991. Mycorrhizas in ecosystems – nature's response to the "law of the minimum". In: Hawksworth DL, ed. *Frontiers in mycology*. Kew Surrey, UK: CAB International, 101–130.

Reynolds HL, Hartley AE, Vogelsang KM, Bever JD, Schultz PA. 2005. Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. *New Phytologist* **167**: 869–880.

Robinson D, Fitter AH. 1999. The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. *Journal of Experimental Botany* **50**: 9–13.

Ryan MH, Angus JF. 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. *Plant and Soil* **250**: 225–239.

SAS. 2004. *JMP statistical discovery software*. Cary, NC, USA: SAS Institute Inc.

Schade JD, Espeleta JF, Klausmeier CA, McGroddy ME, Thomas SA, Zhang L. 2005. A conceptual framework for ecosystem stoichiometry: balancing resource supply and demand. *Oikos* **109**: 40–51.

Seastedt TR, Briggs JM, Gibson DJ. 1991. Controls of nitrogen limitation in tallgrass prairie. *Oecologia* **87**: 72–79.

Shi GX, Liu Y, Johnson NC, Olsson PA, Mao L, Cheng G, Jiang S, An L, Du G, Feng H. 2014. Interactive influence of light intensity and soil fertility on root-associated arbuscular mycorrhizal fungi. *Plant and Soil* **378**: 173–188.

Smith SE, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annual Review of Plant Biology* **62**: 227–250.

Sterner RW, Elser JJ. 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton, NJ, USA: University Press.

Thompson JP. 1994. Inoculation with vesicular-arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum usitatissimum* L.) by improving P and Zn uptake. *Soil Biology & Biochemistry* **26**: 1133–1143.

Tu C, Booker FL, Watson DM, Chen X, Rufty TW, Shi W, Hu S. 2006. Mycorrhizal mediation of plant N acquisition and residue decomposition: impact of mineral N inputs. *Global Change Biology* **12**: 793–803.

Wilson GWT, Hartnett DC. 1998. Interspecific variation in plant responses to mycorrhizal colonization in prairie grasses and forbs. *American Journal of Botany* **85**: 1732–1738.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Percentage tissue N and P in Expt III (mycorrhizal (AM) and nonmycorrhizal (NM) plants grown with light and fertilizer treatments).

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