Common mycorrhizal networks amplify size inequality in Andropogon gerardii monocultures

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Introduction

Arbuscular mycorrhizal (AM) fungi can influence plant community composition. They may do so by increasing plant productivity (van der Heijden et al., 1998) through improved host acquisition of limiting mineral nutrients, such as relatively immobile phosphorus. Mineral nutrient acquisition is enhanced by means of extensively branched networks of AM fungal hyphae that forage throughout the soil. Because AM fungi have little host specificity, such hyphal networks can interconnect and distribute mineral nutrients among many plant individuals regardless of species (Chiariello et al., 1982); hence the networks are called common mycorrhizal networks (CMNs). Interconnections via CMNs can potentially influence seedling establishment (Eissenstat & Newman, 1990; Kytöviita et al., 2003; Janouskova et al., 2011) by influencing competition for mineral nutrients (Hartnett et al., 1993; West, 1996). For example, seedlings connected to large plants by CMNs may grow less than those not connected, which suggests resource pre-emption across CMNs (Allsopp & Stock, 1992; Hartnett et al., 1993; Janouskova et al., 2011). However, little is known about whether CMN interconnections influence competition among individuals that are similar in age and size.

Common mycorrhizal networks can influence the distribution of mineral nutrients among networked plants (Chiariello et al., 1982; Wilson et al., 2006; He et al., 2009), but whether CMNs confer similar access to mineral nutrients upon all interconnected plants or intensify competition among them is poorly understood. In an early field experiment that employed radioactive phosphorus as a tracer, Chiariello et al. (1982) found that CMN interconnected individuals obtained phosphorus from a donor with shoot excised regardless of their species, size or proximity to the donor. Chiariello et al. (1982) suggested that mineral nutrients taken up by CMNs are ‘shared’ among interconnected plants with no clear pattern as to which individuals obtain the most mineral nutrients. By contrast, in an in vitro culture experiment, roots that provided carbon to CMNs had twice as many arbuscules and received up to 10 times more phosphorus than did carbon-limited roots (Lekberg et al., 2010). This latter experiment suggests that CMNs might make competition for phosphorus among interconnected individuals asymmetric, with strong carbon-donor root systems disproportionately obtaining phosphorus from CMNs.

It once was thought that carbon flow across AM fungus CMNs might facilitate the growth of shaded, suppressed plants (Francis & Read, 1984; Grime et al., 1987), but it now appears that with...
the exceptions of mycoheterotrophic and mixotrophic plant species, AM fungi simply may shunt carbon acquired from carbon-rich individuals to storage locations within the roots of suppressed individuals. While labeling studies have shown that carbon from one plant frequently can be detected in the root system of another (Fitter et al., 1998), recent in vitro research suggests that the labeled carbon remains within intraradical fungus tissue (Pfeffer et al., 2004). Moreover, AM fungi may transfer mineral nutrients preferentially to host individuals that are strong carbon suppliers, and, reciprocally, hosts may reward AM fungi that are strong phosphorus suppliers (Lekberg et al., 2010; Hammer et al., 2011). Hammer et al. (2011) showed that the AM fungus Glomus intraradices accumulated phosphorus when connected to a carbon-limited host, but did not when its host provided sufficient carbon to the fungus. The fungus accumulated up to seven times more phosphorus in its spores and nine times more phosphorus in its hyphae under reduced host carbon than under carbon-sufficient conditions (Hammer et al., 2011). Another in vitro study suggested that carbon-limited plants attached to CMNs may serve as protected storage sites for AM fungi, because the fungi within their roots accumulate labeled carbon in the form of storage lipids (Lekberg et al., 2010). Although the lack of shoots in these in vitro experiments precludes transpiration, shoot phosphorus sinks and diurnal changes in carbon supply, such functional differences between carbon suppliers and carbon-poor whole plants interconnected by AM CMNs might exacerbate competition between large and small plants and accelerate the development of a size hierarchy.

Plants that tend to recruit near members of their own species (Harper, 1977) are likely to compete intraspecifically, and within such cohorts, competition can lead to the development of a size hierarchy. Dense seedling cohort size distributions change through time in a typical manner from symmetric, normal distributions shortly after germination to right-skewed, asymmetric ones of many small and a few large plants as they age. Dissimilar germination times (Weiner, 1990), intrinsic natural variation in individuals’ exponential growth rates (Koyama & Kira, 1956), and mortality as populations self-thin (Mohler et al., 1978), can contribute to size inequality. Dominance and suppression, which are recognized as reflecting asymmetric competition, can also contribute to size differences within cohorts (Ford & Diggle, 1981; Weiner & Thomas, 1986; Weiner, 1990). Asymmetric competition is thought primarily to take place above ground because large individuals pre-empt light acquisition by shading small individuals, thereby disproportionately obtaining the resource (Weiner, 1990). Alternatively, belowground competition is considered predominantly symmetric (Weiner, 1990) with resources obtained in proportion to root system size. However, if CMNs functionally associate phosphorus supply with photosynthetic provision by hosts, then CMNs might translate aboveground asymmetric competition for light into belowground asymmetric competition for phosphorus, thereby amplifying size inequality within a cohort.

Size inequality arising in consequence of competition usually increases with elevated plant density because competitive interactions begin quickly at high density (Weiner & Thomas, 1986). Competitive interactions can also be accelerated by inoculation with AM fungi, which may result in greater size inequalities in mycorrhizal populations than in those without mycorrhizas (Allsopp & Stock, 1992; Shumway & Koide, 1995; Facelli & Facelli, 2002). For example, Facelli & Facelli (2002) found mycorrhizas to increase size inequality of Trifolium subterraneum, and they attributed size inequality to asymmetric competition for phosphorus through mycorrhizas. Allsopp & Stock (1992) investigated density-dependent intraspecific competitive interactions among separate populations of Otholobium birtum and Aspalathus linearis and attributed greater size inequality after mycorrhizal inoculation to early germinants experiencing rapid mycorrhiza formation and consequent growth that resulted in the suppression of other individuals. Although Shumway & Koide (1995) did not find mycorrhizas to contribute to size inequalities of whole-plant dry weight (DW), they did find that mycorrhizas contributed to inequality in fecundity.

In contrast to the preceding studies, others (Turner & Rabinowitz, 1983; Ayres et al., 2006) have found neither elevated plant density nor mycorrhizal inoculation to increase size inequality within populations. Turner & Rabinowitz (1983) observed that size distributions of crowded Festuca paradoxa did not differ from those of isolated plants. They attributed a lack of right skew at high density to minimal aboveground competition because of F. paradoxa’s erect, graminoid growth form, and they contended that competition was principally below ground and symmetric. Ayres et al. (2006) investigated the effects of both density and AM fungi on size distributions of Plantago lanceolata and found high density populations to have more equitable size distributions than those experiencing little competition, with mycorrhizas having no effect on size inequality. Ayres et al. (2006) speculated that belowground competition among dense, mycorrhizal P. lanceolata was symmetric and the distribution of resources among them was relatively uniform, which is similar to the conclusions of Turner & Rabinowitz (1983).

None of the previously cited studies directly investigated CMNs. Instead, when involving AM, they compared inoculated plants with those without mycorrhizas. Alternatively, we decided to inoculate all plants with AM fungi and to isolate root systems while preventing or allowing CMNs to form, and repeatedly severing CMNs. We thereby assessed the effects of CMNs on size inequality of high density seedling populations of Andropogon gerardii, a strongly mycotrophic (Hartnett et al., 1993; Hartnett & Wilson, 1999), dominant mid-western prairie grass. By extrapolating in vitro findings that hint that CMNs might unequally distribute mineral nutrients among networked plants (Pfeffer et al., 2004; Lekberg et al., 2010; Hammer et al., 2011; Kiers et al., 2011), we hypothesized that CMNs would mediate intraspecific competition and consequently amplify plant size inequality.

Materials and Methods

We constructed wooden box microcosms as model systems in which to examine intraspecific interactions among young A. gerardii Vitman seedlings. We imposed three treatments upon
different microcosms with three replicate microcosms for each treatment: intact CMNs, severed CMNs and no CMNs (designated ‘controls’). Every seedling was grown individually in a Ray Leach Cone-tainer (2.5 cm diameter × 12.1 cm length; 49 ml volume; Tangent, Oregon, USA) which confined roots and prevented direct interactions among neighboring root systems. The control treatment comprised cone-tainers without modification, but cone-tainers that were intended to allow CMNs to form were modified by cutting two 2 × 5 cm slots in their opposite sides. We glued silk screen, nylon mesh cloth (40 μm pores) over both slots. Mycorrhizal fungus hyphae could grow freely through openings in the mesh, but roots could not. For the severed CMNs treatment, we manually rotated each cone-tainer through two complete revolutions twice a week, watering immediately after rotating to re-establish hydrological continuity. Intact CMN cone-tainers and controls were not rotated.

Microcosm establishment

The microcosms were 52 cm × 52 cm × 10 cm deep with plank sides and plywood bottoms. In each microcosm, we arranged cone-tainers in a 12 row by 12 column square array (Fig. 1). To precisely position each cone-tainer, the plywood bottom was drilled (1.9 cm diameter holes) to accept the conical bottoms of the cone-tainers, which thereby could drain externally. Surrounding the central 100 (10 rows by 10 columns) treatment plants were 44 nonmodified cone-tainers intended to mitigate aboveground edge effects. Each cone-tainer was 2.5 cm away from each of its four nearest neighbors.

We filled cone-tainers with a homogenized soil mixture of two parts relatively infertile sandy flatwoods soil from Archbold Biological Station (27°11′2.41″N, 81°20′55.66″W) and one part University of Miami Gifford Arboretum (25°43′26.03″N, 80°16′47.48″W) fertile soil (Table 1). This soil mixture ensured relatively low mineral nutrient availability in order to encourage competition among seedlings. The soil mixture had a pH of 7.3, a cation exchange capacity of 0.039 mEq g⁻¹ and a bulk density of 1.4 g ml⁻¹. In order to limit seedlings’ ability to acquire mineral nutrients elsewhere than within cone-tainers, we filled the interstices between them with infertile silica sand (Table 1). The sand consisted of a 2:1 mixture of 30–65 grade medium sand and 6–20 grade fine sand from Surface Prep Supply Co. (Miami, FL, USA). The interstitial sand had a pH of 8.1, a cation exchange capacity of 0.009 mEq g⁻¹ and a bulk density of 1.6 g ml⁻¹. The sand mixture in interstices tightly conformed to the cone-tainer sides and nylon mesh.

We inoculated every cone-tainer with AM fungi by collecting fine roots of Stenotaphrum secundatum (Walt.) Kuntze from a lawn in the Gifford Arboretum and then cutting the roots into 1–2 cm pieces by hand. We mixed these root pieces uniformly throughout the soil with which we filled the cone-tainers. This inoculum predominantly comprised Sclerocystis rubiformis, Glomus clarum and several unidentified AM fungal species of the genus Glomus sensu lato.

We fostered potential CMN formation through a pretreatment during which we grew transplanted *A. gerardii* in all cone-tainers within the microcosms for 8 wk after inoculation. At that time, *A. gerardii* seeds (Easy Wild Flowers Nursery, Willow Springs, MO, USA) were sown directly into the cone-tainers, which contained the predecessor, pretreatment plants. We began counting days after germination (DAG) when at least one germinant had appeared in every cone-tainer. Fourteen DAG, we clipped the pretreatment plants below their basal meristems to eliminate them, and by similar clipping left only one most vigorous germinant in each cone-tainer. Thus, the microcosms had a total of 10 wk of pretreatment plant growth during which to establish interconnecting CMNs, similar to the time allowed for CMNs to establish in other studies (Johnson et al., 2001; Walder et al., 2012).

All nine microcosms were randomized on benches in a greenhouse at the University of Miami. We randomized them again 42 DAG. Microcosms were watered daily by hand. A preliminary experiment suggested that *A. gerardii* might become nitrogen deficient in the soil mixture, so at 42 DAG, we began to add 10 ml of a 30 ppm KNO₃ solution to every individual cone-tainer once a week until harvest. Initial ammonium and nitrate concentrations of the soil mixture are shown in Table 1.

Measurements and harvest

Beginning 14 DAG, we measured weekly the length of the longest leaf of each experimental seedling (excluding those in the
buffer rows) from the leaf sheath to the leaf tip. We harvested at 94 DAG to prevent plants from becoming root-bound in the cone-tainers. We clipped shoots directly above the basal meristem and dried them to constant weight at 60°C. The dried tissues for each individual were weighed before composing them into eight groups, or samples, in order to ensure that sufficient tissue was available within each sample for element analysis by the Kansas State Agronomy Soil Testing Laboratory (Manhattan, KS, USA). Rather than randomly assigning individuals to each of the eight groups, we composited individuals according to whole-plant DW. Each sample was compiled by rank ordering whole-plant DWs of all surviving plants from the three replicate microcosms to every second group which resulted in 31 among the groups (e.g. four extra plants were distributed as one to every second group) which resulted in 31–35 plants per octile. Thus, for example, the upper (eighth) octile contained the largest 12.5% of all plants within a treatment.

We removed root systems from the cone-tainers, rinsed them in gently running water over a 1 mm sieve, and preserved them in 50% ethanol until we finished the harvest. The root systems were then blotted dry and each root system was weighed to determine its total moist weight. After randomly removing a subsample of fine roots from each root system and preserving them in 50% ethanol for later assessment of mycorrhizal colonization, we again weighed the remaining roots before placing them in an oven at 60°C to dry to constant weight. We weighed the dried roots and used the DW to moist weight ratio to calculate the DWs of entire root systems.

For assessment of percentage colonized root length by AM fungi, we composited root systems into the identical eight octiles per treatment as for shoot tissue. We cleared the roots in 10% KOH at room temperature for 5 d, acidified them in 5% HCl for 30 min and then placed them in 0.05% Trypan blue in lactoglycerol for 15 h at room temperature to stain AM fungi. For each octile, we mounted 25 1–2 cm root segments on microscope slides and scored mycorrhizal colonization by using the magnified gridline intersection method (McGonigle et al., 1990), examining 250 intersections per octile.

**Table 1** Soil mineral nutrient concentrations and contents of the sand mixture surrounding cone-tainers in a microcosm and for the soil within the 100 cone-tainers in a microcosm

<table>
<thead>
<tr>
<th>Soil characteristic</th>
<th>Concentration (ppm)</th>
<th>Content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interstitial sand</td>
<td>Soil (within cone-tainers)</td>
</tr>
<tr>
<td>Ammonium</td>
<td>2.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1.0</td>
<td>15.6</td>
</tr>
<tr>
<td>Olsen phosphorus</td>
<td>1.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Potassium</td>
<td>7.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>155</td>
<td>1505</td>
</tr>
<tr>
<td>Magnesium</td>
<td>25.0</td>
<td>68.0</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

¹Samples were analysed by the Kansas State University Soil Testing Laboratory, Manhattan, KS, USA.

**Statistical analyses**

We analysed differences in percentage colonized root length by AM fungi among treatments with a one-way analysis of covariance (ANCOVA) using octile position (first through eighth) as the covariate after examining heteroscedasticity with Levene’s test. To compare the relationships between mean whole-plant DW per octile and per cent colonized root length among treatments, we used least-squares linear regression. We also used ANCOVAs to detect treatment differences in mean leaf tissue element concentrations and contents (= concentration × total whole leaf dry weight of an octile/number of individuals in the octile). We used least-squares linear regression to compare the relationships between whole-plant DW per octile and concentrations of phosphorus and manganese. For both elements, because the control and severed CMNs treatments did not differ from one another, we combined their data for comparison with the intact CMNs treatment.

To compare plant growth throughout the experiment, we used a one-way, repeated measures analysis of variance (ANOVA) of longest leaf length per microcosm (n = 9) followed by a least significant difference (LSD) post-hoc test at α = 0.05. We used Levene’s test to examine heteroscedasticity. We similarly used one-way, repeated measures ANOVAs to investigate longest leaf length size hierarchy differences among treatments over time after establishing that the distributions of whole-plant DWs at harvest did differ among treatments (all replicates combined within treatments) with three pairwise Kolmogorov–Smirnov tests that were Bonferroni-corrected (α = 0.0166). We examined the following size hierarchy descriptors: standard deviation, Gini mean of differences, Gini coefficient and Lorenz coefficient of asymmetry for longest leaf lengths. We also calculated all these descriptors for aboveground and whole-plant DW and tested them for differences among treatments by one-way ANOVAs and LSD post-hoc tests after using corrected Kolmogorov–Smirnov tests to establish that distributions differed. All the aforementioned statistical analyses were conducted with STATISTIX v. 9.0 (Analytical Software, Tallahassee, FL, USA).

We calculated the size hierarchy descriptors Gini mean of differences, Gini coefficient and Lorenz coefficient of asymmetry for each microcosm with a Wolfram Mathematica v. 8.0
(Champaign, IL, USA) notebook (Damgaard & Weiner, 2000; http://mathworld.wolfram.com/GiniCoefficient.html). The Gini mean of differences is a measure of dispersion. It is the arithmetic average of the differences between all possible pairs of individuals within a population (Weiner & Solbrig, 1984). Gini coefficients represent the inequality of a distribution, with a minimum value of 0 indicating that all plants within a population are uniform in size and a maximum value of 1 indicating maximum inequality (Damgaard & Weiner, 2000). The Gini coefficient is based on the Lorenz curve that graphically represents the distribution of a population by ranking individuals from smallest to largest and then plotting the cumulative percentage of a size parameter against the cumulative percentage of individuals (Weiner, 1985).

A population of uniformly sized individuals would produce a straight line, while inequality in the population causes the line to curve below the line of equality. Inequality within a population is reflected by the Lorenz asymmetry coefficient, with values below or above 1.0 indicating right or left skew, respectively (Damgaard & Weiner, 2000).

We wished to examine our data for evidence of asymmetric competition, such as dominance by large plants being associated with suppression of their neighbors (i.e. a negative correlation between plant and neighbor size). In order to summarize the patterns of covariation among whole-plant DWs within our treatments, we performed separate, but procedurally identical, principal components analyses (PCA) for each treatment using the software PC-ORD v. 6.07 (McCune & Mefford, 2011). For each treatment, we examined associations between all surviving individuals (=‘targets’) and PCA first axes derived from variance/covariance cross-products matrices. We used covariances in order to center variables while giving weight to divergent values. We ranked ordered by DW the four nearest neighbors of each target and separately rank ordered the four diagonal neighbors of each target (see Fig. 1). We then used these rank categories for both distances as eight ‘neighbor’ variables. For the lowest ranks, at both distances, dead plants resulted in zeros and we eliminated three variables with >15% zeros (among the 900 experimental plants of all treatments) because zeros were not informative for our analyses (i.e. zeros did not capture time of death and hence, did not reflect how long a neighbor and target might have competed before the neighbor’s death). Five neighbor variables were retained: three nearest neighbors excluding only the smallest and two diagonal neighbors excluding the two smallest. We tested the significance of PCA axes by randomization tests with 999 runs.

After performing PCA, we rotated each ordination so that Axis 1 was positively associated with the second-largest, nearest neighbor (the middle of the three nearest-neighbor variables), and then calculated Pearson correlations between the targets and Axis 1.

Results

Mycorrhizal colonization and leaf tissue composition

Mycorrhizal colonization differed among treatments ($F_{2,20} = 60.27$, $P < 0.0001$). *Andropogon gerardii* seedlings with intact CMNs had the greatest mean colonization (71.1%). Severing CMNs significantly reduced mean colonized root length to 65.2%. Control, nonmodified cone-tainers further significantly reduced colonization to 47.9%. When we regressed mean whole-plant DW by octile against per cent colonized root length (Fig. 2) relativized by treatment mean, the regression was significant ($F_{1,22} = 8.20$, $P = 0.009$). Although the slopes for the severed CMNs and control treatments did not differ ($F_{1,12} = 0.05$, $P = 0.827$), the slope for the intact CMNs treatment differed from those of both the control and severed treatments ($F_{1,12} = 9.67$, $P = 0.009$, $F_{1,12} = 12.29$, $P = 0.004$, respectively).

Among the elements that we assessed (Fig. 3), only phosphorus and manganese concentrations differed significantly among treatments (P: $F_{2,20} = 18.05$, $P < 0.0001$; Mn: $F_{2,20} = 18.94$, $P < 0.0001$). All three treatments differed from one another by LSD post hoc test for both phosphorus and manganese, and seedlings with intact CMNs had the highest mean concentrations followed successively by the controls and those with severed CMNs (Fig. 3). All treatments combined showed a decrease in plant size with increasing phosphorus concentration ($F_{1,22} = 5.90$, $P = 0.024$), but showed an increase in plant size with increased manganese concentration ($F_{1,22} = 13.10$, $P = 0.002$). For phosphorus, severed CMNs and the control treatments slopes did not differ ($F_{1,12} = 0.67$, $P = 0.430$; Fig. 4a). When those two treatments were combined, however, their slope differed significantly from that of the intact CMNs treatment ($F_{2,20} = 27.45$, $P = 0.0001$). For manganese also, the severed CMNs and the control treatments slopes did not differ ($F_{1,12} = 0.48$, $P = 0.501$), but when combined, their slope was significantly exceeded by that of the intact CMNs treatment ($F_{2,20} = 10.58$, $P = 0.004$). Mean element contents for intact CMNs shoots significantly exceeded those with severed CMNs.

![Fig. 2 Andropogon gerardii mean whole-plant dry weight (DW; g) per octile group vs per cent colonized root length (%CRL) for control (open diamonds), severed common mycorrhizal networks (CMNs; tinted squares) and intact CMNs (closed triangles) treatments. The slopes of the linear regressions differed among treatments ($F_{1,18} = 7.20$, $P = 0.005$) with the intact CMNs treatment (DW = $1.05 \times \%CRL - 66.9$) differing significantly from both severed CMNs (DW = $0.19 \times \%CRL - 7.4$) and control treatments (DW = $0.16 \times \%CRL - 1.5$), which did not differ from one another ($F_{1,12} = 0.05$, $P = 0.803$).]
for all elements except iron and zinc (see the Supporting Information, Table S1) when not Bonferroni corrected.

Longest leaf lengths

At the first measurement 14 DAG, mean longest leaf length within microcosms did not differ significantly ($F_{2,6} = 4.19$, $P = 0.073$) among treatments, but during the entire 94 d experiment, there was a treatment main effect on mean longest leaf length (Table 2, Fig. 5). Mean longest leaf lengths of seedlings with intact CMNs exceeded those of seedlings in the severed CMNs and control treatments. There also was a treatment $\times$ time interaction (Table 2, Fig. 5).

Treatment produced significant main effects on size hierarchy descriptors based on longest leaf lengths for standard deviation and Gini mean difference (Table 2). The intact CMNs standard deviation and Gini mean difference were greater than those of the severed CMNs and control treatments. Treatment interacted significantly with time for the Gini ratio (Table 2). The Lorenz asymmetry coefficient did not differ among treatments either as main effects or interaction with time. Fig. 6 illustrates that while size hierarchy distributions did not differ among treatments 21 DAG, by 94 DAG the distribution of longest leaf lengths for plants with intact CMNs differed from those with severed CMNs (Kolmogorov–Smirnov two tailed test statistic $= 0.21$, $P = 0.0001$) and from the controls (Kolmogorov–Smirnov two tailed test statistic $= 0.18$, $P = 0.0002$), but the last two treatments’ distributions did not differ from one another (Kolmogorov–Smirnov two tailed test statistic $= 0.04$, $P = 1.00$; Fig. 6).

Dry weights

At harvest, mean shoot DWs differed significantly among treatments (Table 2) with the intact CMNs treatment exceeding the other two treatments, which were identical. Size distributions of shoot DW for the intact CMNs treatment also differed from the severed CMNs (Kolmogorov–Smirnov two tailed test statistic $= 0.31$, $P = 0.0001$) and control treatments (Kolmogorov–Smirnov two-tailed test statistic $= 0.25$, $P = 0.0001$). Severed CMNs and control treatments size distributions did not differ from one another (Kolmogorov–Smirnov two tailed test statistic $= 0.06$, $P = 0.717$). Shoot DW standard deviations, Gini mean differences and Gini ratios all differed significantly among treatments (Table 2). The intact CMNs treatment had greater mean standard deviation and Gini mean differences than the severed CMNs and control treatments, which did not differ from one another. For the Gini ratio of shoot dry weight, the intact CMNs treatment differed significantly from the severed CMNs treatment, but neither differed significantly from the controls, which were intermediate (Table 2).

Whole-plant DW size hierarchies differed among treatments similarly to shoot DWs. The intact CMNs treatment differed significantly from both severed CMNs (Kolmogorov–Smirnov...
two-tailed test statistic = 0.31, $P = 0.0001$) and controls (Kolmogorov–Smirnov two-tailed test statistic = 0.24, $P = 0.0001$), which did not differ from one another after Bonferroni correction Kolmogorov–Smirnov two-tailed test statistic = 0.13, $P = 0.0270$). Whole-plant DW means, standard deviations, Gini mean differences and Gini ratios (Table 2) were affected significantly by treatments. For those descriptors, the intact CMNs treatment exceeded the severed CMNs and control treatments, which did not differ from one another (Table 2).

The PCA results for each treatment are summarized in Table 3. Randomization tests showed first axes to represent more variation than expected by chance for all three PCAs, but the second axis was significant only for the intact CMNs treatment. Rotation of ordinations maintained 90.4%, 90.5% and 99.0% orthogonality between Axes 1 and 2 for control, severed CMNs and intact CMNs treatments, respectively. Rotation improved the percentage of variance represented by Axis 1 for the control and severed CMNs treatments, but resulted in Axis 2 representing more variance than Axis 1 for the intact CMNs treatment. Nevertheless, Axis 1 continued to represent 41.7–59.3% of the variance among neighbors for all treatments. As intended, rotation caused the second-largest, nearest neighbor to be strongly associated with Axis 1 in all three PCAs (Pearson’s $r$ ranged from 0.725 to 0.834). For the severed CMNs and intact CMNs treatments, however, the largest nearest-neighbors were the most strongly associated with Axis 1. For the controls, the third-largest, nearest neighbor was most strongly associated with Axis 1. Neighbors on the diagonals always had the weakest associations with Axis 1. Target plant correlation with the first axes of the three PCAs was positive for the control ($r = 0.045$) and severed CMNs ($r = 0.171$) treatments, but was negative ($r = -0.220$) for the intact CMNs treatment.
Discussion

Our study suggests that size hierarchy development within *A. gerardii* monocultures was influenced by hyphal interconnections in the form of CMNs. Although we lack direct evidence for CMNs, three results strongly support that CMNs formed in our microcosms: despite similar initial inoculation of all treatments, plants in the ‘intact CMNs’ treatment had the greatest colonized root length; the most likely source of the potentially limiting mineral nutrient, manganese, was container soil; and

![Figure 6: Size frequency distributions of *Andropogon gerardii* seedlings based on longest leaf lengths 21 d and 94 d after germination (DAG), shoot dry weight (DW) and whole-plant DW at harvest 94 DAG for control, severed and intact common mycorrhizal networks (CMNs) treatments. Superimposed normal curves facilitate visual comparison but do not imply that the distributions are normal. By 94 DAG, the distribution of longest leaf lengths for plants with intact CMNs differed from those with severed CMNs (Kolmogorov–Smirnov two-tailed test statistic = 0.21, *P* = 0.0001) and from the controls (Kolmogorov–Smirnov two-tailed test statistic = 0.18, *P* = 0.0002), but the last two treatments’ distributions did not differ from one another (Kolmogorov–Smirnov two-tailed test statistic = 0.04, *P* = 1.00). At harvest, shoot and whole-plant dry weight of plants with intact CMNs significantly differed from both those with severed CMNs and controls (Shoot DW: Kolmogorov–Smirnov two-tailed test statistic = 0.31, *P* = 0.0001, and Kolmogorov–Smirnov two-tailed test statistic = 0.25, *P* = 0.0001, respectively; Whole-plant DW: Kolmogorov–Smirnov two-tailed test statistic = 0.31, *P* = 0.0001, Kolmogorov–Smirnov two-tailed test statistic = 0.24, *P* = 0.0001, respectively) the last two of which did not differ significantly from one another after Bonferroni correction (Shoot DW: Kolmogorov–Smirnov two tailed test statistic = 0.06, *P* = 0.717; Whole plant DW: Kolmogorov–Smirnov two-tailed test statistic = 0.13, *P* = 0.0270).

![Table 3: Principle components analysis (PCA) proportion of variance represented by the first two axes (*r*² = coefficient of determination), correlations (Pearson’s *r*) with Axis 1 for the five PCA summarized *Andropogon gerardii* neighbor variables, and the correlation between Axis 1 and target *A. gerardii* whole-plant dry weights for control, severed common mycorrhizal networks (CMNs) and intact CMNs treatments.](image)

1The probability is from a randomization test with 999 runs.
2Axis 1 was rotated to maximize congruence with the second-largest, nearest neighbor for each PCA.
competitive interactions suggested by a negative correlation between plant DWs and those of their nearest neighbors could only be found under conditions in which CMNs, if formed, were likely to have remained intact.

Plants grown in the intact CMNs treatment had greater colonized root length than those with severed CMNs or controls, and of the last two treatments, colonization of severed CMNs roots exceeded that of the controls (Fig. 2) even though the sand between cone-tainers was never inoculated. The elevated mycorrhiza formation by plants in both slotted cone-tainer treatments was probably a consequence of hyphal spread among cone-tainers. Nevertheless, repeated severing somewhat retarded hyphal spread among cone-tainers, resulting in less root colonization, on average, than when hyphae were not severed.

**CMN influence on mineral nutrition**

High root colonization of plants with intact CMNs by itself is unlikely to have accounted for that treatment’s significantly highest mean plant DW because there was substantial overlap in both mean per cent colonized root length and whole-plant DWs of small individuals in both of the slotted cone-tainers treatments (Fig. 2). Instead, the steep increase in dry weight of intact CMNs plants with small increases in percentage colonized root length suggests that hyphae interconnecting cone-tainers improved mineral nutrition and plant growth. Hyphal networks of the intact CMNs treatment likely extended into neighboring cone-tainers where they could obtain disproportionate manganese for large host individuals. Although both cone-tainer soil and interstitial sand had low concentrations of available manganese, its concentration in the soil was 17 times higher than that of the sand, and the total manganese content in all cone-tainer soil potentially accessible to a CMN was approximately four times that in all of the sand within a microcosm (Table 1).

Plants in the intact CMNs treatment had significantly higher manganese and phosphorus concentrations than those in the other two treatments. However, as phosphorus concentration increased, mean whole-plant DW per octile decreased (Fig. 4a), suggesting a dilution effect of plant DW (Johnson et al., 1980; Estrada-Luna et al., 2000). By contrast, as manganese concentration increased, intact CMNs mean whole-plant DW per octile increased markedly (Fig. 4b), suggesting that manganese and not phosphorus was the primary growth-limiting mineral nutrient. Other studies also have found AM to increase host manganese concentrations (Ratti et al., 2010; Baslam et al., 2011). Ratti et al. (2010) grew Catharanthus roseus in treatments inoculated with different Glomus species vs controls lacking AM and found inoculation to increase plant manganese concentration, chlorophyll content and total plant DW. Baslam et al. (2011) similarly found that AM increased the manganese concentration and improved the growth of Lactuca sativa. Manganese plays a key role in electron transport in photosynthesis (Raven et al., 2005), so the increased concentration of manganese might positively feedback to AM fungi by enhancing host provision of fixed carbon, which could increase both root colonization and extraradical mycelium spread. In return, that might enhance the supply of mineral nutrients to hosts (Lekberg et al., 2010; Kiers et al., 2011). Such positive feedbacks likely contributed to the greater inequality in size distributions that we found for plants with intact CMNs vs those lacking persistent AM fungus interconnections.

**Size hierarchy development and competition**

Our model system separated the effects of CMNs from those of root interactions and demonstrated that intact CMNs contribute to skewing of A. gerardii seedling size distributions. Every size hierarchy descriptor that we found to differ significantly among treatments, whether derived from leaf lengths or DWs, distinguished plants with intact CMNs from those of the other two treatments, which consistently did not differ from one another. Although severing CMNs may have disrupted competition for water and mineral nutrients acquired via mass flow along hypha surfaces, the lack of statistically significant differences between the controls in nonmodified cone-tainers and the severed CMNs treatment suggests that mass flow into modified cone-tainers after hyphal severing did not substantially influence our results. It also suggests that even though glomeromycotan fungi can exhibit hyphal anastomoses (Giovannetti et al., 1999) and wound healing (Gerdemann, 1955), rotation twice weekly was sufficient to disrupt CMN function.

Both longest leaf length distributions and DW distributions (Fig. 6) revealed size hierarchy differences, although leaf lengths were less sensitive in revealing those differences than were dry weights. Nevertheless, leaf length measurements revealed size hierarchy shifts through time, and despite not reflecting numbers of leaves, differences in longest leaf length distributions agreed well with both shoot and whole-plant DW distribution differences. For example, among the size hierarchy descriptors based upon the Lorenz curve, the Gini mean of differences and Gini coefficient revealed significant treatment main effects or interactions with time for both leaf lengths and DWs. By contrast, Ayres et al. (2006) found no effect of mycorrhizas on whole-plant DW Gini coefficients for dense P. lanceolata, but their dense monocultures comprised only 25% as many individuals in total as ours, which might have made an advantage to just a few individuals hard to detect.

The greater inequality of size distributions that were found with intact CMNs reflected more large plants in the presence of intact CMNs than in the other two treatments. Across all three treatments, the smallest plants remained similar in size (Fig. 6) even as they continued to grow throughout the experiment (data not shown). Nevertheless, our target–neighbor PCAs indicate that intact CMNs did mediate a negative association between plants and their neighbors that did not appear when belowground interactions across CMNs either were prevented (controls) or disrupted (severed CMNs). Such suppression of their neighbors by dominant, large plants likely amplified the size inequality within the intact CMNs treatment, and furthermore, it suggests that belowground competition was asymmetric across intact CMNs.
Conclusions

We conclude that CMNs contributed to seedling population size inequality through positive feedbacks between mycorrhizal formation, mineral nutrient uptake and host growth. Although our model system was less controlled than recent root organ culture work it did provide physiological and environmental realism to common mycorrhizal network function. Our results are consistent with studies that have found AM fungi to contribute to size inequality as a result of differences in mycorrhizal colonization (Allsopp & Stock, 1992) and rapid uptake of mineral nutrients by AM fungi (Allsopp & Stock, 1992; Shumway & Koide, 1995; Facelli & Facelli, 2002). In our model system, even though the largest plants of the control and several CMNs treatments had the highest mean colonized root lengths of their treatments, they neither attained the size of the largest plants with intact CMNs nor were they associated with small neighbors. Instead, with intact CMNs, the negative association between targets and neighbors suggests competitive dominance and suppression uniquely across CMNs (Allsopp & Stock, 1992; Janouskova et al., 2011).

Our study prevented direct root system interactions among plants and thereby may have unmasked the contribution of CMNs to belowground competition. Both Turner & Rabinowitz (1983) and Ayres et al. (2006) failed to find differences in size hierarchies, which they attributed to little or no aboveground asymmetric competition and to symmetric competition below ground. In our study too, aboveground competition among A. gerardii seedlings was unlikely to have contributed to the observed differences in size hierarchies among treatments because of a graminoid growth form combined with a relatively small difference in mean longest leaf length among treatments (i.e. only c. 3.5 cm at the time of maximum difference). Despite this lack of strong, differential, aboveground effects we found that size hierarchies did differ among treatments. It is possible that direct, symmetric competitive interactions among root systems – which we prevented – tend to conceal the asymmetric effects of CMNs.

In nature, plants are likely inter-connected by CMNs, but neighboring root systems often overlap. Although many factors may influence whether belowground or aboveground, symmetric or asymmetric competition predominates among plants, our study suggests that CMNs can promote asymmetric competition below ground. Thus CMNs may have consequences for plant fitness (Weiner, 1990), natural selection and community assembly for plant species that recruit in dense, monospecific seedling stands.

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References


Supporting Information

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

Table S1 Mean element content differences in shoot tissues among intact common mycorrhizal network (CMNs), severed CMNs, and control treatments as indicated by ANCOVA main effects (F statistics and associated probabilities, P)

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