

LETTER

An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest

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Abstract

Tropical forests are renowned for their high diversity, yet in many sites a single tree species accounts for the majority of the individuals in a stand. An explanation for these monodominant forests remains elusive, but may be linked to mycorrhizal symbioses. We tested three hypotheses by which ectomycorrhizas might facilitate the dominance of the tree, *Oreomunnea mexicana*, in montane tropical forest in Panama. We tested whether access to ectomycorrhizal networks improved growth and survival of seedlings, evaluated whether ectomycorrhizal fungi promote seedling growth via positive plant–soil feedback, and measured whether *Oreomunnea* reduced inorganic nitrogen availability. We found no evidence that *Oreomunnea* benefits from ectomycorrhizal networks or plant–soil feedback. However, we found three-fold higher soil nitrate and ammonium concentrations outside than inside *Oreomunnea*-dominated forest and a correlation between soil nitrate and *Oreomunnea* abundance in plots. Ectomycorrhizal effects on nitrogen cycling might therefore provide an explanation for the monodominance of ectomycorrhizal tree species worldwide.

Keywords

Decomposition, Juglandaceae, mycorrhizal networks, mycorrhizal symbiosis, Panama, stable isotopes.

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INTRODUCTION

Tropical forests are renowned for high local species richness, often exceeding 100 tree species ha⁻¹ (Wright 2002). Nonetheless, exceptions to the general pattern of high diversity and low relative abundance exist throughout the tropics. These ‘monodominant’ communities, in which a single tree species accounts for > 60% of basal area (Hart *et al.* 1989), include *Dicymbe corymbosa* forests in Guyana, *Gilbertiodendron dewevrei* forests in central Africa and some dipterocarp forests in Southeast Asia (e.g. *Dryobalanops aromatica*, *Shorea curtisii*). Several potential mechanisms that promote monodominance in tropical forest have been proposed, but a general explanation remains elusive (Peh *et al.* 2011a).

Mechanisms to explain monodominance have focused either on the disturbance regime of the forest, with low disturbance rates favouring competitive exclusion (Connell & Lowman 1989; Hart *et al.* 1989), or on intrinsic traits of the species that confer a competitive advantage, including low palatability to herbivores, slow rates of leaf litter decomposition, high seedling shade tolerance, and large seed size (Hart *et al.* 1989; Torti *et al.* 2001). However, a feature of many monodominant species, both temperate and tropical, is that they form associations with ectomycorrhizal (EM) fungi (Connell & Lowman 1989). Only 6% of neotropical tree species are estimated to form associations with EM fungi, while 94% associate with

arbuscular mycorrhizal (AM) fungi (Table S1). In contrast, of the 22 tree species reported as monodominant by Peh *et al.* (2011a), 10 (45%) are EM and 12 (55%) are AM. As a consequence, mechanisms have been sought that could account for how EM-associated plants achieve monodominance at sites with similar soil conditions and disturbance regimes as those that support diverse communities of AM-associated trees (Connell & Lowman 1989; Dickie *et al.* 2014).

Two mechanisms have been proposed for the EM facilitation of local monodominance. First, EM networks consisting of hyphal connections linking plants could facilitate the transfer of water, carbon (C) and nutrients from adults to seedlings (Simard *et al.* 2012). Ectomycorrhizal networks are predicted to increase juvenile survival, disproportionately increasing their abundance near adult trees (Teste *et al.* 2009; Simard *et al.* 2012). Consistent with this hypothesis, seedlings of the monodominant species *Paraberlinia bifoliolata* in Cameroon and *Dicymbe corymbosa* in Guyana grew more slowly and died more frequently when isolated from potential EM networks using exclosures (Onguene & Kuyper 2002; McGuire 2007). However, these studies provided no direct evidence that EM networks transfer nutrients or photosynthate. Moreover, neither study included a non-mycorrhizal plant species to assess exclosure effects unrelated to mycorrhizal associations.

Second, recent reviews hypothesise that monodominance may arise through microbially mediated positive plant–soil

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feedbacks (McGuire 2014). Plant–soil feedbacks (PSF) mediated by EM fungi could lead to monodominance in two ways. First, the build-up of beneficial EM fungi around adult host trees might favour the growth and survival of conspecific seedlings relative to those of neighbouring non-ectomycorrhizal seedlings. This positive plant–soil feedback is expected to promote the dominance of EM plant species (Bever *et al.* 1997). Second, EM fungi could weaken the strength of negative PSF produced by species-specific pathogens and increase the survivorship rates of EM seedlings around conspecific adult trees (Bever 2003). Plant species that exhibit weak negative feedbacks often dominate the community (Klironomos 2002; Mangan *et al.* 2010), yet no experimental study has examined the strength of PSF in tropical EM tree species.

In addition to network effects and altered plant–soil feedback, EM fungi might also facilitate monodominance by altering N cycling, with detrimental effects on competing AM plant species. Recent research has highlighted mycorrhizal association as an important functional trait influencing species interactions and ecosystem processes (Phillips *et al.* 2013; Averill *et al.* 2014). Here, we propose that EM associations facilitate monodominance via the ‘microbial competition for N hypothesis’ (referred to as the ‘organic nutrient-use hypothesis’ in Dickie *et al.* 2014). Ectomycorrhizal fungi have the enzymatic capability to access organic N directly from soil organic matter, including the litter layer (Hobbie & Högberg 2012), implying that they compete directly with saprotrophs for N (Orwin *et al.* 2011; Hobbie & Högberg 2012; Averill *et al.* 2014). This enhanced competition is expected to reduce litter decomposition and N mineralisation rates in the presence of EM fungi compared to AM fungi (Connell & Lowman 1989; McGuire *et al.* 2010; Phillips *et al.* 2013). Slower decomposition rate and reduced N availability could, in turn, favour EM plants when competing with AM plants (Orwin *et al.* 2011; Phillips *et al.* 2013). Consistent with this hypothesis, lower concentrations of total N, ammonium and nitrate have been reported below stands of monodominant forest relative to nearby mixed forest (Torti *et al.* 2001; Read *et al.* 2006; Brookshire & Thomas 2013; Dickie *et al.* 2014).

Here, we evaluated these three potential mechanisms mediating monodominance in a lower montane tropical forest in Panama. We used a combination of field and growing house experiments to test for mycorrhizal networks, plant–soil feedback, and changes in the N cycle in forest dominated by *Oreomunnea mexicana* (Juglandaceae), a widely distributed EM tree species that forms monodominant forest. We predicted that if dominance is promoted by mycorrhizal networks, then disruption of hyphal networks would negatively affect *Oreomunnea* seedling growth and survival and alter leaf tissue and leaf sugar C isotope ratios. In contrast, if dominance is mediated by plant–soil feedback, then we predicted that *Oreomunnea* seedling growth would either be increased in the presence of soil inoculum from beneath *Oreomunnea* trees relative to inoculum from competing species (positive PSF), or that the strength of negative PSF in the presence of conspecific inoculum would be weaker for *Oreomunnea* than for competitors. Finally, if dominance is mediated via the ‘microbial competition for N hypothesis’, then we predicted that mineral N supply would be reduced inside *Oreomunnea*-dominated forest, and that bulk

soil stable $\delta^{15}\text{N}$ isotope ratio – an integrated measure of the N cycle – would be smaller inside than outside *Oreomunnea*-dominated forest, indicating a tighter N cycle due to depletion of N from the soil organic matter pool by EM fungi.

METHODS

Study site

The study was located in a primary lower montane forest (1000–1400 m a.s.l.) in the Fortuna Forest Reserve in western Panama (hereafter Fortuna; 8°45' N, 82°15' W). Mean annual temperature ranges from 19 to 22 °C, and annual rainfall from 5800 to 9000 mm (Andersen *et al.* 2012). Tree communities at Fortuna are diverse, containing between 61 and 153 species ha⁻¹ (> 10 cm DBH), with high compositional turnover reflecting variation in both rainfall and soils. *Oreomunnea mexicana* is a canopy tree distributed between Mexico and Panama at elevations from 900 to 2600 m. The distribution of *Oreomunnea* at Fortuna is patchy, with populations occurring on both high and low fertility soils (Corrales *et al.* 2015). On low fertility rhyolite-derived soils, mixed forest is interspersed within *Oreomunnea*-dominated forest, where it accounts for up to 70% of individuals and basal area (Andersen *et al.* 2010; Corrales *et al.* 2015). Dominance by *Oreomunnea* appears unrelated to particular plant functional traits previously associated with monodominance: foliar N, foliar phosphorus (P) and C : N ratios are close to community averages for the site (Adamek 2009) and seeds are small (c. 100 mg). However, *Oreomunnea* forms EM associations (Corrales *et al.* 2015), in contrast to almost all co-occurring tree species at Fortuna. Other EM tree species found at low abundance at the study area are *Quercus insignis*, *Q. cf. lancifolia* and *Coccoloba* spp.

Experimental assessment of EM network effects

We established a hyphal exclusion experiment to test whether *Oreomunnea* seedlings benefit from EM hyphal connections to neighbouring plants. *Roupala montana* (Proteaceae), a non-mycorrhizal tree species that co-occurs with *Oreomunnea*, was used to assess the non-mycorrhizal treatment effects of exclusions on seedling growth. *Oreomunnea* and *Roupala* seedlings were planted in mesh exclusions inside and outside a ~ 25 ha *Oreomunnea*-dominated forest. Individual seedlings were transplanted 2 m from each of 20 randomly selected *Oreomunnea* trees (blocks) inside the *Oreomunnea* forest and 10 similar-sized heterospecific trees 30 m outside the edge of the forest. Nearest-neighbour focal trees inside the *Oreomunnea*-dominated forest were on average 40 m apart; trees outside were on average 45 m apart.

Treatments consisted of: (1) fungal hyphal exclusions with 0.5 μm mesh to prevent seedlings from connecting to EM networks; (2) root exclusions with 35 μm mesh to exclude roots of neighbouring plants, but allow hyphae access to the transplanted seedling (this treatment was used to assess the effect of reduced root competition in the analysis of EM network effects); (3) transplants without an enclosure, to assess the effect of soil disturbance on seedling growth (the seedlings

and surrounding soil was removed and replaced in a similar way to treatments 1 and 2); (4) control: A naturally occurring *Oreomunnea* seedling of similar size growing next to the other treatments, but left untouched for comparison (inside *Oreomunnea* forest only). A total of 202 seedlings were established, 112 *Oreomunnea* (30 replicates \times 3 enclosure treatments, and 22 control seedlings) and 90 *Roupala* (30 replicates \times 3 enclosure treatments, no control). Mesh enclosures were assembled following Nottingham *et al.* (2010) from 16 cm diameter, 20 cm deep PVC piping, perforated along the sides and covered on the bottom and sides with the nylon mesh corresponding to each treatment (Plastok, Birkenhead, UK).

Seedlings were transplanted from a nearby forest into the enclosure treatments between August and October of 2013 and grown for 297–345 days. Previous studies that have reported effects of EM networks on seedling mortality and RGR grew plants for between 5 and 12 months (Onguene & Kuyper 2002; McGuire 2007). We therefore expected that 10–11 months would be sufficient time to detect differences in our experiment. Seedlings were harvested and the relative growth rate (RGR, $\text{mg g}^{-1} \text{day}^{-1}$) was calculated as the natural log of final dry mass minus the natural log of initial dry mass divided by the number of days in the experiment. To estimate initial seedling biomass, 20 and 15 seedlings of *Oreomunnea* and *Roupala* from outside of the experiment were harvested, and height, leaf number and leaf area measured to develop biomass models (Table S2). In a subset of the surviving *Oreomunnea* seedlings growing inside ($n = 32$) and outside ($n = 8$) a sample of root tissue was saved to evaluate EM colonisation by clearing in 10% KOH and staining with trypan blue. Colonisation of 10 randomly chosen 2.0-cm root sections per seedling was assessed using the grid-line intersect method (McGonigle *et al.* 1990). Fresh leaf tissue was frozen immediately in liquid N for isotopic analysis. In tropical trees with long leaf life spans, foliar isotope ratios can reflect nutrient uptake over months or years, and may mask any signal of fungal C dependency (Hynson *et al.* 2012). We therefore analysed leaf soluble sugars, which represent recently acquired C. Sugars were extracted using ion exchange resins for sugar purification following Hynson *et al.* (2012). In addition, leaf tissue from a subset of the surviving *Oreomunnea* seedlings growing inside ($n = 16$) and outside ($n = 12$) *Oreomunnea*-dominated forest was analysed for C and N concentrations and stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) by continuous flow isotope ratio mass spectrometry using an elemental analyser (Costech Analytical, Valencia, California 4010) coupled to a Delta-V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Run precision for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was typically $< 0.2\text{‰}$.

Plant–soil feedback experiment

To determine whether *Oreomunnea* shows positive or negative PSF compared with co-occurring species, seedlings of five tree species were grown in a fully factorial greenhouse experiment. The species included were two EM plant taxa, *Oreomunnea mexicana* (Juglandaceae) and *Quercus insignis* (Fagaceae), and three AM species, *Guarea pterorhachis* (Meliaceae), *Cupania*

seemannii (Sapindaceae) and *Nectandra purpurea* (Lauraceae). Species were chosen based on seed availability and contrasting abundance in permanent 1-ha forest plots. Species relative abundance of individuals with a diameter at breast height (DBH) > 10 cm ranged from 0.3% for *Quercus* to 7.7% for *Oreomunnea*.

Seedlings were grown in different soil treatments: each species was grown either in their own soil inoculum ('conspecific' soil treatment), where pots received an inoculum of live soil from underneath conspecifics representing 6% of the total soil volume (120 cm^3), or in pots inoculated with live soil from one of each of the other four species separately ('heterospecific' soil treatment). Seven seedlings of each species were grown in each of the 'heterospecific' treatments and 10 were grown in the 'conspecific' treatment. To control for differences in abiotic conditions associated with the live soil, two additional replicates of each treatment were established in which the inoculum was sterilised (Fig. S4). A total of 240 sampling units were used for the full experiment ('heterospecific' treatments = 5 species \times 4 inoculum types \times 7 replicates = 140 seedlings; 'conspecific' treatments = 5 species \times 10 replicates = 50 seedlings; sterilised inoculum controls = 10 replicates \times 5 species = 50 seedlings).

Between June and October 2013, seeds of the five species were collected from the forest floor, surface sterilised with 1% NaClO and grown in sterile sand for 2 months prior to the start of the experiment. *Oreomunnea* seeds were also soaked in 1% HCl for 1 h prior to planting to break dormancy. Each species was grown in a 2-L pot (14 cm \times 11 cm \times 13 cm depth) filled with steam-pasteurised soil collected from three relatively nutrient-rich sites in the Fortuna Reserve. Seedlings were planted during November and December 2013 and grown under 18% full sun in a growing house at the Fortuna Forest Reserve. Inoculum was collected from underneath five adult individuals of each species (about 500 g of soil per tree) and inoculum from each tree was used separately to keep track of individual inoculum sources. *Nectandra* seedlings were grown for 3 months, while the remaining species were grown for 5–6 months depending on growth rate. Plants were watered once or twice per week and did not receive additional nutrients.

At harvest, a subsample of root tissue was saved to evaluate EM colonisation using the same method described above. The roots of all plants were washed and all plant parts were dried at 70 °C for 48 h and weighed to quantify total biomass. To calculate leaf area, photographs of all fresh leaves of each plant were analysed using ImageJ (Schneider *et al.* 2012). For individual plants, the RGR was calculated as above. Leaf area ratio (LAR) was calculated by dividing total leaf area by total biomass. To estimate the initial seedling biomass, 14–20 seedlings per species were harvested, and height, number of leaves and leaf area measured to develop biomass models (Table S2).

Nutrient availability and uptake inside and outside *Oreomunnea*-dominated forest

To determine whether *Oreomunnea* forest influences N cycling in a way that is consistent with the 'microbial competition for N hypothesis', we compared resin-extractable nitrate, ammo-

nium and phosphate inside and outside the same *Oreomunnea*-dominated forest used for the EM enclosure experiment. Twenty resin bags containing 5 g of mixed-bed anion and cation exchange resins (Dowex Marathon Mr-3 Supelco, Bellefonte, PA, USA) sealed inside 220 µm polyester mesh were buried 2 cm beneath the soil surface 0.5–1 m from 10 trees inside and 10 trees outside the *Oreomunnea* forest. Bags were buried during August 2014 at the same locations where mesh cores were installed. After incubation *in situ* for 18 days, the resin bags were collected, rinsed with deionised water to remove adhering soil, extracted with 75 mL of 0.5 M HCl, and then nitrate (+ nitrite), ammonium and phosphate were determined by automated colorimetry on a Lachat QuikChem 8500 (Hach Ltd., Loveland, CO, USA). In addition, soils collected inside ($n = 10$) and outside ($n = 8$) *Oreomunnea* forest were analysed for C and N isotope ratios and total concentrations as described above using a Flash HT analyser (Thermo Scientific, Waltham, MA, USA) coupled to a Delta-V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

Finally, we examined whether there was a relationship between tree abundance and soil properties. Abundance was calculated for the two EM tree species (*Oreomunnea mexicana* and *Quercus insignis*) and the four most abundant AM tree species (*Ardisia* sp., *Dendropanax arboreus*, *Cassipourea guianensis* and *Eschweilera panamensis*) as the sum of individuals > 5 cm DBH in 20 × 20 m² subplots located in two permanent 1-ha forest plots at the study site ($n = 18$ subplots; plots = Honda A and Honda B in Andersen *et al.* 2010). Soils data specific to each subplot were collected in 2008 to 10 cm depth, and included soil ammonium (µg N g⁻¹ soil dry mass), nitrate (µg N g⁻¹), total N (%), total C (%), total P (µg P g⁻¹) and litter mass (g m⁻²) (Table S3).

Statistical analysis

For the mesh enclosure study, a two-way ANOVA was used to compare seedling RGR, isotopic data and EM colonisation among treatments inside and outside *Oreomunnea* forest, with location (inside vs. outside) and mesh treatment (0.5 µm, 35 µm, transplant, and control) as fixed effects and replicates (trees) as a random effect. The mortality rates of seedlings were compared using contingency tables and a Chi-squared test of independence. Resin extractable nitrate, ammonium and phosphate were not normally distributed, and were compared between locations (inside vs. outside) using a Wilcoxon test.

We used ANCOVA to analyse the effects of inoculum source and plant species (and their interaction) on plant growth using the SAS procedure PROC MIXED. Estimated initial biomass was included as a covariate. Within this model, we used *a priori* contrasts to isolate the inoculum source × species interaction of each possible pair of species (Bever *et al.* 1997) to determine the strength and direction of plant–soil feedback. In the case where seedlings performed better in conspecific inoculum relative to that of heterospecifics, the interaction coefficient (i.e. pairwise feedback) would be positive. Average feedback strength of a given species was then determined by calculating the average of all pairwise interaction terms that involved those species (Bever *et al.* 1997). The relationship

between tree species abundance and strength of average feedback was examined using linear regression. The relative abundance of each species was calculated for trees > 10 cm DBH in permanent plots established where the species inoculum was collected (*Oreomunnea mexicana* = Honda A, *Cupania seemannii* and *Nectandra purpurea* = Pinola, *Quercus insignis* and *Guarea pterorhachis* = Hornito) (J.W. Dalling, personal communication).

Soil extractable C, N and δ¹⁵N were compared inside and outside the *Oreomunnea* forest using one-way ANOVA. The relationship between the number of trees or basal area in subplots and soil variables in permanent plots was analysed using a simple linear regression after (log + 1) transformation of the independent variable (individuals and basal area).

RESULTS

EM network effects

From an initial total of 202 seedlings established in the experiment (112 *Oreomunnea* and 90 *Roupala*), 173 survived until the end of the experiment: 86 *Oreomunnea* and 87 *Roupala*. Of the 86 surviving *Oreomunnea* seedlings, 70 seedlings grew inside and 16 outside *Oreomunnea* forest. There were no significant effects of hyphal and root enclosure treatments on growth rate of *Oreomunnea* seedlings, with the exception of faster growth in the root enclosure treatment (0.35 µm mesh) compared to the control (undisturbed) treatment ($F_{3,78} = 2.67$, $P = 0.05$) when including seedlings both inside and outside *Oreomunnea* forest (Fig. 1a). Therefore, excluding hyphae (0.5 µm mesh) did not reduce seedling growth as predicted. *Roupala* did not show significant differences among treatments ($F_{2,78} = 0.39$, $P = 0.68$; Fig. 1b).

Oreomunnea mortality was significantly lower inside (9%) vs. outside *Oreomunnea* forest (34%; $n = 112$, $\chi^2 = 19.8$, d.f. = 1, $P < 0.001$), but there was no significant difference in its growth rate ($F_{1,78} = 1.05$, $P = 0.31$; Fig. 1c). *Roupala* grew significantly faster outside *Oreomunnea* forest ($F_{1,78} = 6.02$, $P = 0.02$; Fig. 1d), but mortality rate did not differ (2% inside and outside; $n = 90$, $\chi^2 = 0.13$, d.f. = 1, $P = 0.71$).

Overall, there was no significant difference in EM colonisation of *Oreomunnea* seedlings among treatments ($F_{3,34} = 0.81$, $P = 0.50$). However, the percentage of EM colonisation of *Oreomunnea* seedlings was significantly higher inside than outside *Oreomunnea* forest ($F_{1,38} = 13.71$, $P < 0.001$; Fig. S1), and there was a significant site × treatment interaction ($F_{1,34} = 9.46$, $P = 0.004$) driven by significantly lower EM lower colonisation ($F_{1,2} = 38.06$, $P = 0.03$) among the few surviving seedlings analysed from the 0.5 µm mesh treatment outside *Oreomunnea* forest.

There were no significant differences among treatments for seedlings growing inside the *Oreomunnea* forest for δ¹³C of the extracted leaf sugars ($F_{3,28} = 1.97$, $P = 0.14$), or for δ¹³C ($F_{3,12} = 1.02$, $P = 0.7$, Fig. 1e) or δ¹⁵N ($F_{3,12} = 0.46$, $P = 0.98$, Fig. 1f) of leaf bulk tissue. *Oreomunnea* seedlings growing inside *Oreomunnea* forest did not differ in foliar δ¹³C ($F_{1,23} = 1.15$, $P = 0.35$, Table 1). However, seedlings growing inside *Oreomunnea* forest were significantly depleted in foliar ¹⁵N compared with seedlings growing out-

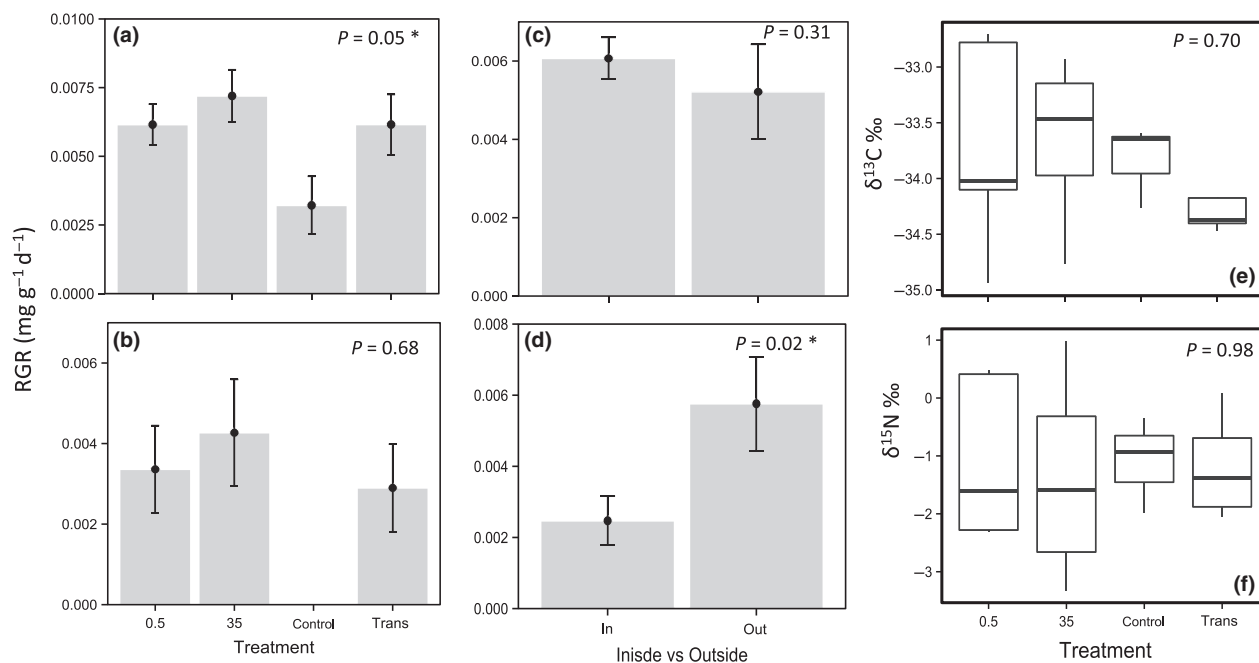


Figure 1 Relative growth rate (RGR) of *Oreomunnea* (a) and *Roupala* (b) seedlings among treatments; RGR of *Oreomunnea* (c) and *Roupala* (d) seedlings inside and outside *Oreomunnea* forest; *Oreomunnea* seedling $\delta^{13}\text{C}$ (e) and $\delta^{15}\text{N}$ (f) of leaf tissue among treatments inside *Oreomunnea*-dominated forest. Exclosure treatment abbreviations: 0.5 μm (0.5), 35 μm (35), transplant (trans), and control. Error bars in the bar plots represent the standard error of the mean. The boxplots were created using the median and the 25th and 75th percentiles and the 95% confidence interval of the median was used for the error bars.

Table 1 Mean C : N, total C, total N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of soils and seedlings, and soil ammonium, nitrate, and phosphate ($\mu\text{g bag}^{-1}$) measured using an 18-day resin bag incubation at sites either inside *Oreomunnea*-dominated forest, or 30 m outside in mixed AM dominated forest.

| Variable | Location | Soil | <i>n</i> | <i>F</i> | <i>P</i> | Seedlings | <i>n</i> | <i>F</i> | <i>p</i> |
|-----------------------|----------|--------|----------|----------|----------|-----------|----------|----------|----------|
| C:N | Inside | 19.30 | | | | 23.68 | | | |
| | Outside | 15.75 | 17 | 26.77 | <0.001 | 24.94 | 23 | 0.45 | 0.51 |
| %C | Inside | 28.19 | | | | 46.22 | | | |
| | Outside | 12.63 | 17 | 21.37 | <0.001 | 41.44 | 23 | 23.10 | <0.001 |
| % N | Inside | 1.46 | | | | 1.98 | | | |
| | Outside | 0.80 | 17 | 15.9 | <0.001 | 1.70 | 23 | 17.33 | <0.001 |
| $\delta^{13}\text{C}$ | Inside | -28.01 | | | | -33.84 | | | |
| | Outside | -28.55 | 17 | 8.65 | <0.01 | -33.38 | 23 | 1.15 | 0.35 |
| $\delta^{15}\text{N}$ | Inside | 0.57 | | | | -1.18 | | | |
| | Outside | 2.84 | 17 | 26.31 | <0.001 | 0.87 | 23 | 6.55 | 0.02 |
| Ammonium | Inside | 4.46 | | | | - | | | |
| | Outside | 10.67 | 19 | W = 19 | 0.02 | - | - | - | - |
| Nitrate | Inside | 4.33 | | | | - | | | |
| | Outside | 12.40 | 19 | W = 9 | <0.01 | - | - | - | - |
| Phosphate | Inside | 0.28 | | | | - | | | |
| | Outside | 0.33 | 19 | W = 38 | 0.31 | - | - | - | - |

side ($F = 17.33$, $P < 0.001$, Table 1) and had significantly greater foliar N concentration ($F_{1,23} = 6.55$, $P = 0.02$, Table 1).

Plant–soil feedback experiment

Monodominance could arise if beneficial EM fungi are restricted to forest dominated by *Oreomunnea* and provide a competitive growth advantage to *Oreomunnea* seedlings. If so, we predicted that *Oreomunnea* would show positive PSF or weaker negative PSF effects relative to co-occurring species.

However, *Oreomunnea* showed the strongest negative PSF among species in the experiment (Fig. 2, Table S4), and the strength of negative PSF was significantly positively correlated with species relative abundance (Fig. 2).

Differences in seedling and soil nutrient status inside and outside *Oreomunnea* forest

There was significantly lower resin extractable ammonium (Wilcoxon test $W = 19$, $P = 0.021$, Table 1) and nitrate ($W = 9$, $P < 0.01$) inside compared to outside the *Oreo-*

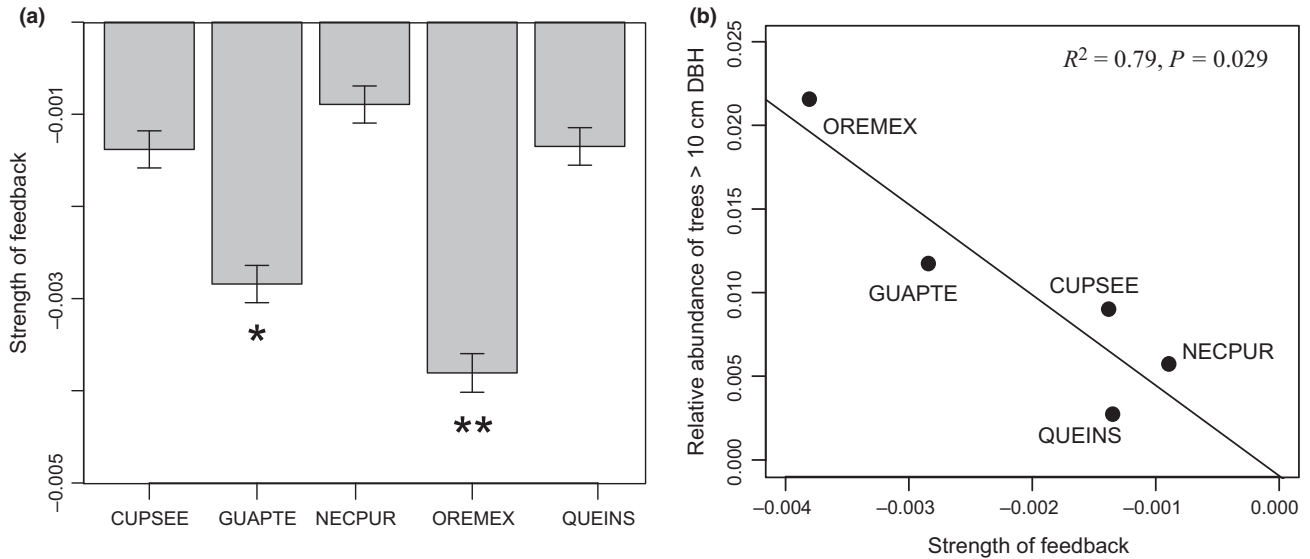


Figure 2 (a) Strength of negative plant-soil feedback effects on relative growth rate in 240 seedlings of five tree species differing in abundance in permanent forest plots. (b) Regression model for the strength of plant-soil feedback vs species abundance individuals > 10 cm DBH in three 1 ha permanent plots. AM species are *Guarea pterorhachis* (GUAPTE), *Cupania seemanii* (CUPSEE), and *Nectandra purpurea* (NECPUR). EM species are *Oreomunnea mexicana* (OREMEX) and *Quercus insignis* (QUEINS). Bars indicate standard errors of the mean. (* $P < 0.05$, ** $P < 0.01$).

munnea forest, but there was no difference in resin-extractable phosphate across the same sites ($W = 38$, $P = 0.31$, Table 1). There were also significantly higher concentrations of total C ($P < 0.001$) and total N ($P = 0.001$, Table 1), and a higher C : N ratio ($P < 0.001$; Table 1) in soils inside compared to outside *Oreomunnea* forest. Soils inside *Oreomunnea* forest were significantly depleted in ^{15}N ($P < 0.001$; Table 1).

The number of *Oreomunnea* individuals > 5 cm DBH in 20×20 m² subplots in two 1-ha plots was significantly negatively associated with the concentration of nitrate ($R^2 = 0.48$, $P < 0.001$), and P ($R^2 = 0.21$, $P < 0.001$) measured in the center of each subplot, but was not significantly correlated with ammonium ($R^2 = 0.06$, $P = 0.15$), total N ($P = 0.79$), total C ($P = 0.65$) or litter mass ($P = 0.44$, Fig. 3). Results were similar when the regression analysis was repeated using *Oreo-*

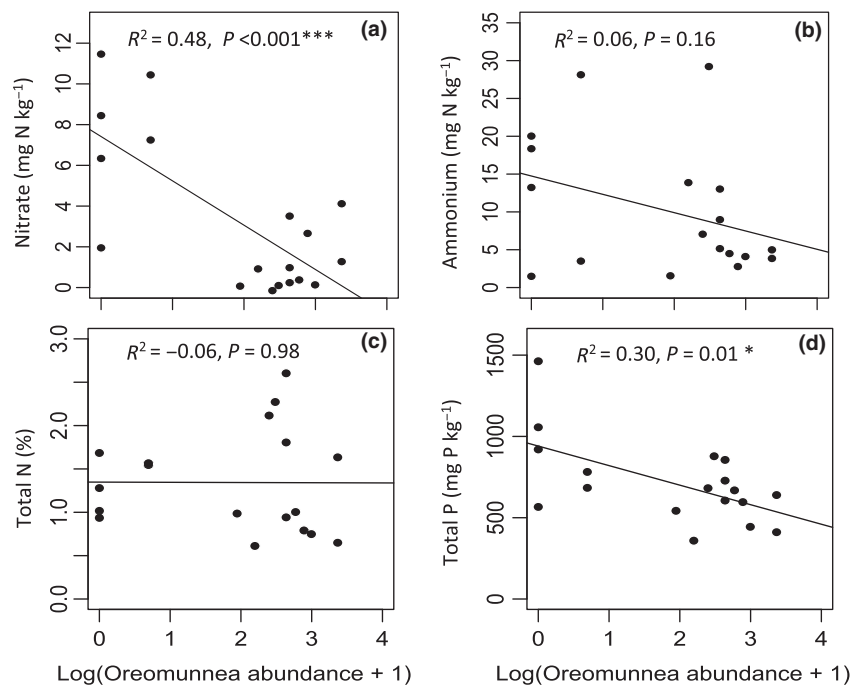


Figure 3 Regression models for the number of *Oreomunnea* individuals ≥ 5 cm DBH in 20×20 sub-plots of two 1 ha permanent plots and nitrate (mg N kg⁻¹), ammonium (mg N kg⁻¹), total N (%), and total P (mg P kg⁻¹) measured in the same sub-plots.

munnea basal area (Fig. S2). *Quercus insignis* abundance was not significantly correlated with any soil nutrients (Fig. S3). However, all the most abundant AM species were negatively associated with either ammonium or nitrate concentrations in the soil (Table S5). *Ardisia* sp., *Cassipourea guianensis* and *Eschweilera panamensis* were also significantly positively correlated with the number of individuals or basal area of *Oreomunnea mexicana* (Table S5).

DISCUSSION

We provide the first simultaneous test of two prominent hypotheses to account for monodominance in tropical forests. Our results suggest that neither the absence of negative PSF nor the formation of EM networks can account for monodominance in our study system. Instead, we provide evidence that *Oreomunnea*-dominated forest is associated with lower availability of soil ammonium and nitrate than nearby forest where *Oreomunnea* is infrequent or absent. This reduced nutrient availability, presumably driven by depletion of readily mineralisable N from soil organic matter by EM fungi, could confer *Oreomunnea* seedlings a competitive advantage over AM or non-mycorrhizal species.

Plant–soil feedback and EM associations

It has been proposed that positive PSF experienced by EM plants underlie monodominance in tropical forests (McGuire 2007, 2014). However, these studies identify EM networks as the mechanism generating positive PSF. Here, we differentiate microbially mediated PSF *sensu* Bever *et al.* (1997) from positive distance-dependent effects of EM networks. To the best of our knowledge, only two studies of microbially mediated PSF have included EM tree species and, in general, negative feedbacks dominate over positive feedbacks (Liu *et al.* 2012; McCarthy-Neumann & Ibañez 2012). In our study, *Oreomunnea* showed the strongest negative PSF among the species tested, despite its high local abundance. This result contrasts with findings from lowland tropical forest and temperate grasslands, where more abundant species show weaker negative PSF (Klironomos 2002; Mangan *et al.* 2010). This is the first PSF experiment ever done including montane tree species and more research in this topic is needed to confirm if this is widespread phenomenon of just a peculiarity of our system. The reduced RGR of *Oreomunnea* when grown in its own inoculum indicates that the negative effect of *Oreomunnea* species-specific pathogens is stronger than any potential positive effects that might arise from more beneficial inoculum sources.

Mycorrhizal network effects

Previous experiments that have tested for the presence of EM networks in tropical monodominant forests reported higher growth and survival for seedlings in contact with the roots of conspecifics and EM mycelium relative to those isolated in mesh enclosures (Onguene & Kuyper 2002; McGuire 2007). In contrast, our results show no evidence of C or N transfer from adults to seedlings through EM networks based on

either changes in seedling growth or survival in response to hyphal enclosure treatments. The only difference in growth rate among treatments was higher RGR in seedlings grown in 35- μ m mesh enclosures compared with control seedlings, which was probably due to release from belowground competition with roots of other plants. However, the fact that we used already established (and EM-infected) seedlings in the experiment could have reduced the likelihood that *Oreomunnea* seedlings joined existing EM networks.

The leaves of plants connected to EM networks have an isotopic composition that is distinct from fully photosynthetic plants (Selosse & Roy 2009; Hynson *et al.* 2012). If plants receive sugars via EM networks, then we would expect that leaf tissue, and in particular leaf soluble sugars, would be enriched in ^{13}C (Selosse & Roy 2009; Hynson *et al.* 2012). Here, we found no differences in the isotopic composition of leaf tissue among enclosure treatments, further suggesting that no EM networks were formed in this system.

Instead, this study shows that seedlings growing inside *Oreomunnea*-dominated forest had higher EM colonisation, lower foliar ^{15}N and higher foliar N concentration. Previous studies have found a correlation between $\delta^{15}\text{N}$ in plants and the degree of EM fungal colonisation (Hobbie & Colpaert 2003). The presence of EM colonisation alters host plant $\delta^{15}\text{N}$ by transferring ^{15}N -depleted compounds to the plant while sequestering ^{15}N -enriched compounds in fungal tissue (Hobbie & Colpaert 2003). In the case of *Oreomunnea* seedlings growing inside *Oreomunnea* forest, the lower ^{15}N of their leaves could have been partially due to lower ^{15}N in the soil. However, a decrease in foliar ^{15}N has been observed in EM plants experiencing increased N limitation (McLauchlan *et al.* 2010; Craine *et al.* 2015), associated with a higher dependency on EM fungi (Craine *et al.* 2015). These results suggest that the patchiness of *Oreomunnea* populations in part reflects reduced mycorrhizal infection outside *Oreomunnea* forest. The lack of appropriate or compatible EM inoculum outside *Oreomunnea* patches could have strongly reduced survivorship rates due to a reduced capacity of seedlings to compete for N with larger AM trees (Nara 2006).

Microbial competition for N as a mechanism facilitating monodominance in tropical forest

A reduction in the decomposition rate of litter, and an increase in organic matter accumulation has been frequently noted under EM-dominated forest (Torti *et al.* 2001; Orwin *et al.* 2011; Phillips *et al.* 2013; Averill *et al.* 2014). The mechanisms underlying this effect are not fully understood, although it has been proposed that direct competition for N between EM fungi and the community of free-living decomposers could reduce litter decomposition rates (Read & Perez-Moreno 2003; Orwin *et al.* 2011). Competition for nutrients is proposed to result in the accumulation of organic matter depleted in N under EM-dominated forest, resulting in a reduction in nutrient availability for AM or non-mycorrhizal plant species, as well as microbial heterotrophs, that rely to a greater extent on inorganic N sources (Phillips *et al.* 2013). Ectomycorrhizal plants growing in soils with low available N may have a competitive advantage over AM plants because

EM fungi are able to acquire N directly from organic matter when supplied with sugar from their host plant (Phillips *et al.* 2013; Lindahl & Tunlid 2015).

Lower N availability could reduce plant diversity (Dickie *et al.* 2014) and ultimately drive monodominance in tropical forests (Fig. 4). The differences in soil ^{15}N in our system along with the higher total C, reflects N limitation and an accumulation of soil organic matter with a higher C : N ratio. We propose that N depletion from the soil organic matter by EM fungi increases N limitation. This results in a tightened N cycle, and is consistent with low rates of nitrification, denitrification and gaseous N losses from the soil organic layer of our study site reported over 2 years of measurements (Koehler *et al.* 2009; Corre *et al.* 2010). Nitrification and denitrification strongly discriminate against ^{15}N , enriching the soil in ^{15}N (Houlton *et al.* 2006). Therefore, soils with low resin-extractable inorganic N, and presumably therefore low mineralisation rates, show reduced $\delta^{15}\text{N}$ of the soil available N pool (Martinielli *et al.* 1999). This is supported by the finding that the abundance of *Oreomunnea*, as well as the most abundant AM tree species, was negatively correlated with inorganic N concentration in the soil in permanent plots.

Several studies have found results consistent with our findings of lower availability of inorganic N inside than outside monodominant forest (Read *et al.* 1995, 2006; Torti *et al.* 2001; Brookshire & Thomas 2013). Further, studies that failed to find differences in soil nutrients did not measure plant available ammonium or nitrate in the soil (i.e. Hart *et al.* 1989; Conway & Alexander 1992; Henkel 2003; Peh *et al.* 2011b).

Finally, differences in N mineralisation between EM- and AM-dominated forests may be a consequence of intrinsic differences in litter quality between EM- and AM-associated

plants. However, using a global database of leaf functional traits, Koele *et al.* (2012) found no relationship between leaf traits and mycorrhizal type after correcting by phylogenetic placement. At Fortuna, *Oreomunnea* is close to the community average for leaf traits associated with decomposition (foliar C : N, Adamek 2009) and $\delta^{15}\text{N}$ (Mayor *et al.* 2014).

We conclude that changes in N availability due to the ability of EM fungi to acquire N directly from organic matter is the likely mechanism underlying *Oreomunnea* monodominance in our study system. This positive density-dependent mechanism creates patterns consistent with previous evidence in EM tree species. Furthermore, the reduction in N availability may explain the occurrence of other monodominant forests, including *Dicymbe corymbosa* in Guyana, *Gilbertiodendron dewevrei* in central Africa and dipterocarp species in Southeast Asia.

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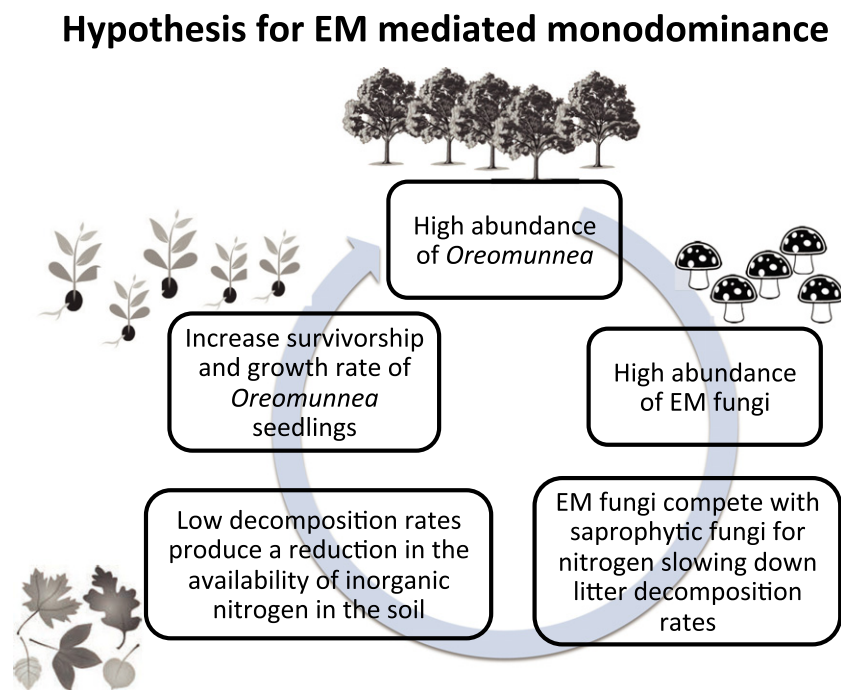


Figure 4 Feedback loop to explain monodominance of *Oreomunnea mexicana* based on the ‘microbial competition for nitrogen hypothesis’. Abbreviations: Ectomycorrhizal (EM).

AUTHORSHIP

AC, SM and JD designed the study. AC and JD performed the research. SM and BT provided support for PSF and isotopes analysis. AC wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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