Do Common Ectomycorrhizal Networks Play a Role in Dampening Competition Amongst Conifer Species?

FRST 498
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Abstract

Niche theory predicts that the coexistence of species is the result of dissimilar demands on the resources available in their ecosystem. This is contradictory, however, to the observation that species with similar needs and even individuals of the same species often live in close proximity to one another. Common mycorrhizal networks (CMNs) have been proposed as a possible explanation of this phenomenon via their ability to acquire and transfer nutrients between plants. To test this hypothesis, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine (*Pinus ponderosa* C. Lawson) seedlings were grown in autoclaved and non-autoclaved soil. Seeds were sown in a grid formation to keep density consistent and the plants were allowed to grow for 34 weeks. To assess resource allocation and the effects of competition, Gini coefficients were calculated for height, basal diameter, dry shoot biomass, and dry root biomass. Gini coefficients tended to be lower in non-autoclaved than autoclaved pots for three out of four variables measured for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), but differences were not statistically significant. There appears to be some evidence that CMNs affect intraspecific competition among Douglas-fir.

Key Words

Common mycorrhizal networks, Niche Theory, competition, autoclave, Gini coefficient, Douglas-fir

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Introduction

In 2001, Neutral Theory was proposed as an explanation for the spatial coexistence of species with similar nutrient requirements (Hubbell 2001). The theory originated from work with species-rich, rainforest plant communities in which many species that appeared to have very few differences in competitive advantage coexisted in close proximity (Hubbell 2006). Classical Niche Theory, which has long been the standard explanation of species distributions, (Bever et al. 2010; Kitching 2013) posits that, in order to coexist, species must demand different levels from available resource pools (e.g. light or available nitrogen) (Silverton and Law 1987). Therefore, classical Niche Theory would predict the observed species composition in the aforementioned rainforest communities to be highly unstable and unlikely to last (Silverton and Law 1987). This was not found to be the case (Hubbell 2006). As an alternative to Niche Theory, Hubbell 2001 assumed that all individuals within large groups of species (e.g. shade tolerant trees) had equal competitive advantage, negating the need for niche differentiation as an explanation in the rainforest communities observed.

Despite its success in explaining species-rich rainforest communities, there are systems in which Neutral Theory appears not to fit (Alder 2004; McGill 2003). Kitching 2013 proposed a hybrid of the two theories, recognizing that there is a point on a spatial scale at which it is appropriate to switch from using Neutral Theory to using Niche Theory. This point varies by ecosystem and is dependent on a variety of factors (Kitching 2013). Nevertheless, with Neutral Theory determined not to be a panacea, there is still a theoretical need within Niche Theory for a mechanism capable of dampening competition in a way that allows for coexistence.

There have been many mechanisms proposed to explain the coexistence of species with overlapping resource needs (Brown 1989; Hastings 1980). Bever et al. 2010 reviews several soil microbiological examples of these. For example, asymmetric reliance of one plant species on a symbiont (e.g. mycorrhizae) to obtain a particular nutrient can cause resource partitioning if that symbiont then increases the plant's need for another nutrient. There is also the possibility that different mycorrhizal species draw resources from different pools of the same nutrient (e.g. nitrogen). This could result in resource partitioning if these species associated unequally with different host species. They also propose differences in species reactions to soil microbes as a potential for positive or negative feedback (Bever et al. 2010). The fourth mechanism they propose is the process of resource sharing through common mycorrhizal networks (CMNs).

CMN's form when a mycorrhizal fungus infects multiple plant hosts in a community. When these connections are made, it often allows for transfer of nutrients between plants through the hyphae of the fungus (Perry et al. 1989; Simard et al. 1997a). Tapping into the CMN of nieghbouring plants can also increase access to nutrients and therefore uptake, without involving interplant nutrient transfer (Teste et al. 2009). There is some debate on the nature and ecological significance of CMNs and nutrient transfer (Robinson and Fitter 1999; Selosse 2006). Bever et al 2010 concluded that there was insufficient evidence to assert that CMNs could provide a biologically significant mechanism to dampen or enhance competition.

In contrast to claims of CMN driven equality, a recent study by Weremijewicz and Janos 2013 showed that seedling size was significantly more unequal when arbuscular

mycorrhizal networks were allowed to form between individuals in soil microcosms. They proposed that the networks drive inequality by favouring good carbon-donors for the acquisition of nutrients (Weremijeicz and Janos 2013). There is evidence, however that ectomycorrhizal networks may differ from those of arbuscular mycorrhizae in their propensity to transfer sugars to plants (Robinson and Fitter 1999) and that they may in fact relieve mutual antagonism between species grown in close proximity (Perry et al. 1989).

To quantify differences in resource allocation, ecologists have relatively recently adopted a statistical measure of dispersion known as the Gini coefficient (Bendel et al. 1989). Commonly used to compare income inequality between countries, the Gini coefficient is based off of the Lorenz curve, which plots cumulative percentage of a population by cumulative percentage of the variable of interest (e.g. individual wealth, height, or biomass). A line of equality exists at a 45°-angle from the origin. This line represents a scenario in which all "wealth" is equally distributed amongst all individuals in the population. The Gini coefficient is the ratio of the area between this line and the observed curve. This means that when all "wealth" is equally distributed, the Gini coefficient is 0. When all "wealth" is owned by one individual, the Gini coefficient is 1 (Weiner and Solbrig 1989).

The objective of the current study was to investigate whether CMNs can dampen competition among ectomycorrhizal conifers. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine (*Pinus ponderosa* C. Lawson) were grown in "stands" of 25 seedlings in pots containing soil that was either left alone (CMN treatment) or autoclaved (non-CMN treatment). The Gini coefficient was used to represent relative differences in resource allocation among conifer seedlings, theoretically reflecting the results of competition. The hypothesis was that pots with common ectomycorrhizal networks would result in lower Gini coefficients than pots where CMNs did not form.

Methods

Soil and Sterilization Treatment

Soil was collected from a forested location on the University of British Columbia Vancouver campus, a region within the Coastal Western Hemlock zone of the Biogeoclimatic Ecosystem Classification System of British Columbia (BEC)(Meidinger and Pojar 1991). The site was dominated by western red-cedar (*Thuja plicata* Donn ex D. Don) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Ponderosa pine does not grow natively in this ecosystem, though it is known to share mycorrhizal species with Douglas-fir (Massicotte et al. 1994; Molina and Chamard 1983). For this reason, the soil was thought to contain suitable mycorrhizal inoculum for both tree species. For the purposes of this study, ectomycorrhizal inoculum will be defined as the spores, hyphae, and/or sclerotia of ectomycorrhizal fungi (Jones et al. 2003).

Once collected, the forest soil was mixed in a 1:1 ratio with potting soil to increase the volume of soil. Half of this mixture was put through an autoclave at 121° C for one hour. Autoclave tape was placed on each autoclave bag to ensure that 121° C had been reached. The purpose of autoclaving was to reduce the amount mycorrhizal fungi inoculum present in the soil. To assess the degree to which autoclaving impacted the initial soil conditions, three samples were taken from the totality of the autoclaved soil and three from the non-autoclaved soil. It is important to note that these are subsamples, as the treatment was not

done specifically to each sample. These sub-samples were sent to the British Columbia Ministry of Environment laboratory in Victoria, BC. Cations and effective cation exchange capacity (CEC) were determined by exchange with 0.1 M barium chloride followed by ICP. Available phosphorus content was assessed using Bray P-1. Nitrogen was mineralized in a 2-week anaerobic incubation at 30°C and the resultant ammonium-N determined colourimetrically.

Experimental Design

The autoclaved soil was used to fill nine 3-gallon plastic planting pots. The remaining non-autoclaved soil was used to fill nine more 3-gallon pots (total of 18 pots). Each pot was sown with 25 seeds placed in a grid formation (roughly 4cm apart) plus four seeds outside the grid to act as buffer trees. Three species combinations (Douglas-fir only (F), ponderosa pine only (P), mixture of Douglas-fir and ponderosa pine (M)) and two CMN treatments (sterilized (S, or without CMN)) and non-sterilized (NS, or with CMN)) were applied in a completely randomized design with three replications (Table 1). Of the 18 pots, six were planted with only interior Douglas-fir, six were planted with only ponderosa pine, and six were planted with a 12:13 mix of either Douglas-fir to pine or pine to Douglas-fir. All pots were planted at the same density. Of the six pots of each species treatment, three contained sterilized (autoclaved) soil and three contained non-sterilized (non-autoclaved) soil (Table 1). Upon completion of the planting, the soil was covered with a light gravel mix to prevent "damping off", a common greenhouse fungal disease. Each individual seedling was labeled according to its position in the grid.

S-F-1	S-F-2	S-F-3
S-P-1	S-P-2	S-P-3
S-M-1	S-M-2	S-M-3
NS-F-1	NS-F-2	NS-F-3
NS-P-1	NS-P-2	NS-P-3
NS-M-1	NS-M-2	NS-M-3

Table 1: Experimental Design. S=Sterilized and NS=Non-Sterilized. F=Douglas-fir, P=Ponderosa pine, M=Mixed species. The number represents the replicate number.

About 1 month after sowing, empty spaces left by seeds that failed to germinate or seedlings that died early were filled with transplanted seedlings of the same age from a pot of reserves. Unfortunately, the soil in the reserve pot was not autoclaved which introduces the possibility of contamination. The roots of transplanted seedlings were brushed off in order to reduce this possibility.

Upkeep Period

The pots were kept in the Horticulture Greenhouse at the University of British Columbia. The position of the pots on the bench was rotated every two weeks.

Each pot was watered every day until most seeds had germinated (about one week after sowing). Watering was then reduced to every two days for about 18 weeks. There was some concern that excessive watering was discouraging mycorrhizal development and so watering was again reduced to every three days for about two months and finally to once a week for the remaining month.

Seedling height was measured for every individual seedling every two weeks. Height was measured from the soil to the tip of the tallest apical meristemic needle. In the ponderosa pine, this included the fascicle bundles growing from the apical meristem, but not those emerging from the stem.

Harvest

After 34 weeks of growth, seedlings were marked with Wite-out® to indicate the soil level and carefully removed from their pots. Basal diameter was measured at the soil level using calipers. The seedlings were then cut at the soil level in order to separate roots from shoots. All shoots were placed in labeled paper bags so that each individual could still be tracked. All roots were washed to remove as much soil as possible. The roots of the nine seedlings that had made up the interior of each grid were placed in labeled, slightly moist Ziploc® bags and put in cold storage. The remaining roots were placed in labeled paper bags. All shoots and roots in paper bags were put in an oven and dried at 70° C for about 72 hours to reduce the chance of molding. They were then stored at room temperature for about seven weeks. No molding was observed.

Morphotyping Method

Douglas-fir roots were morphotyped to confirm presence of mycorrhizae and to determine if mycorrhization rates differed between sterilization treatments. Five roots per pot were randomly selected from all six Douglas-fir pots. Due to time constraints pine and mixed species pots were not morphotyped. Fifty root tips were then randomly selected from each root and placed under a Stemi SV 11 stereomicroscope. Tips were divided into separate morphotypes, stored in Eppendorf® tubes filled with water, and frozen. Upon completion of morphotyping, all Douglas-fir roots were placed in paper bags and prepared for oven drying.

Dry Biomass

All roots and shoots from the pure Douglas-fir pots were placed in a drying oven for 88 hours at 70° C. This was the second time through the oven for all non-morphotyped roots and shoots. The mass was measured for each root and shoot. Mass was not measured for any of the pine or mixed species treatments due to time constraints.

Statistical Analysis

Student's t-tests were used to compare soil chemistry between autoclaved and non-autoclaved soil. For three variables (K, pH by H_2O, CEC), the assumption of normality was not met and so the Wilcoxon rank sum test was used to determine if the distributions occupied the same location.

When data was available for all species levels (height and basal diameter), two-way Analysis of Variance (ANOVA) was used to compare treatment means (α=0.05). Bonferrini correction was used when appropriate. One-way ANOVA was used to compare CMN treatments for Douglas-fir only (shoot and root biomass). Data transformations were necessary for many of the variables to meet assumptions of equal variance and normality required for ANOVA.

Results

Soil Tests

Mn concentrations were significantly greater in the autoclaved soil (0.43 CMol+/Kg) than in the non-autoclaved soil (0.08 CMol+/Kg) (p=6.4e-06). When pH was determined using the $CaCl_2$ method, it was also significantly greater in the autoclaved soil (4.68) than in the non-autoclaved soil (4.58) (p=0.02). Anaerobic mineralizable nitrogen was significantly lower in the autoclaved soil (22.9 mg/Kg) than in the non-autoclaved soil (45.3 mg/Kg) (p=0.005). Fe and Na tended to be greater in the autoclaved than non-autoclaved soil (p=0.06 and p=0.08, respectively). pH using H_2O also tended to be slightly greater in the autoclaved than non-autoclaved soil (p=0.08). Ca tended to be lower in the autoclaved soil (p=0.06). All remaining soil variables were statistically similar in the two CMN treatments (Table 2).

Variable	Autoclaved (x) Mean	Non-Autoclaved (y) Mean	Significant Difference?
Al (CMol/kg)	1.16	1.06	No (p=0.46)
Ca (CMol/kg)	5.32	6.05	No, possible trend (p=0.06)
Fe (CMol/kg)	0.07	0.04	No, possible trend (p=0.06)
K (CMol/kg)	0.27	0.31	No (p=0.1)*
Mg (CMol/kg)	1.75	1.89	No (p=0.34)
Mn (CMol/kg)	0.43	0.08	Yes (p=6.4e-06)
Na (CMol/kg)	0.22	0.16	No, possible trend (p=0.08)
CEC (CMol/kg)	9.23	9.59	No (p=0.82)*
%C	8.21	8.38	No (p=0.81)
%N	0.51	0.49	No (p=0.74)
%S	0.047	0.054	No (p=0.17)
pH with H_2O	4.98	4.78	No, possible trend (p=0.08)*
pH with CaCl2	4.68	4.58	Yes (p=0.02)
Available. P (mg/kg)	114.0	121.67	No (p=0.32)
Mineral. N (mg/kg)	22.9	45.3	Yes (p=0.005)

Table 2: Results of soil tests. An "*" next to a p-value indicates that the value was derived from a non-parametric Wilcoxon rank sum test.

Mycorrhization

A total of 4 distinct morphotypes were observed in the non-autoclaved pot, with a minimum of 2 morphotypes per pot. The average "mycorrhizal tip/total tip" ratio was 0.20. It should be noted that the possibly immature nature of the observed mycorrhizas made identification somewhat challenging. There were no mycorrhizal tips observed in the autoclaved soil.

Gini Coefficients

The Gini coefficients of height and basal diameter were calculated for each pot. Gini coefficients of biomass were calculated only for Douglas-fir. When grown in the autoclaved soil, the seedlings developed a Gini coefficient of height of 0.19. This was not affected by growth in non-autoclaved soil, which resulted in a Gini coefficient of 0.18 (p=0.34) (Figure 1). The Gini coefficient of height did vary among some species levels, however. The Douglas-fir and mixed species pots had similar Gini coefficients of 0.20 and 0.25, respectively (where p<0.0167 suggests significance due to Bonferroni correction, p=0.039), but ponderosa pine pots grew more equally (0.11) than the Douglas-fir and mixed species pots (p=0.00002 and p=0.000001, respectively).

The two mixed species treatments (autoclaved and non-autoclaved) developed significantly greater Gini coefficients for basal diameter (where p<0.003 suggests significance due to Bonferroni correction) than the other treatments (p<0.0003 for all pairs), though they were not different from each other (p=0.50). The two ponderosa pine treatments differed from neither each other (p=0.97) nor the non-autoclaved Douglas-fir treatment (p=0.1 and p=0.09). The two Douglas-fir treatments also did not differ from one another, though it should be noted that the Gini coefficient was 0.187 for autoclaved Douglas-fir and 0.139 for non-autoclaved Douglas-fir (p=0.009) suggesting a strong trend (Figure 1).

The Gini coefficient for shoot biomass of Douglas-fir tended to be greater in autoclaved soil (0.395) than non-autoclaved soil (0.287), indicating that stands were more variable in autoclaved soil (p=0.08) (Figure 1).

The Gini coefficients for root biomass also tended to be greater for autoclaved than

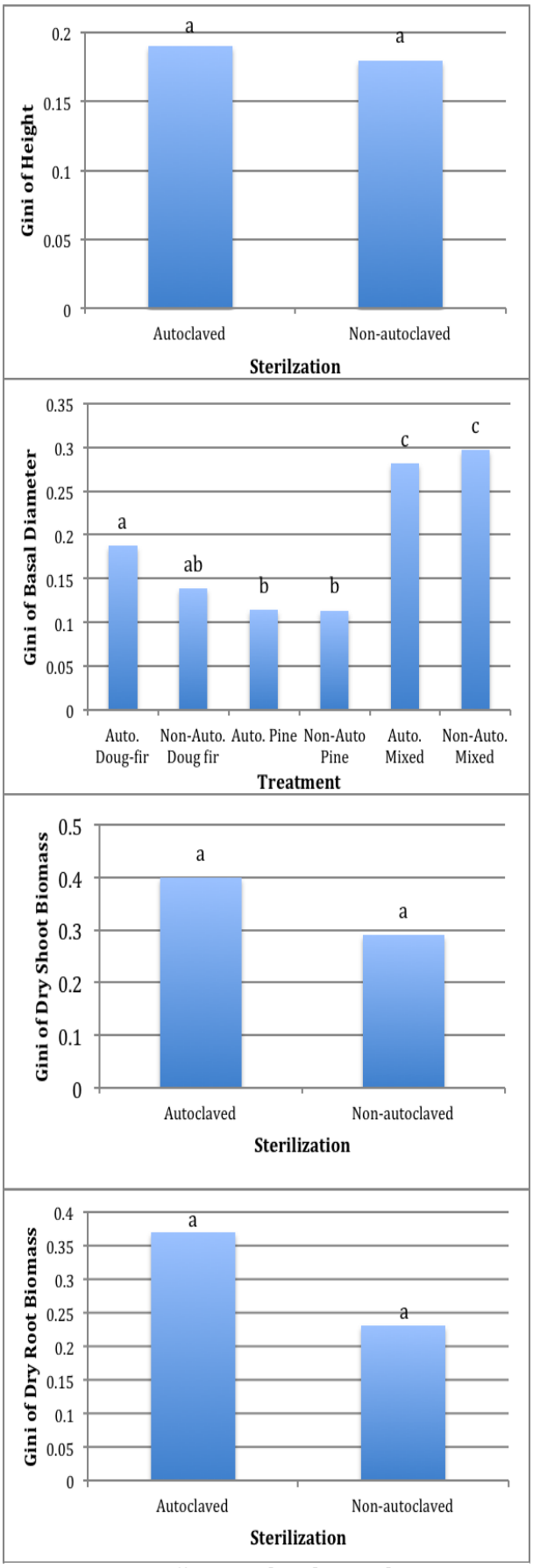

Figure 1: Gini coefficient values by sterilization. Different letters represent significant difference with Bonferroni correction considered.

non-autoclaved soil (autoclaved=0.369 and non-autoclaved= 0.231) (p=0.0515).

Pot Averages

Average values of each variable were calculated for each pot. Seedlings grown in the autoclaved soil reached an average height of 13.7 cm. Growing seedlings in non-autoclaved soil resulted in significantly shorter seedlings with an average height of 12.0 cm (p=0.024) (Figure 2). The pure Douglas-fir and mixed species seedlings reached similar average heights (11.56 cm and 11.66 cm respectively) (p=0.90). The pure ponderosa pine seedlings were on average 15.36 cm tall. This is taller than both pure Douglas-fir (p=0.0005) and mixed species (p=0.0006) average heights.

The average basal diameter of the seedlings grown in autoclaved soil was 0.22 cm. Seedlings grown in non-autoclaved soil had a significantly lower average basal diameter of 0.19 cm (p=0.012). There were also significant differences between all species compositions (Douglas-fir was 0.15 cm, mixed species 0.21 cm, and pine 0.26 cm) (p<0.002 for all species compositions).

Average shoot biomass tended to be greater in the autoclaved (0.34 g) than the non-autoclaved (0.22 g) treatment (p=0.064). Average root biomass of the seedlings did not vary significantly between autoclaved (0.28 g) and non-autoclaved (0.23 g) pots (p=0.58).

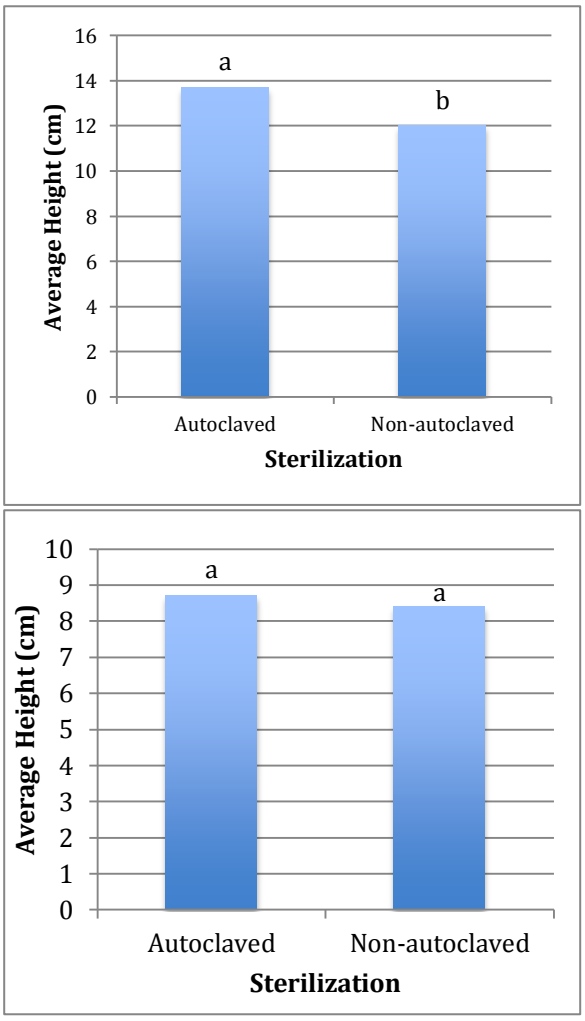

Figure 2: Average heights by sterilization treatment. Different letters represent significant difference with Bonferroni correction considered. Top: Average height at harvest (34 weeks). Bottom: Average height at 16 weeks.

Height at 16 weeks

Gini coefficients and pot averages were calculated for height of seedlings at 16 weeks. This age was chosen because it was close to halfway through the experiment. By 16 weeks, there was not yet a difference in average height between sterilization treatments (autoclaved= 8.7 cm, non-autoclaved=8.4 cm, p=0.669) (Figure 2). All species levels had different average heights (F=6.31, FP=8.27, P=11.12)(p<0.00002). There was no difference in the average Gini coefficients for height between sterilization treatments (autoclaved=0.150 and non-autoclaved=0.149) (p=0.96). However, height Gini differed between mixed species (0.21) and Douglas-fir (0.13) (p=0.00002) and between mixed species and pine (0.11) (p=0.000003), not pine and Douglas-fir (p=0.16).

Discussion

Mycorrhizal networks likely linked seedlings in pots containing pure stands of interior Douglas-fir based on the common occurrence of four ectomycorrhizal morphotypes among seedlings over 20% of their root tips. The presence of mycorrhizal networks appeared to dampen intraspecific competition based on strong trends in reduced Gini coefficients for basal diameter, shoot biomass, and root biomass in non-autoclaved compared with autoclaved pots. Hence, mycorrhizal networks appeared to result in increased seedling size equality within stands. By contrast, height growth of interior Douglas-fir was not affected by sterilization, which is not surprising considering that height growth is generally insensitive to inter-tree competition (Oliver and Larson 1997).

There was less evidence that mycorrhizal networks affected the competitive response of ponderosa pine to individuals of the same species or to interior Douglas-fir because Gini coefficients for neither height nor basal diameter Gini coefficients differed significantly between sterilization treatments. It may be that fungal species appropriate for association with ponderosa pine were not available in our study despite the previous evidence for shared mycorrhizal associates of Douglas-fir and ponderosa pine (Massicotte et al. 1994; Molina and Chamard 1983). The purpose of the mixed species level was to determine whether interspecific competition would be dampened by shared association with mycorrhizal networks. Unfortunately we were unable to show that both species were associated with the same mycelial network or even infected by the same species due to time constraints. There is no evidence to suggest that mycorrhizal association influenced interspecific competition.

Soil manganese concentration increased, while mineralizable nitrogen decreased following autoclaving of the field soil. Lopes and Wollum 1976 also observed increases in Mn after autoclaving, which they attributed to the reducing conditions of an autoclave. They propose that the saturated steam and pressure creates an environment in which formerly oxidized forms of Mn are reduced and therefore more likely to be extracted (Lopes and Wollum 1976). This may also explain the possible difference in exchangeable Fe levels in this study, as Fe would follow a similar pattern (Lopes and Wollum 1976). The decrease in anaerobic mineralizable nitrogen after autoclaving conflicts with previous studies that suggest that partial sterilization causes a "flush" of decomposition and thus, mineral nitrogen. Proposed theories to explain this "flush" involve sterilization releasing certain inhibitors (i.e. toxins or competing organisms) or freeing previously unavailable nutrient sources (i.e. waxy films preventing bacterial attack) (Jenkinson 1966). It may be that in the case of our study, autoclaving so reduced the size of the anaerobic bacterial population that it was unable to recolonize the soil. For these reasons, it does not seem prudent to use mineralizable nitrogen as a proxy for plant available nitrogen as it appears rather to be reflecting a reduction in the anaerobic bacterial population. This corresponds well with the lack of mycorrhization on the Douglas-fir grown in autoclaved soil. The differences in pH ($CaCl_2$) and residual acidity are quite minimal and not likely to alter the effects of this study. There was concern that autoclaving would cause structural changes in the soil organic matter that would alter the cation exchange capacity (CEC) of the soil. This does not appear to have happened in this study, as there was no significant difference in CEC between the soils.

It must be recognized that there are some unfortunate side effects of autoclaving soil. The first is that autoclaving kills the biological community of the soil indiscriminately; it does not specifically target fungi and it has been known to fail to denature enzymes that are

important in nutrient cycling (Carter et al. 2007; Tanaka et al. 2003). It is possible then, that any effect observed in the sterilization treatments is due to either the absence of non-fungal organisms or the unbalanced presence of certain enzymes. Additionally, Berns et al. 2008 found that autoclaving caused destabilization of soil aggregates, presumably due to the destruction of binding polysaccharides, as well as changes in the relative frequencies of organic functional groups. There have also been reported increases in soluble organic matter (Salonius et al. 1967) and differences in exchangeable cation concentrations, particularly manganese (Lopes and Wollum 1976; Wolf et al. 1989). Several studies found marked increases in the abundance of mineral nitrogen after autoclaving (Jenkinson 1966; Lopes and Wollum 1976; Ramsay and Bawden 1983). Because only half of the soil in the experiment was autoclaved, these changes to the initial conditions of the soil would only be experienced by seedlings grown in autoclaved soil. There was concern that this would cause an initially increased growth rate in these seedlings that would not occur in the seedlings grown in non-autoclaved soil and that this difference in growth rate would establish a level of inequality that would develop independently of mycorrhizal association.

Despite the differences in soil chemistry observed in our study, the seedlings grown in autoclaved soil do not appear to have undergone any increased growth rate due solely to initial soil conditions. At the age of 16 weeks, the seedlings in both sterilization treatments were of essentially equal height, suggesting that seedlings in autoclaved soil had not yet been influenced by differences between the soils. It is unlikely that any difference in nutrient content of the soils would have taken 16 weeks to have a biologically significant effect on plant growth. It seems safe then, to attribute any growth differences seen between sterilization treatments by time of harvest to primarily mycorrhizal activity.

There are several possible explanations for the difference in equality that autoclaving the soil appears to have led to in the Douglas-fir pots. Song et al. 2010 suggested it is possible for stress hormones triggered by *Glomus mosseae* (Nicol. & Gerd) to travel between tomato plants (*Lycopersicon esculentum*, Mill. cv. Jin Bao) via arbuscular mycorrhizal networks. There may be a similar system by which plant hosts use mycelial networks passively to communicate inequalities in nutrient distribution and respond by reallocating nutrients where they are needed. This does not seem particularly likely, however, as there would be little benefit to the fungus to allow uncontrolled nutrient movement through its hyphae. A more likely explanation would be that the fungus disproportionately acquires carbon from the best carbon-donors, which are likely the largest, most photosynthetically active individuals (Nehls et al. 2007; Weremijewicz and Janos 2013). It would then use this carbon for its own growth, possibly resulting in a decreased growth rate in these large individuals. If the growth rate of the smaller individuals did not decrease, the diameter and biomass distributions in the pot could equalize.

It may also be that the smaller trees are parasitizing the fungus in a manner similar to that of mycoheterotrophic orchids. These orchids derive their carbon either partially or exclusively from ectomycorrhizal fungi which presumably receive their carbon from photosynthetically active trees that are part of the same CMN (Stockel et al. 2011). If this process were happening in accordance with the previously proposed decrease in growth rate of large individuals and it resulted in an increase in growth rate of small individuals, the potential to equalize the seedlings would increase. An understandable concern raised by studies that suggest net movement of carbon into poor carbon-donor plants is that there is little incentive for fungi to stay in these relationships (Pfeffer et al. 2004; Robinson and Fitter 1999). However, if the fungus is generally gaining carbon from good carbon-donors, it

may be that the cost of disassociating with a poor donor and finding a better host is higher than maintaining the relationship (Selosse et al. 2006). Massicotte et al 1994 showed that particular species of the fungal genus *Rhizopogon* would expand their host range when grown in polycultures of their primary host and a tree species that did not act as host when grown in monolculture. This suggests that the primary host tree species was able to provide enough energy to maintain a relationship that was not possible in its absence. A similar process may be occurring between good carbon-donor and bad carbon-donor plants. There may also be some mechanism the plant uses to force the fungus to stay in the relationship. For example, Nehls et al. 2007 discusses evidence of up-regulation of *Amanita muscaria* carbon importers in response to extracellular monosaccharide concentration. Fungus to plant movement of carbon has been shown to occur in the field (Simard et al. 1997a) and so presumably a carbon *exporter* protein exists, similar to the importers, that releases fungal sugars into the plant-fungal interface. It is not entirely implausible that a plant could introduce some mechanism that controlled the regulation of those exporters, influencing sugar flow out of the fungus. Nehls et al. 2007 also suggests there is evidence of rapid control of plant carbon transporters via phosphorylation. This may provide a simpler explanation, in which seedlings initially remove very little carbon from the plant-fungus interface, encouraging fungal infection and development. When the need for carbon later arises, due to photosynthetic deficiency, the seedlings may activate their carbon transporters, increasing their uptake of carbon from the fungal interface and diminishing the sugar resources available to the fungus. Experiments with transgenic poplar plants showed that plants with no ability to deactivate their carbon importers experienced a significant fungal defection from the mutualism (Nehls et al. 2007). This suggests the importance of plant control of these transporters to the plant-fungal relationship.

It is understandable that the plants grown in the non-autoclaved soil reached lesser average height, diameter, and potentially shoot biomass than the plants in the autoclaved soil. It is likely that the fungi acted as a net sink of carbon as this is a common phenomenon in mycorrhizal-plant relationships (Bidartondo et al. 2001; Dosskey et al. 1990; Nehls et al. 2007; Selosse et al 2006). This supports the proposal that nutrient transfer to mycorrhizae may act to reduce the growth rate of certain plants, though it cannot be determined if this occurred disproportionately to good carbon-donors.

One possible explanation of the differences between Weremijewicz and Janos 2013 and this study is that the plant hosts they used form associations with arbuscular mycorrhizae, while this study was ectomycorrhizal. There is little evidence that arbuscular mycorrhizas partake in fungal-plant carbon transfer (Robinson and Fitter 1999) and so it may be that the availability of carbon flow somehow balances the unequal reallocation of other nutrients (i.e. phosphorus and manganese). There may have also been differences in fungal density. It is plausible that the needs of individual fungi change in certain density conditions, triggering different allowances of parasitism or tendencies of favouritism (Bever et al. 2010). There may also be some environmental factor that influenced the difference between the studies. Preiss et al. 2010 showed that varying the light conditions of mycoheterotrophic orchids resulted in differing levels of plant-fungus carbon and nitrogen transfer. There is also evidence that local CO_2 concentration can influence mycorrhizal infection rate and propensity to contribute to plant growth (Choi et al. 2005).

An important concern raised by our conclusions is that there is little proof that the results were specifically mycorrhizal. As mentioned above, there is the possibility that there is some other soil microbial mechanism providing for the seemingly dampened competition in

the non-autoclaved soil (Bever et al. 2010). Our study did not test whether specific morphotypes were found disproportionately on poor carbon-donors, which could result in resource partitioning. However, considering the low diversity of fungal morphotypes, differences in resource acquisition between fungal species were not likely the primary cause of reduced competition.

Conclusion

There does appear to be some evidence that ectomycorrhizal networks can play a biologically significant role in dampening competition and allowing for coexistence of similar species. Gini coefficient averages were not significantly lower between sterilization treatments, but three of four variables measured indicated strong trends to this end. Lack of height difference after sixteen weeks suggests that concerns over uneven soil conditions were not relevant and autoclaving appeared to successfully eliminate mycorrhizal inoculum.

The possibility of reduced intraspecific competition via CMNs has interesting implications with regard to stand dynamics. It is unknown how long the equalizing effect of CMNs remains relevant in terms of stand development. If the pattern persists as the trees grow, it may be that ecologically significant differences arise in the dynamics between seedlings regenerating in undisturbed soil with intact CMNs and seedlings regenerating in disturbed soils (i.e. clear-cuts resulting from timber harvest). For example, competition for light could be less of a limiting factor to growth in trees grown in undisturbed soils. If this is the case, it may be an important factor to consider in the development of growth and yield models for Douglas-fir.

Replication of this study in various environmental conditions (e.g. light, CO_2, soil water content) may provide insight as to why observed trends were not stronger.

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