ECOLOGY LETTERS

Ecology Letters, (2021) doi: 10.1111/ele.13737

LETTER

The mycorrhizal tragedy of the commons

Nils Henriksson,[1]* Oskar Franklin,[2] Lasse Tarvainen,[3] John Marshall,[1] Judith Lundberg-Felten,[4] Lill Eilertsen[4] and Torgny Näsholm[1]

Abstract

Trees receive growth-limiting nitrogen from their ectomycorrhizal symbionts, but supplying the fungi with carbon can also cause nitrogen immobilization, which hampers tree growth. We present results from field and greenhouse experiments combined with mathematical modelling, showing that these are not conflicting outcomes. Mycorrhizal networks connect multiple trees, and we modulated C provision by strangling subsets of *Pinus sylvestris* trees, assuming that carbon supply to fungi was reduced proportionally to the strangled fraction. We conclude that trees gain additional nitrogen at the expense of their neighbours by supplying more carbon to the fungi. But this additional carbon supply aggravates nitrogen limitation via immobilization of the shared fungal biomass. We illustrate the evolutionary underpinnings of this situation by drawing on the analogous *tragedy of the commons*, where the shared mycorrhizal network is the commons, and explain how rising atmospheric CO_2 may lead to greater nitrogen immobilization in the future.

Keywords

Carbon, forest, immobilization, mycorrhiza, nitrogen, trade.

Ecology Letters (2021)

INTRODUCTION

In boreal forests, ectomycorrhizal fungi (EMF) contribute significantly to tree nitrogen (N) acquisition, which is frequently the growth-limiting factor in this biome (Högberg *et al*. 2017). But mycorrhizal N is acquired at the cost of photosynthetic carbon (C) (Colpaert *et al*. 1996; Corrêa *et al*. 2008). This is the basis for mycorrhizal trade, but despite the fact that it is one of the most widespread and influential symbioses in boreal forest ecosystems, the ecological nature of this exchange has not been settled (Johnson *et al*. 1997; Alberton *et al*. 2005; Franklin *et al*. 2014; Terrer *et al*. 2019). This represents a critical weak point in any predictions of ecosystem responses to future perturbations of C or N dynamics (Alberton *et al*. 2007; Högberg *et al*. 2017).

Ectomycorrhizal symbioses can vary from mutualism to parasitism, depending on prevailing growth conditions (Johnson *et al*. 1997; Alberton *et al*. 2007; Ågren *et al*. 2019). Under N limitation, host plants have been observed to continue supplying their ectomycorrhizal partner with C, and even increasing the C investment, despite diminishing N returns (Corrêa *et al*. 2008, 2010). If N availability is amended via fertilization, however, EMF transfer a greater proportion of their absorbed N (Näsholm *et al*. 2013). N is thus withheld under conditions of limiting availability, and the host tree cannot unlock it by supplying the EMF with more C, because such an investment results in further diminishing N returns. The eco-evolutionary explanation is that each fungal individual competes with other EMF symbionts of the same plant and can gain a larger share of the plant's C supply by increasing its N export, until its own remaining N matches its C supply (Näsholm *et al*. 2013). Conversely, a larger C flux from the plant allows the fungus to use a greater proportion of the N it absorbs from the soil, as dictated by the stoichiometric requirements of fungal biomass and growth (Alberton *et al*. 2007; Näsholm *et al*. 2013; Franklin *et al*. 2014). Enhanced EMF growth may initially increase N uptake and, by extension, export to host plants but N availability eventually becomes limiting, whereas N immobilization in fungal biomass continues, leading to a negative feedback on the plant's N uptake (Corrêa *et al*. 2010; Näsholm *et al*. 2013). This sequence has been suggested as a mechanism for observed progressive N limitation in forests under increased atmospheric CO_2 concentrations (Alberton *et al*. 2007; Högberg *et al*. 2017). The presence of such an N-immobilizing feedback loop raises the question of how the symbiosis can remain stable over evolutionary time scales and how it has survived natural selection.

Here, we present an ecological framework to reconcile the observed plant and fungal behaviours summarized above, by recognizing the dual scale of the ectomycorrhizal symbiosis. The critical point is that multiple fungi can colonize the roots of a given tree and that several trees can be connected to the same fungal individual, creating common mycorrhizal networks (Southworth *et al*. 2005). Trees have evolved to maximize their own competitive benefit from the symbiosis at the individual tree scale, but this maximisation also has consequences for other trees with whom they share EMF partners at the *network scale*.

The classic paper titled *The Tragedy of the Commons* (Hardin 1968) presents a theory of over-exploitation of shared resources which effectively illustrates the evolutionary underpinnings which have led to the widespread success of a symbiosis in which one partner is in fact maintaining its own resource limitation: Trees do not coordinate their carbon investments within the

[1]*Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå SE-90183, Sweden*
[2]*International Institute for Applied Systems Analysis, Schlossplatz 1, Laxenburg A-2361, Austria*
[3]*Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg SE-40530, Sweden*
[4]*Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre Swedish University of Agricultural Sciences, Umeå SE-90183, Sweden*
**Correspondence: E-mail: nils.henriksson@slu.se*

shared fungal network, but act to increase individual advantages over their neighbours. In Hardin's example, a common pasture was depleted by several herdsmen who all increased the number of cattle they kept there. From each herdsman's perspective, this is the rational course of action, because the cost of the degraded pasture is divided among all users, whereas the individual herdsman receives the entire reward of an extra head of cattle. Analogously, all host trees are competing for enhanced shares of mycorrhizal nutrients, but their combined efforts serve to aggravate the overall nutrient immobilization in fungal biomass.

Hypothesis

We hypothesize that common EMF symbionts reward the host plants that supply the most C by allocating a greater proportion of their total exported N to them. Conversely, the hosts, which share multiple EMF symbionts, reward the partners that supply the most N by releasing a greater proportion of their total C export to those networks. The 'tragedy' from the plant viewpoint arises when the total C export to all fungi is so high that it leads to N immobilization, in which case the proportions cease to matter and all plants suffer. The 'tragedy' from the fungal viewpoint arises when exporting more N would reduce their own growth, but exporting less N would reduce their competitiveness for plant C (Näsholm *et al.* 2013; Franklin *et al.* 2014).

This hypothesis has specific and predictable implications for plant N uptake in response to C export (mathematically formulated in the Materials and Methods section of this article): A positive linear relationship at the individual plant level and a saturating or hump-shaped relationship at the community level (Fig. 1, alternative 3). As stated, the hypothesis should be rejected if a similar relationship between N uptake and C export was observed at both individual and community levels (Fig. 1, alternatives 1, 2). Such a result would imply a lack of inter-plant competition for N through a common network.

We conducted two experiments, (in the greenhouse and field conditions), where belowground C flux was reduced by

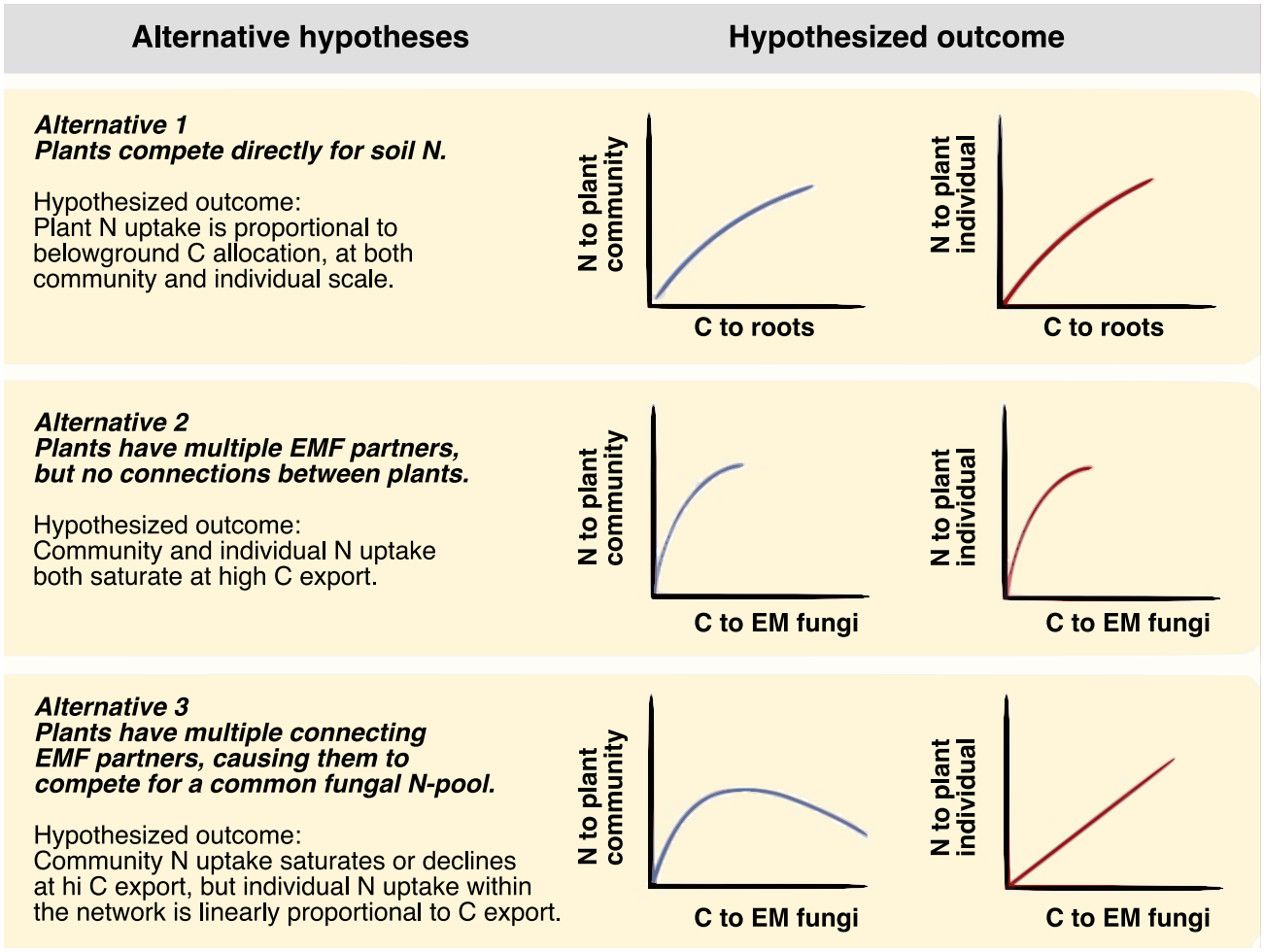

Figure 1 Descriptions and expected outcomes for three alternative hypotheses. (1) Each plant takes up soil N directly rather than via ectomycorrhizal fungi (EMF). This precludes N immobilization in EMF biomass and leads to a continuously increasing N uptake with C export, both at individual and community N levels. (2) Plants have EMF symbionts but no common EMF network. While the EMF provide N to the plants, they also immobilize N, which leads to a stronger saturation of plant N uptake than in alternative 1. (3) A common EMF network. This allows each plant to gain N at the expense of the other plants in the network by exporting more C to the EMF, resulting in a share of community N uptake proportional to each plant's contribution to ectomycorrhizal C supply. This individual plant behavior drives N immobilization that results in declining N export at the community scale.

shading and/or stem strangling. Strangling is a treatment whereby belowground C flux is physically restricted by blocking phloem transport. It has been proven to consistently control plant C export to roots and EMF (Björkman 1944; Henriksson *et al*. 2015). Strangling a subset of seedlings growing in the same pot accomplishes two things: (1) the total belowground C flux is decreased, and (2) each seedling's relative contribution to that flux is altered. Shade treatments were also applied to uniformly reduce total C availability to belowground structures in a subset of pots.

MATERIALS AND METHODS

Seedling experiment

Preparation of mycorrhizal inoculum

A culture of *Suillus variegatus* was prepared based on the protocol described in (Vuorinen *et al*. 2015) with a few modifications. Briefly, ½ MMN medium, was prepared. The media contained 1.25 g L^{-1} glucose, 5 g L^{-1} malt, 0.5 g L^{-1} $(NH4)_2HPO_4$, 0.5 g L^{-1} KH_2PO_4, 0.15 g L^{-1} $MgSO_4°7H_2O$, 0.05 g L^{-1} $CaCl_2*2H_2O$, 0.025 g L^{-1} NaCl, 0.02 g L^{-1} Fe-EDTA, 0.02 g L^{-1} Thiamine-HCl and the pH value adjusted to 5.8 with NaOH-HCl. As inoculum, plugs (5 × 5 mm) from the actively growing peripheral zone of *S. variegatus* mycelia, growing on solid ½ MMN agar plates, were used. The mycelia were first cultured in 250 mL ½ MMN medium in 1 L Erlenmeyer flasks, sealed with cotton and aluminium foil for 16 days in a dark incubator sat at 23°C and rotation speed at 100 r/m. Following this, the liquid culture was homogenized and mixed with silica powder (Sipernat 22S; Algol Chemicals AB, Malmö, Sweden) moistened with ½ MMN medium to a moisture content of 70%, by weight (250 g silica in each container). The containers were placed in a dark and ventilated space and the mycelium was allowed to grow for another 28 days.

Seedling growth conditions

On May 10, 2018 (DOY 130, Table S2), 2-year-old *Pinus sylvestris* seedlings were bare-rooted, weighed and potted in a soil mix containing 10% *S. variegatus* inoculum and 90% soil (50-50 mixture of peat and commercial non-fertilized potting soil). No measures were taken to exclude fungal species already present on the roots of the seedlings. Each pot (23 cm x 17 cm x 6 cm) contained six seedlings, planted as shown in Fig. 1b. Seedling fresh weight was measured before planting. Seedlings were randomly selected to pots and there was no significant difference in initial fresh weight among seedlings that would subsequently be allotted to different treatments ($P = 0.74$).

To allow the seedlings to establish themselves in their pots, they were kept in controlled greenhouse conditions for 53 days. They were then transferred outdoors, first to partial shade for 10 days, to avoid damage to the needles from sudden exposure to direct sunlight, and then into the open. After 18 days in direct sunlight, the experimental treatments were initiated. Thus, the experimental treatments were begun on July 30, 2018 (DOY 211), 81 days after the seedlings were potted. Finally, all the pots were transferred back inside the greenhouse for the final month of the study. This was done to avoid the loss of ^{15}N tracer in autumn rains. All pots were watered daily throughout the experiment duration.

Shading and strangling treatments

Half of the pots were covered by individual shade tents constructed of DeWitt UV PE-knitted shade cloth (Agriculture Solutions, LLC, Strong, ME, USA), reducing incoming photosynthetic radiation (PAR) by 78.8 ± 4.8% (mean ± SD).

Within each pot, the six seedlings could be either strangled or left unstrangled, and the treatments were designed so that 0, 1, 5 or 6 seedlings were strangled. Thus, there were four levels of the strangling treatment, and two levels of the light treatment (Fig. 1). This resulted in a total of eight factorial combinations of treatments that were replicated five times in a blocked design.

Seedlings were strangled by tightly wrapping iron wire (0.7 mm diameter) around the stems below the lowest branch (Björkman 1944). This method, and modified versions for large trees, have been shown to effectively reduce belowground C flux in *P. sylvestris* and *P. ponderosa* (Björkman 1944; Henriksson *et al*. 2015). In Björkman (1944) strangling of 3-year-old pine seedlings for one entire season was shown to strongly reduce root soluble carbohydrate levels as well as ectomycorrhizal colonization rate (<5% of root tips, compared to c. 65% in control seedlings). In that publication, the strangling wire was removed from a subset of the seedlings after 3 months, resulting in intermediary levels of both measurements.

^{15}N application

Thirty-two days after the initiation of shade and strangling treatments, ^{15}N was applied to the soil surface of each pot (DAY 243), in the form of KNO_3 (Larodan AB, Karolinska Institutet Science Park, Stockholm, Sweden). The total added quantity corresponded to 0.3 g $^{15}N/m^2$, which was applied in three doses over the course of 6 days to avoid flushing the system with nitrogen. Each pot thus received a total of 0.012 g ^{15}N.

To avoid loss of ^{15}N from the bottom of the pots, as well as isotopic contamination between pots, the pots were placed in individual trays before isotopic label was applied, and remained in these for the duration of the study. Any water that drained out the bottom was used to re-water the same pot using a syringe.

Final harvest and sampling

Three weeks after the ^{15}N labelling (on DOY 269–276), the seedlings were harvested. All 240 seedlings (6 seedlings × 40 pots) were washed and their roots separated. The needles, roots, stem and buds of each seedling were separated, and weighed. For strangled seedlings, the wire was removed before weighing the stem. The material was then dried at 60°C for 48 h before being weighed again.

The dried needles, roots and stem of each seedling were milled in a chamber mill (IKA-Werke GmbH & Co.KG, Staufen im Breisgau, Germany). Using isotope ratio mass spectrometry, the total C and N contents (% C and % N) and the isotopic enrichment of ^{15}N were analysed for each plant compartment.

The current setup allows quantification of the ^{15}N abundance in the entire seedling biomass, rather than relying on foliage concentration, which is commonly used as a measure of N uptake in field conditions (Hasselquist *et al.* 2016). By measuring each seedling compartment separately (needles, roots, stem), we avoid problems in distinguishing actual N mobilization from a potential shade avoidance response in the trees (Henry & Aarssen 1997), which could shift internal N partitioning towards the foliage.

Statistical analyses

Seedling dry weights, elemental and isotopic composition and photosynthetic rates were compared using a standard least squares means model where light level and the number of strangled seedlings per pot were considered as fixed effects in a factorial design. Where the initial tests yielded *F*-scores < 0.05, Student's *t*-test or Tukey's HSD were used post hoc, to perform pairwise comparisons within groups. The statistical analyses were carried out in JMP (JMP® pro 15.0.0; SAS Institute Inc., Cary, NC, USA).

Field experiment

The study site

The experiment was conducted in a 15- to 20-year-old, naturally regenerated *P. sylvestris* stand located in northern Sweden (64°14′ N, 19°46′ E and 175 m above sea level). The soil is weakly podsolized sandy silt sediment, and the field layer consists mainly of lichens, with infrequent occurrences of *Calluna vulgaris* and *Vaccinium vitis-ideae*. The organic mor layer is 1–3 cm thick, and has a C-N ratio of 37 ± 1 and pH of 4.0 ± 0.1 (Hasselquist *et al.* 2016).

The trees were between 3 and 5 m tall with stem diameters at breast height of 7.4 ± 2.6 cm (mean ± 1 SD). The site is very N-poor and has an uneven stand density, including bald patches as well as patches where the trees grow closer together.

Study design

We selected 23 circular plots with the radius of 2 m, using a tree as the centre. All trees growing within this area were considered as part of the plot. Plots contained 5–11 trees (7.1 ± 1.7 mean ± SE) and had a total basal area at breast height of 3.2 ± 1.2 dm^2 (mean ± SE). The plots were placed around trees growing in the denser patches of the site, so that they were naturally semi-discrete in the landscape. We had two reasons for doing this: First, the higher tree-density in these patches allowed us to assume that these trees were occupying the same soil volume and were more likely connected to the same mycorrhizal network. Second, the surrounding low-density areas should help reduce the influence from trees whose stems grew outside the plot boundary.

We designed four plot-level treatments using stem strangling to reduce the trees' belowground C-transport (detailed description in Henriksson *et al.* 2015). In Henriksson *et al.* (2015) canopy ^{13}C labelling and subsequent isotopic analysis of phloem carbohydrates showed that none of the $^{13}CO_2$ absorbed after strangling was transported past the strangling point to the lower part of the stem. The experiment described in that publication was performed on similar trees to this study, and in the same study area. Plots were considered to have two types of trees – the centre tree, and neighbour trees –which could be either strangled or not strangled. In other words, we either strangled the stems of all trees, none of them, only the centre tree, or all except the centre tree (Fig. 2). The plots were divided into six blocks and then randomly assigned one of the four treatments. One of the blocks only had space for three plots, and thus one of the treatments (all trees strangled except one) was only replicated five times, but all the rest were replicated six times. In each plot, two trees were selected for needle sampling (3 weeks after ^{15}N-application), the central tree and one neighbour tree.

Mean basal area did not differ between plot treatments ($P = 0.14$, ANOVA), although the variance was unequal: the basal area of control plots varied more than the other treatments ($P = 0.001$, Levine's test). The number of trees per plot was not significantly different between treatments ($P = 0.23$, ANOVA).

Sampling and treatments

All strangling treatments began on July 21, 2015 (DOY 202). On August 10 (DOY 222), we applied 2.62 g of ^{15}N-labelled KNO_3 (0.39 g ^{15}N) dissolved in water, which could be detected in needle samples taken 3 weeks later. This N form was chosen for the high C requirement associated with its reduction and assimilation, which should lead to lower efficiency of N immobilization by free-living soil microbes than would be the case with N sources like ammonium or organic N. The application was equivalent to 0.02 kg ^{15}N ha^{-1} and the solution corresponded to 2 L m^{-2}, which was evenly distributed from above and allowed to soak into the soil, over a circular area with a radius of 2.5 m (plot radius + 0.5 m), in order to treat a larger proportion of edge trees' root systems. The isotopic enrichment of N in the foliage of trees that received different treatments could then be compared to detect changes in uptake patterns among the treatments.

Model description

Our hypothesis is formulated in terms of a model that describes the C-N exchange between plants and mycorrhizal fungi, both at the stand (or network) level and from the perspective of an individual plant. It was tested and evaluated based on the data from the pot experiment, which allowed better control and isolation, and more complete quantification of the ^{15}N uptake by all plants than was possible in the field experiment. Strangling of a seedling predictably reduces its C provision below ground, to roots and the fungal network (Henriksson *et al.* 2015). Thus, we assume that the C supply to fungi is proportional to root biomass but that it is reduced by strangling according to a strangling factor estimated by the model.

Stand level C-N exchange

The growth of mycorrhizal fungi is fuelled by C supply by the plants ($\dot{C}_s$), which we consider in relative terms (compared to the mean of control plants) because its absolute value cannot be estimated and is not important for our conclusions. We assume that C supply to fungi from an individual plant ($\dot{C}_{si}$) is proportional to its root mass (C_{ri}) (Rouhier & Read 1998; Neumann & Matzner 2013) and further reduced by strangling

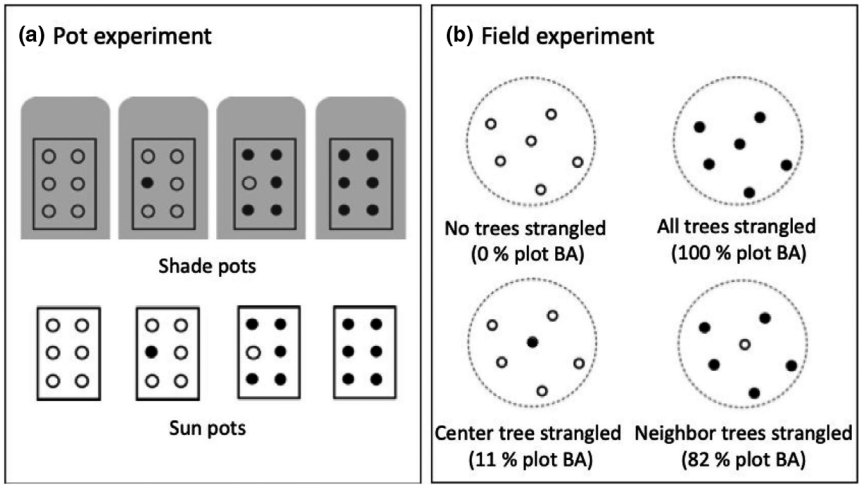

Figure 2 Schematic representation of the treatments in the two experiments. Panel (a) shows the design of the pot study. Each pot contained six seedlings. Half of the pots were shaded from above (top row). To achieve unequal belowground carbon flux among seedlings sharing the same pot, a subset of them were strangled to block phloem transport (filled circles). Out of the six seedlings, either one, five, all, or none were strangled. Panel (b) shows the strangling scheme in the field study. The circular plots had a radius of 2 m, and contained 5–11 trees. In both panels, filled circles denote strangled trees/seedlings, and open circles denote non-strangled trees/seedlings.

by a constant factor (see Supporting Information for model parameterization).

We assume that fungal N uptake ($\dot{N}_u$), is a saturating function of fungal growth (which is proportional to its biomass):

$$\dot{N}_u = \frac{N_a \dot{C}_s}{\dot{C}_s + \dot{C}_{sh}}. \quad (1)$$

In eqn 2 N_a = soil N availability (maximum potential N uptake) and $\dot{C}_{sh}$ = half-saturation $\dot{C}_s$.

N immobilization in fungal biomass is:

$$\dot{N}_f = \dot{C}_s \cdot I_f. \quad (2)$$

The N immobilization factor, $I_f = \mathrm{N{:}C}_f \cdot e_f$, where $\mathrm{N{:}C}_f$ = N:C ratio of fungal biomass and e_f = C use efficiency of fungal growth.

Combining eqns 1 and 2, N export to plants ($\dot{N}_p$), can be written as:

$$\dot{N}_p = \dot{N}_u - \dot{N}_f = \frac{N_a \cdot \dot{C}_s}{\dot{C}_s \cdot + \cdot \dot{C}_{sh}} - \dot{C}_s \cdot I_f. \quad (3)$$

Competition for N among individual plants

We assume that the fungi are attached to all plants in a pot and deliver N to each plant depending on its C supply relative to its competitors, which is postulated by eco-evolutionary theory (Wyatt *et al.* 2014) and indicated by experiments (Kiers *et al.* 2011; Fellbaum *et al.* 2014). This N competition effect was estimated in terms of N uptake of a plant i ($\dot{N}_{pi}$) relative to mean N uptake of all plants in the pot ($\bar{\dot{N}}_p$) as:

$$\frac{\dot{N}_{pi}}{\dot{N}_p} - 1 = d \cdot \left[\left(\frac{\dot{C}_{si}}{\bar{\dot{C}}_s} \right)^z - 1 \right]. \quad (4)$$

In eqn 4, $\dot{C}_{si}$ = C supply from plant i, $\bar{\dot{C}}_s$ = mean C supply from all plants, and d = degree of fungal N export discrimination according to plant C supply. d is approximately equal to the marginal tree N gain per C supply for a tree, or the marginal C gain from each tree per N export for a fungus. Theoretically, $d = 1$ maximizes the total C a fungus receives from all its tree partners, because a larger or smaller d would mean that the fungus could increase C supply by redistributing N supply among its tree partners. The parameter z allows for a potential non-linear effect of individual C supply on N uptake (for $z \neq 1$), e.g. due to N limitation of fungal partners.

The modelled relationships between individual plant scale and network scale C and N exchange are illustrated in Fig. 3. Detailed descriptions of parameter estimation and model testing are presented in the Supporting Information for this article.

RESULTS

We found that seedlings growing in shaded communities (78.8 ± 4.8% reduction of PAR, mean ± SD) for 2 months had 36% smaller dry mass at the time of harvest than seedlings in sun pots ($P < 0.0001$, Fig. 4a). Phloem strangling of individual seedlings did not affect their biomass, but it did cause a decrease in root/shoot ratio ($P < 0.0001$, Fig. 4b).

Three weeks before harvest, 12 mg traceable nitrogen isotope ^{15}N was applied to the soil surface of each pot. Shading led to 50% higher recovery of applied ^{15}N in seedling biomass (1.07 mg ^{15}N per seedling, compared to 0.71 mg ^{15}N per seedling, Fig. 4c; $P < 0.0001$). Thus, a total of 54% of applied ^{15}N was accounted for in plant biomass in shaded pots, and only 36% in sun pots. Furthermore, strangled seedlings received significantly less ^{15}N than non-strangled ones (Fig. 4 c). This supports the stoichiometric model of mycorrhizal C-N exchange, which predicts that decreased C export to fungi mobilizes soil N to the plant host and *vice versa*.

In shaded seedlings, ^{15}N allocation to foliage was higher than for seedlings grown in full sun ($P < 0.0001$, Fig. 4d). Strangling individual seedlings had the opposite effect on ^{15}N allocation, compared to shading – more N remained in the

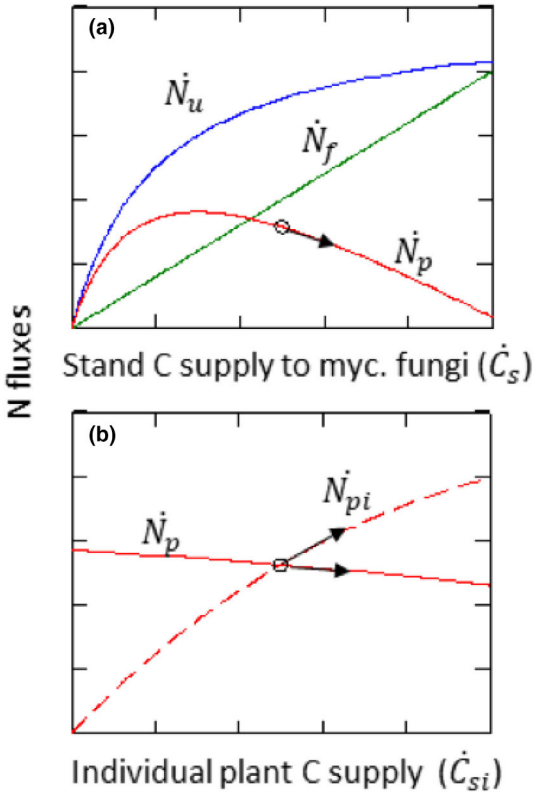

Figure 3 The two-level model of mycorrhizal interaction. (a) At the network scale, plants supply C ($\dot{C}_s$) to mycorrhizal fungi, who take up N ($\dot{N}_u$), use (immobilize) what they need for growth ($\dot{N}_f$), and export the rest to plants ($\dot{N}_p$). (b) At the individual level, each plant can increase its own share of mycorrhizaderived N ($\dot{N}_{pi}$) by increasing its C supply ($\dot{C}_{si}$), despite a small negative effect on total N uptake ($\dot{N}_p$). However, each plant's negative influence on ($\dot{N}_p$) affects all other plants as well. Thus, the effects add up at the network scale and reduce N return for all (arrow in panel a). Parameter estimation and model testing is detailed in Supporting Information. Briefly, ($\dot{N}_p$) and ($\dot{N}_{pi}$) are derived from ^{15}N data in the greenhouse experiment. C supply to fungi from an individual plant ($\dot{C}_{si}$) was assumed to be proportional to its root mass (C_{ri}) (Rouhier & Read 1998; Neumann & Matzner 2013) and further reduced by strangling by a factor (see Supporting Information).

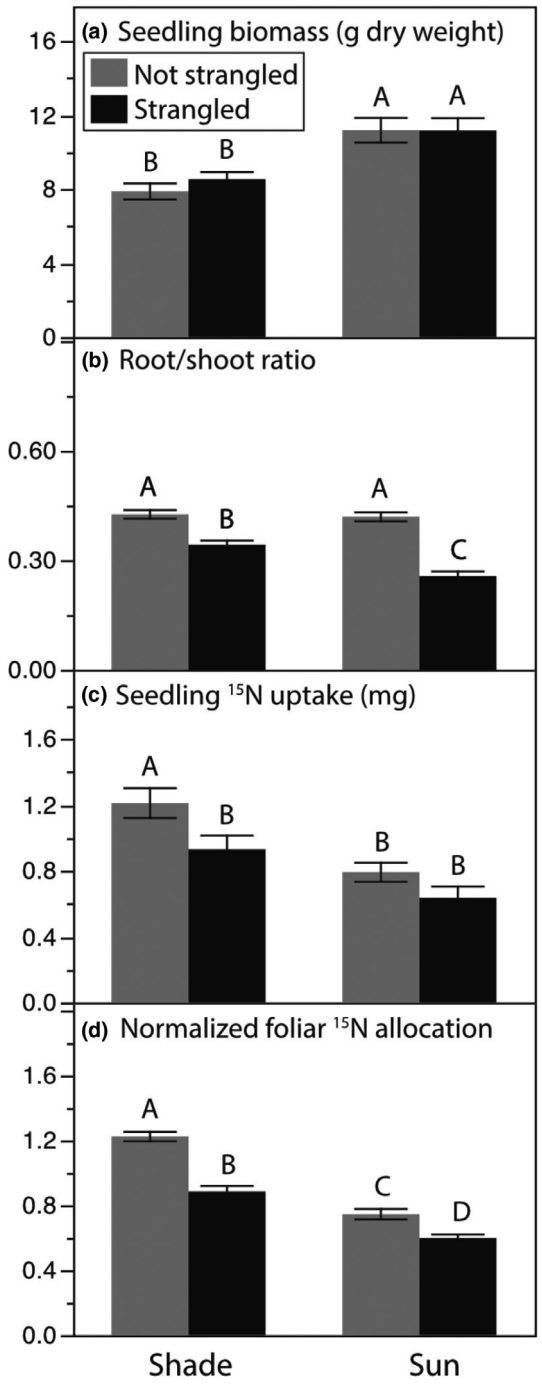

Figure 4 Seedling biomass in grams of dry weight (a); Root-to-shoot ratio (b); total amount of ^{15}N recovered in seedlings (mg seedling^{-1}) (c); and ^{15}N allocation to foliage, normalized for needle biomass fraction. It is thus a unitless fraction: (^{15}Nneedle/^{15}Nseedling)/(DWneedles/DWseedling) (d). Shaded seedlings allocated a greater proportion of their ^{15}N to foliage than sun seedlings, but strangling resulted in lower allocation to foliage. Within each panel, different letters denote statistically significant differences ($P < 0.05$). All panels show mean values with error bars corresponding to 1 SE.

mycorrhizal roots of the seedlings, which included both a fungal and a plant component ($P < 0.032$).

In the field, we found that reducing belowground C flux, by strangling one tree per plot, improved the total mobilization of applied ^{15}N label to the trees (Fig. 5a,b). In such plots, the strangled tree received less N than its neighbours (Fig. S3). Strangling a greater subset of trees, thereby further impairing the capacity for plot-scale belowground C export, caused total ^{15}N uptake to fall significantly, as was observed in plots where 82–100% of tree basal area was strangled (Fig. 5a,b).

Both shading and strangling reduce the belowground C flux that fuels the activity of mycorrhizal fungi. Based on the greenhouse experiment, we developed a model to test the connection between belowground C export and plant N acquisition at both the *individual tree-scale* (seedling) and at the *network-scale* (whole pot). The model explained 58% of the variation among individuals in the same plot, and 25% of the variation between pots in plant N uptake. In addition to the measured effects on root mass, the model indicated that C flux to mycorrhiza per root mass was significantly reduced by strangling (by 55%) but not by shading (Supporting Information).

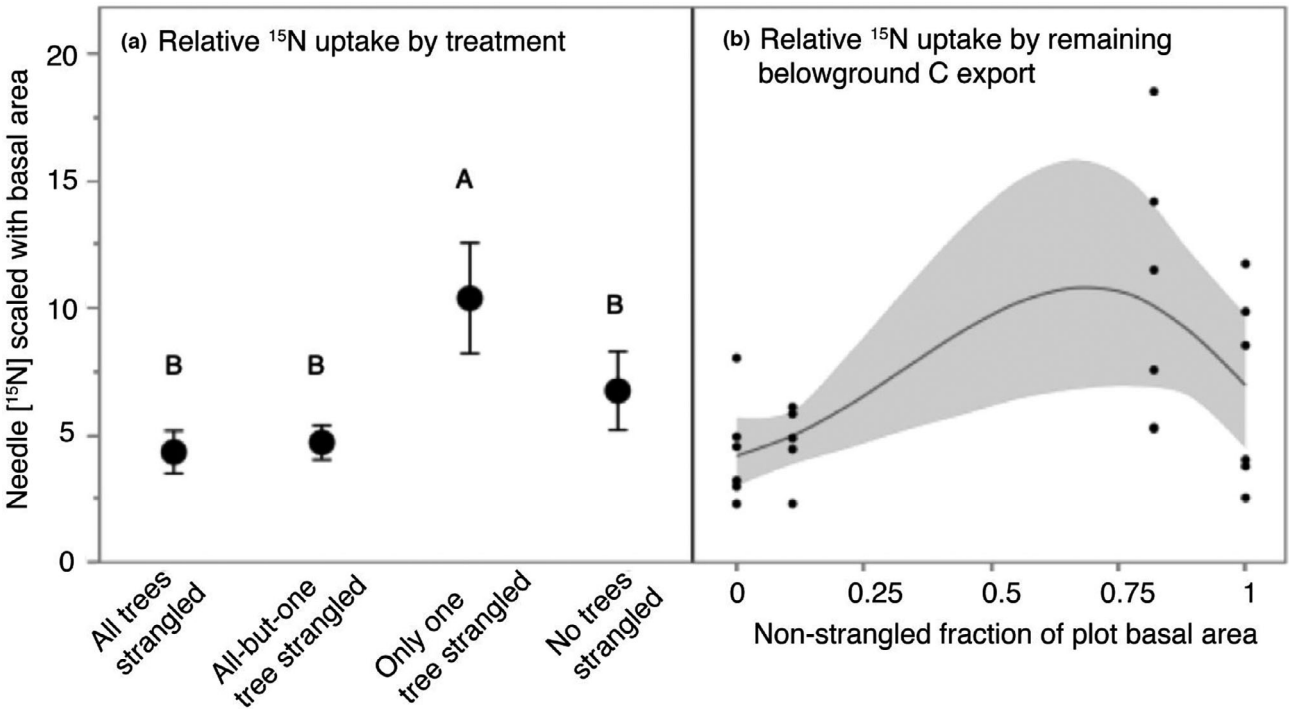

Figure 5 ^{15}N uptake by *Pinus sylvestris* plots in the field. Needle ^{15}N concentration has been scaled up using tree basal area, and the sum for each plot is presented as a relative comparison of total N mobilization. Total plot C provision to the mycorrhizal network is assumed to have been reduced in proportion to the fraction of tree basal area that was strangled in each plot. Panel (a) shows the uptake (mean ± 1 SE) of plots where varying numbers of trees were strangled. Different letters denote statistically significant difference between treatments (ANOVA, Tukey-Kramer, $P < 0.05$). In panel (b) the same data is plotted against a continuous *x*-axis showing the fraction of plot basal area which could still conduct C to belowground structures (i.e. non-strangled trees). The curve shows the predicted mean ^{15}N uptake with a shaded confidence of fit ($P = 0.05$). This curve can be compared to Fig. 6a.

The model, supported by our measurements, shows that the greatest N-mobilization for plant use occurred at an intermediate level of belowground C export (Fig. 6a). Initially, N uptake and export to plants increases steeply with C supply to mycorrhizal fungi, but the rate of increase gradually declines as hyphae fill up the soil and N becomes limiting. At the same time, N immobilization in fungal biomass increases linearly with C supply, which eventually results in declining net N export to plants. Thus, the model corroborates the hypothesis that maximum *network-scale* N mobilization occurs at an intermediate level of *network-scale* C export. At the individual tree-scale, the marginal increase in the share of N that the plant receives per share C supplied is approximately equal to one (0.95), as theoretically predicted (Fig. 6b). This drives a competition for N among trees where each tree at first gains N by fueling fungal growth, but later the whole community suffers from N immobilization in the shared fungal network.

DISCUSSION

We show that belowground C allocation to can fuel N immobilization, reducing the amount of N to be distributed among the trees. But we also found that individual trees received nutritional benefits in proportion to their carbon contribution to the fungal network in accordance with our hypothesis (Fig. 1, alternative 3). This apparent incongruity can be explained by invoking the concept of the *tragedy of the commons*, as described by Hardin in 1968 (Hardin 1968).

Our estimates of plant C export to EMF are constrained by root measurements and a strangling effect (previously shown to predictably reduce below-ground C export (Henriksson *et al.* 2015). The only free parameter affecting the modelled C export was the magnitude of the strangling effect per root biomass. As an additional test, we used respiration measurements to make independent estimates of the C export to EMF at the pot (community) level, which were well correlated with the model results and suggesting that the model explains 39% of the variation in EMF respiration (Supporting Information). The underlying details of the strangling effect on C export are not relevant for our conclusions but may include reduced EMF colonization of roots (Björkman 1944), and reduced growth of extraradical mycelia extending from strangled roots. Either way, the strangled plants cannot have been completely ejected from the EMF network, or their N uptake would not fall on the same line as the non-strangled plants in Fig. 6b.

The most plausible alternative scenarios and their implications are displayed in Fig. 1: (1) direct N uptake without EMF and (2) N uptake via EMF but without a common network. In alternative 1, plant N uptake would increase with below ground C allocation to roots and would tend to saturate, but never decline, at higher C allocation. Competition would lead to slightly less saturation at the individual than at the community level (Franklin *et al.* 2014). Alternative 2 would lead to initially increasing but eventually saturating plant N uptake with C export, due to linearly increasing

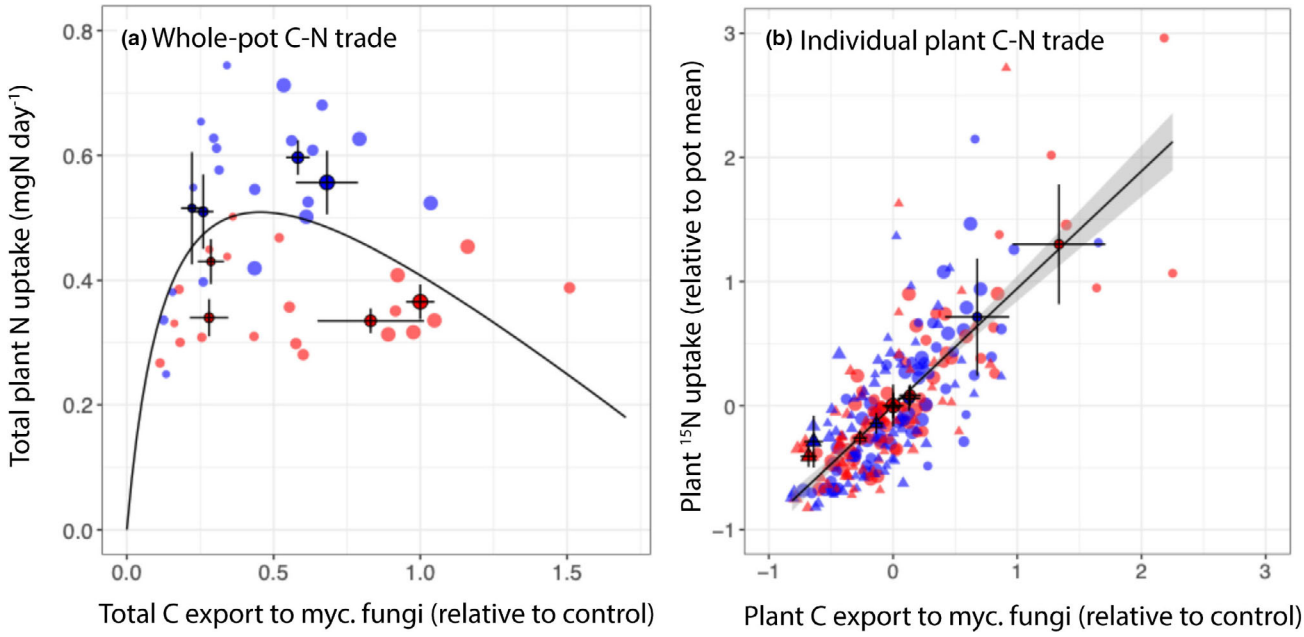

Figure 6 Modelled relationship between plant C export to common mycorrhizal fungi and fungal N mobilization to its hosts. Panel "a" shows estimated pot-scale N uptake based on measured ^{15}N vs. modeled pot C supply to mycorrhizal fungi relative to control pots (no strangling and no shading). Blue circles = shaded pots and red circles = non-shaded pots. Symbol size indicate the number of plants strangled in the pot: 0 (largest) > 1 > 5 > 6 (smallest). Symbols with black border are mean values per treatment with error bars (standard error). The black line shows predicted plant N uptake (Fig. 3; Materials and Methods), $r^2 = 0.25$. Panel "b" shows measured individual plant-scale ^{15}N uptake vs. modelled C supply to mycorrhizal fungi of individual plants relative to pot mean values. Triangles denote strangled seedlings and circles represent non-strangled seedlings. Grey line with shading is a linear regression with confidence interval, where the slope = 0.95 is the partner discrimination parameter d in the model (see Materials and Methods), $r^2 = 0.58$.

fungal growth and N immobilization with C export. The response would be similar at both the individual level and the community level as there is no strong inter-plant competition for EMF-derived N. Only if plants take up and compete for N via a common EMF network (alternative 3 in Fig. 1) is it possible to obtain the contrasting results shown in Fig. 6, i.e. a linear increase in plant N uptake with C export at the individual level (scaling exponent = 1.038, Supporting Information) and a hump-shaped relationship at the community level. The linearity of the individual response was tested, resulting in a. We conclude that the most reasonable interpretation of this data is the presence of common EMF network (Nara 2006; Beiler *et al.* 2010) in which multiple fungi connect the host plants to each other (Franklin *et al.* 2014).

A stable evolutionary strategy for a multi-partner trade network is achieved when individuals allocate resources among symbionts of the other species in proportion to the relative benefits they receive from each partner. This is called proportional discrimination and has been applied to modelled mycorrhizal networks (Wyatt *et al.* 2014). The fact that our model (where plant N uptake was measured and relative C contribution to fungi was modelled) resulted in a linear proportionality (with slope 0.94, not significantly different from 1) between relative C investment and N uptake strongly indicates the presence of a common mycorrhizal network.

In our experiment, seedlings were potted in soil containing inoculum of the EMF *Suillus variegatus*. This was done to minimize differences in EMF community composition because we focused on the response of a given mycorrhizal system to environmental changes rather than on differences between EMF species. As mentioned in the Methods section, seedlings were 2 years old at the time of planting, and any EMF species already present on the root systems from their time at the nursery were not excluded, potentially leading to some variation in EMF species composition among plants. However, the basic principles of C-N trade in our model should be valid for EMF species in general, and we expect species differences to influence absolute values of N uptake but not the qualitative patterns of C-N exchange observed.

Rising atmospheric CO_2 could significantly increase mycorrhizal fungal biomass (Treseder 2004), which could drive progressive N limitation in forests via mycorrhizal immobilization (Alberton *et al.* 2007; Alberton & Kuyper 2009; Steidinger *et al.* 2019). Our results support this notion. In fact, the field-studied Scots pines were observed to export higher-than-optimal quantities of C under untreated conditions, in agreement with Hasselquist *et al.* (2016) (Fig. 5). Therefore, any increase in the C supply to EMF should further exacerbate the network-scale N limitation in the studied forest stand (Figs 3 and 6a). However, this may not be the case in locations where soil N availability is greater, or in situations where increased C supply allows EMF to have access to more energy-demanding N sources, such as complex organic substrates.

Although a global model meta-analysis of elevated CO_2 experiments (Terrer *et al.* 2019) concluded that rising atmospheric CO_2 would continue to stimulate plant growth in boreal forests in general, empirical evidence for N-poor boreal forests is scant. Of the two experiments from forests similar to ours included in the meta-analysis, one showed a small negative CO_2 effect (Sigurdsson *et al.* 2013). In support of our

prediction, Alberton *et al*. (2005) observed that, in laboratory conditions, the growth-enhancing effect of elevated CO_2 was greater in the fungal component of an ectomycorrhizal symbiosis than it was in the plant. They concluded that this should eventually cause increased plant-fungus competition for N, and suggested this as a mechanism for future progressive N limitation, in line with our model predictions. This further suggests that the risk of losing forests dominated by ectomycorrhizal tree species due to climate change (Steidinger *et al*. 2019) may be unfounded, as that study did not account for increasing N limitation (relative to C) caused by rising CO_2, which should reinforce the stability of the ectomycorrhizal symbiosis (Franklin *et al*. 2014).

That the *tragedy of the commons* mechanism has gone almost unnoticed by scientists until now may be due to many mycorrhizal C-N trade experiments having used setups containing a single plant, paired with a fungal partner (Colpaert *et al*. 1996; Corrêa *et al*. 2010). Such a design cannot capture network-scale drivers of EMF–plant interactions. Arbuscular mycorrhiza connected to multiple host plants can preferentially supply nutrients to the plants presenting greater C-sources for the fungi (Weremijewicz & Janos 2013; Fellbaum *et al*. 2014; Weremijewicz *et al*. 2016). However, this study is the first to show the link between plant–plant competition within EMF networks and the resulting N immobilization as measured at the system level.

ACKNOWLEDGEMENTS

The authors thank the staff of SLU Stable Isotope Laboratory for isotopic analyses for services provided and their valuable input on isotopic analyses, as well as Karin Grensjö, Amez Jaafar Hussein and Hyungwoo Lim for help with seedling harvest and sample preparations. The work presented in this publication was funded from the following sources: Grant awarded to N.H.: Anna och Gunnar Vidfelts fond (Skogssällskapets närstående stiftelser). Grants awarded to T.N.: Knut och Alice Wallenbergs Stiftelse (# 2015.0047), The Kempe Foundations, and The Swedish University of Agricultural Sciences (T4F, Bio4Energy).

AUTHORSHIP

N.H. performed the experiment, analysed data, and drafted the manuscript. O.F. developed the model based on experimental data. L.T., J.M., T.N., J.F. and L.E. contributed to the experimental design and interpretation of data. All authors contributed to the manuscript text.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ele.13737.

DATA AVAILABILITY STATEMENT

The data supporting the results and the model script will be archived in an appropriate public repository, and the data DOI will be included in the article.

REFERENCES

Ågren, G.I., Hyvönen, R. & Baskaran, P. (2019). Ectomycorrhiza, friend or foe? *Ecosystems*, 22, 1561–1572.

Alberton, O., Kuyper, T.W. & Gorissen, A. (2005). Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO_2. *New Phytol*., 167, 859–868.

Alberton, O., Kuyper, T.W. & Gorissen, A. (2007). Competition for nitrogen between *Pinus sylvestris* and ectomycorrhizal fungi generates potential for negative feedback under elevated CO_2. *Plant Soil*, 296, 159–172.

Alberton, O. & Kuyper, T.W. (2009). Ectomycorrhizal fungi associated with *Pinus sylvestris* seedlings respond differently to increased carbon and nitrogen availability: implications for ecosystem responses to global change. *Glob. Change Biol*., 15, 166–175.

Beiler, K.J., Durall, D.M., Simard, S.W., Maxwell, S.A. & Kretzer, A.M. (2010). Architecture of the wood-wide web: *Rhizopogon* spp. genets link multiple Douglas-fir cohorts. *New Phytol*., 185, 543–553.

Björkman, E. (1944). Strangling effect on mycorrhiza establishment in pine. *Svensk botanisk tidsskrift*, 38, 1–14.

Colpaert, J.V., Van Laere, A. & van Assche, J.A. (1996). Carbon and nitrogen allocation in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* L. seedlings. *Tree Physiol*., 16, 787–793.

Corrêa, A., Hampp, R., Magel, E. & Martins-Loução, M.-A. (2010). Carbon allocation in ectomycorrhizal plants at limited and optimal N supply: an attempt at unraveling conflicting theories. *Mycorrhiza*, 21, 35–51.

Corrêa, A., Strasser, R.J. & Martins-Loução, M.A. (2008). Response of plants to ectomycorrhizae in N-limited conditions: which factors determine its variation? *Mycorrhiza*, 18, 413–427.

Fellbaum, C.R., Mensah, J.A., Cloos, A.J., Strahan, G.E., Pfeffer, P.E., Kiers, E.T. *et al*. (2014). Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytol*., 203, 646–656.

Franklin, O., Näsholm, T., Högberg, P. & Högberg, M.N. (2014). Forests trapped in nitrogen limitation – an ecological market perspective on ectomycorrhizal symbiosis. *New Phytol*., 203, 657–666.

Hardin, G. (1968). The tragedy of the commons. *Science*, 162, 1243–1248.

Hasselquist, N., Metcalf, D.B., Inselsbacher, E., Stangl, Z., Oren, R., Näsholm, T. *et al*. (2016). Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. *Ecology*, 97, 1012–1022.

Henriksson, N., Tarvainen, L., Lim, H., Tor-Ngern, P., Palmroth, S., Oren, R. *et al*. (2015). Stem compression reversibly reduces phloem transport in *Pinus sylvestris* trees. *Tree Physiol*., 35, 1075–1085.

Henry, H.A.L. & Aarssen, L.W. (1997). On the relationship between shade tolerance and shade avoidance strategies in woodland plants. *Oikos*, 80, 575.

Högberg, P., Näsholm, T., Franklin, O. & Högberg, M.N. (2017). Tamm review: on the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *For. Ecol. Manage*., 403, 161–185.

Johnson, N.C., Graham, J.-H. & Smith, F.A. (1997). Functioning of mycorrhizal associations along the mutualism–parasitism continuum*. *New Phytol*., 135, 575–585.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E. *et al*. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333, 880–882.

Nara, K. (2006). Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytol*., 169, 169–178.

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S.G., Campbell, C. *et al*. (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytol*., 198, 214–221.

Neumann, J. & Matzner, E. (2013). Biomass of extramatrical ectomycorrhizal mycelium and fine roots in a young Norway spruce stand – a study using ingrowth bags with different substrates. *Plant Soil*, 371, 435–446.

Rouhier, H. & Read, D.J. (1998). Plant and fungal responses to elevated atmospheric carbon dioxide in mycorrhizal seedlings of Pinus. *Environ. Exp. Bot*., 40, 237–246.

Sigurdsson, B.D., Medhurst, J.L., Wallin, G., Eggertsson, O. & Linder, S. (2013). Growth of mature boreal Norway spruce was not affected by elevated [CO_2] and/or air temperature unless nutrient availability was improved. *Tree Physiol.*, 33, 1192–1205.

Southworth, D., He, X.-H., Swenson, W., Bledsoe, C.S. & Horwath, W.R. (2005). Application of network theory to potential mycorrhizal networks. *Mycorrhiza*, 15, 589–595.

Steidinger, B.S., Crowther, T.W., Liang, J., Van Nuland, M.E., Werner, G.D.A., Reich, P.B. *et al.* (2019). Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature*, 569, 404–408.

Terrer, C., Jackson, R.B., Prentice, I.C., Keenan, T.F., Kaiser, C., Vicca, S. *et al.* (2019). Nitrogen and phosphorus constrain the CO2 fertilization of global plant biomass. *Nat. Clim. Chang.*, 9, 684–689.

Treseder, K.K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytol.*, 164, 347–355.

Vuorinen, I., Hamberg, L., Müller, M., Seiskari, P. & Pennanen, T. (2015). Development of growth media for solid substrate propagation of ectomycorrhizal fungi for inoculation of Norway spruce (*Picea abies*) seedlings. *Mycorrhiza*, 25, 311–324.

Weremijewicz, J. & Janos, D.P. (2013). Common mycorrhizal networks amplify size inequality in *Andropogon gerardii* monocultures. *New Phytol.*, 198, 203–213.

Weremijewicz, J., Sternberg, L.D.S.L.O.R. & Janos, D.P. (2016). Common mycorrhizal networks amplify competition by preferential mineral nutrient allocation to large host plants. *New Phytol.*, 212, 461–471.

Wyatt, G.A.K., Kiers, E.T., Gardner, A. & West, S.A. (2014). A biological market analysis of the plant-mycorrhizal symbiosis: mycorrhizal symbiosisas a biological market. *Evolution*, 68, 2603–2618.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Marc-André Selosse
Manuscript received 28 January 2021
First decision made 26 February 2021
Manuscript accepted 5 March 2021