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Does successful forest regeneration require the nursing of seedlings by nurse trees through mycorrhizal interconnections?

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1. Current consensus among scientists

Trees invariably form mycorrhizal symbioses, usually with several fungal species at the same time. The fungi receive carbon (C) as sugars from tree photosynthesis, while the fungi provide the trees with nutrients and water from the soil.

Mycelia of mycorrhizal fungi can form a network connecting trees of the same and other species (Selosse et al., 2006). So called mycoheterotrophic plants (Selosse et al., 2006), some of which lack chlorophyll, are wholly or partly dependent on the C supplied by other plants through mycorrhizal networks. Unlike fully chlorophyllous (green) plants, mycoheterotrophs spend no C fixed through photosynthesis by themselves or less C in the case of partial mycoheterotrophs, for access to soil nutrients and water.

Studies of mycoheterotrophs and of autotrophic (green) plants by the use of isotopes show that C (Simard et al., 1997) and nitrogen (N) (Arnebrant et al., 1993) can be transported from one plant to another. The mycorrhizal network between trees, described as the *wood-wide web*, can function as conduits through which C-compounds and nutrients are transported to other individuals of the same species or to other species. Thus, older trees, nurse trees, may feed young tree seedlings (nurse trees are called mother trees if there is a genetic relation) through the fungal network. If vital in forest regeneration, this service should be considered when systems of forest management are selected. Selective felling systems would provide great opportunities for seedlings to be connected to a mycorrhizal network fueled by C from big trees. In contrast, rotational forestry based on regeneration in clear-fellings allow only seedlings near forest edges to benefit from such connections.

There is widespread agreement that mycorrhizal fungal connections occur between forest trees, tree seedlings and other plants, which can connect to the same mycelium. There is also consensus that C and nutrients can pass through these fungal networks. But how quantitatively important are such flows, and do they play a decisive role in forest regeneration? Does scientific and other empirical evidence support the notion that older trees facilitate regeneration, or of competition from the big trees (Fig. 1)? Here, we focus on evidence from northern temperate and boreal forests, in which the low supply of N from the soil limits plant production and where tree-belowground competition for N need consideration.

2. Contrasting evidence in the scientific literature and from practical experience

Direct evidence of transport of C or N between individuals come from studies using stable isotopes to trace the transport from plant X to plant Y. A natural way of introducing labeled C is to let a plant assimilate isotopically labelled CO_2 through its photosynthesis. It is technically difficult and costly to label large trees this way. Hence, most isotope labelling studies have used small plants interconnected by a common mycorrhizal mycelium.

An important aspect is if plants X and Y only exchange equal amounts C, or if there is a net transfer of C in one direction. Simard et al. (1997)

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solved this challenge by exposing X to CO_2 labelled by the stable isotope ^{13}C and Y to CO_2 labelled by the radioactive isotope ^{14}C. They found that there was indeed a net transfer in one direction. Subsequently, they also found that shading one of the labelled plants affected the net transfer, because the labeled plant produced less photosynthate C and could export less.

But are the net quantities transferred from trees large enough to significantly improve the growth of seedlings (situations depicted as A_1 and A_2 in Fig. 1)? We note that most labelling studies have used small seedlings and that the quantities transferred have been comparatively small. Simard et al. (2012) estimate that autotrophic plants receive 0 – 10 % of their C via mycorrhizal networks. It is unclear if this estimate is a net transfer, i.e. if it accounts for a C transfer in the other direction, as it is difficult to detect a transfer of labelled C from small seedlings to the much larger C pool of big trees.

One exception stands out, but it was a study of transfer of C between large trees (Klein et al., 2016), not from trees to seedlings. A patch of trees was labelled by $^{13}CO_2$ at a low tracer level (the atom % ^{13}C of the labelled air differed very little from that of ambient air). The labelling was not short (like in Simard et al., 1997), but a 5 years long exposure to elevated levels of CO_2 (around 550 ppm as compared to an ambient of around 390 ppm). Tracer from the labelled trees was found in roots of adjacent non-labelled trees. Klein et al., estimated that as much as 40 % of the C in their finest roots came from the labelled trees. However, the study did not directly measure simultaneous transfer of C in the other direction, but attempted to estimate this using a model calculation. Nor was it just a labelling study as it was confounded by an experimental treatment (elevated [CO_2]) of the donor trees only.

Mycorrhizal fungi on roots of older trees serve as a source of inoculum helping establishing seedlings to quickly become mycorrhizal. If this occurs and is beneficial for the seedlings (without necessarily invoking a transfer of C or nutrients from the older trees), this would also lead to the situation depicted in the examples A_1 and A_2.

Evidence in line with the situation in B_1 and B_2 in Fig. 1 come from observations and experiments in boreal forests. Trenching of plots (around 10 m^2 in size) under trees in forests showed that isolation from living tree roots and mycorrhizas leads to elimination of production of fruit bodies of ectomycorrhizal fungi, but prolific regeneration of tree seedlings (Romell & Malmström 1945). Trenching does not alter the incoming light received by the seedlings, which suggest that the successful regeneration of seedlings was the result of alleviation from competition from the large trees for below-ground resources like nutrients and water.

Many other trenching, harvesting, and girdling experiments demonstrated similar strong benefits to understory plants (including tree seedlings) when connections with large trees were severed (see Coomes & Grubb 2000 for a review of trenching experiments).

For example, girdling of all trees on 900 m^2 large experimental plots lead to no immediate loss of needles of the trees (consequently no change in light conditions), but to intense regeneration of tree seedlings away from the edges with non-girdled trees (Axelsson et al., 2014). The competition zone with sparse regeneration near non-girdled trees (Photo 1) coincided with the distribution of sporocarps of ectomycorrhizal fungi, which drastically declined in abundance from the nearest non-girdled trees towards the central area of the plots with girdled trees, in which very few ectomycorrhizal sporocarps were found (Göttlicher et al., 2008).

Thus, trenching and girdling experiments suggest that in boreal forests, the presence of tree roots of large trees and their symbiotic mycorrhizal mycelium is negative for the regeneration of small tree seedlings of the same species. This contradicts the idea that trees nurse their offspring via mycorrhizal connections in these forests. Clearly, mycorrhizal network connections occur, but available evidence does not show they are beneficial for small seedlings.

Recent work labeling soil nitrogen with ^{15}N and tree photosynthate with ^{13}C (Högberg et al., 2010) have enabled studies of the dynamics of the C-for-N exchange rate in ectomycorrhizal symbiosis in boreal forest. Such studies have shown that under conditions of very low soil N supply, the trees allocate more C to roots and mycorrhizal fungi, and the fungi become strong immobilizers of N from the soil (Näsholm et al., 2013). In other words, they keep relatively more N for their own use and transfer less to the trees than under N-rich conditions. This strong N

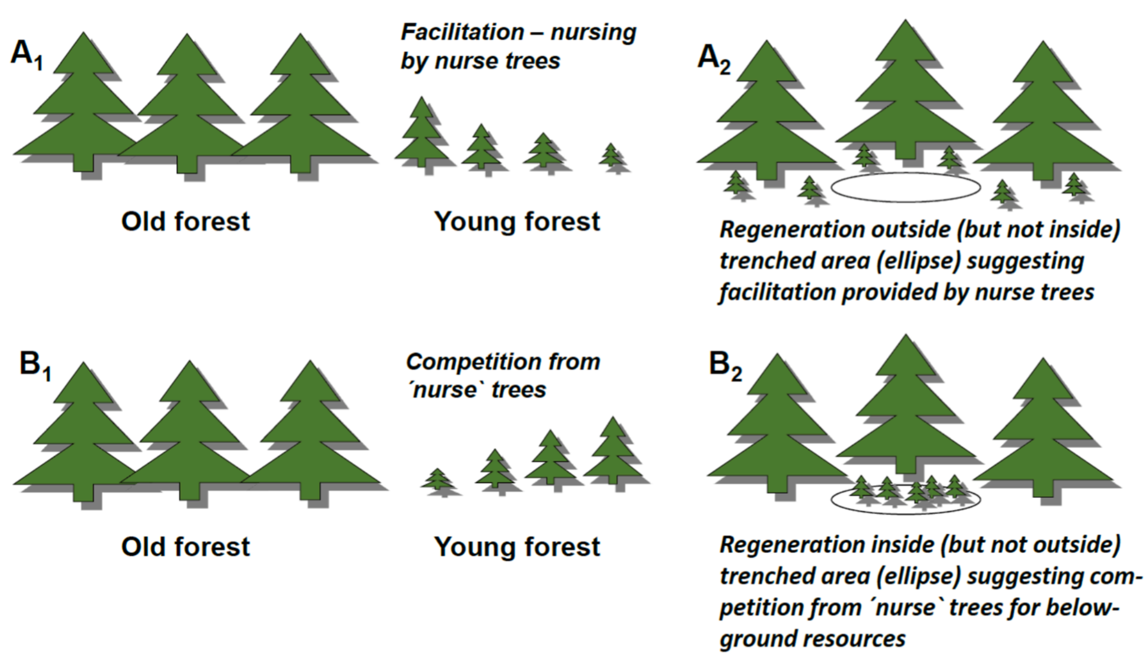

Fig. 1. Conceptualization of the contrasting options A and B describing the relation between tree seedlings and adjacent trees. A, services provided by older trees ("nurse trees") through mycorrhizal network connections facilitate the recruitment and growth of young tree seedlings at forest edges (A_1) and inside forests (A_2). B, mycorrhizal interconnections to older trees do not provide an apparent help to adjacent tree seedlings; their poor growth suggests that the older trees compete with them for soil resources at forest edges (B_1) and inside forests (B_2), where trenching experiments suggests that tree seedlings suffer from competition from old trees and their mycorrhizal fungi.

Photo 1. Dense natural regeneration of tree seedlings to the right, where all trees were girdled 20 years earlier and poor regeneration of tree seedlings close to non-girdled trees to the left, where green field-layer plants are mainly ericaceous dwarf shrubs. The prolific regeneration to the right commenced when girdled trees still retained a full canopy of green needles (Axelsson et al., 2014), and was thus not an effect of alleviation of competition for light; the photo is taken along a S-facing forest edge. The forest is a nitrogen-poor boreal pine forest (at 64°N). Photo: M.N. Högberg.

immobilization mechanism explains why the big trees have little N to share with connected seedlings under the N-poor conditions prevailing in most boreal forests.

The experience from practical forestry in boreal settings is also that tree seedlings commonly establish much better in open clear-fellings than inside forest or near forest edges (Lundqvist 2017). This kind of evidence paved the way for the replacement of selective felling forestry by rotational forestry in the boreal forests of Sweden and Finland, in particular.

3. Key points where more evidence is needed before we can have more confidence

The evidence from field experiments and practical forestry in boreal (and other) forests suggest that competition rather than nursing appears to be the role in these forests. However, this does not exclude the possibility that a mycorrhizal network has a positive effect on the forest regeneration in some situations. The outcomes depicted in Fig. 1 could be context-dependent. We, thus, call for more experimental quantitative evidence from field settings located across major forest biomes and studies of more species.

Such experiments should aim at identifying the role of the presence of mycorrhizal roots of adjacent older trees, but also other limiting factors for regeneration like light, nutrients and water (e.g., see Petriţan et al., 2011).

Trenching experiments eliminate the below-ground competition from the older trees for nutrients and water (Coomes & Grubb 2000). Such experiments are technically simple and inexpensive to carry out and do not need much space (a suitable size is around 5–10 m^2 for each trenched area). Effects of the treatment on soil moisture can easily be detected using modern soil probes, and the water potential of the leaves of seedlings should also be monitored. Effects on nutrient supply can be measured by harvesting seedlings (in- and outside trenches) and determine their contents of nutrients of interest, e.g. N in high-latitude forests and N and P in low-latitude forests.

Trenched areas can be located in transects from inside forests into recent clear-fellings and in forests subject to selective felling management to provide indications on the potential role of the variations in incoming light. Interactive effects of removal of below-ground competition and variations in light and nutrient supply can be studied in factorial experiments, in which lamps provide additional light in dense forests and nutrient additions are used to vary the nutrient supply. In open areas without or with few older canopy trees, screens can reduce the incoming light (e.g., see Hasselquist et al., 2016). Such trenching experiments also provide opportunities for educational demonstrations and discussions of processes influencing tree growth.

Wherever possible, detailed physiological studies using isotopic labelling, i.e. ^{15}N in the case of N should be used to reveal the effects of treatments on the flow of N from soils through mycorrhizal roots to the tree canopy (Hasselquist et al., 2016). Unfortunately, phosphorus is one of few elements with only one stable isotope and analogues studies would need to use radioactive P isotopes, which are problematic from the points of view of radiation safety and their short half-life.

Finally, although the experience from N-poor boreal forests contradicts the notion that older trees nurse their off-spring via direct mycelial interconnections, the build-up of a significant stock of N in mycorrhizal mycelia (in response to the large flow of C from the trees) may be still be viewed as nursing, albeit a much less direct nursing. The withdrawal of N from other competing species and its release to the benefit of the offspring when the old trees die due to harvests or natural causes can be seen as a form of inheritance, heritage, or delayed nursing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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