Tree Carbon Partitioning, Respiratory Efficiency, and Nitrogen Acquisition

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Cover: Strangled *Pinus sylvestris* trees.
(photo: Meredith Blackburn)

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Abstract

Tree growth in boreal systems is frequently limited by nitrogen (N) availability, and a significant portion of photosynthetically assimilated carbon (C) is partitioned to N-acquisition by roots and mycorrhizal fungi.

A new method for reversibly halting tree belowground C flux was developed. The method, termed *stem compression*, consists of applying pressure around the stem to collapse the conducting phloem and was shown to efficiently stop belowground C transport in Scots pine trees. Stem compression reduced tree N uptake by 32%, but N uptake of uncompressed trees also suffered - if they were surrounded by compressed trees. Conversely, a single compressed tree did not significantly affect N uptake by nearby uncompressed trees. This indicates that belowground C transport mediates N uptake, but also that within-community C status influences competition for N.

In the second part of the thesis, a method using the oxygen isotope discrimination technique for partitioning between the cytochrome oxidase (COX) and alternative oxidase (AOX) respiration was developed and applied to Scots pine roots from two forest stands. All plants examined to date contain AOX, as well as many other organisms. Its energy yield is only 1/3 of what is gained from COX, and pathway partitioning can shift in response to stress, such as nutrient deficiency.

Alternative oxidase partitioning was measured at two temperatures (6°C and 20°C). At 6°C significant fraction of fine root respiration (c. 20%) occurred via the AOX pathway. This fraction was found to decrease to c. 12% in response to elevated temperature and improved soil N availability. The potential influence of AOX on C sequestration coupled with responsiveness to environmental conditions make AOX relevant in light of changes in climate and forest management.

Taken together, the results point toward two feedback systems where C and N acquisition can act to reinforce each other: One mechanism where belowground C partitioning mediates uptake of soil/mycorrhizal N; and another where high soil N availability can trigger a shift in AOX partitioning, leading to increased biosynthetic efficiency of roots, potentially allowing more efficient use of the belowground C flux.

Keywords: Stem compression, Pinus sylvestris, stable isotope, isotopic discrimination, alternative oxidase, respiration, carbon partitioning, nitrogen uptake, carbon use efficiency,

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Henriksson, N., Tarvainen, L., Lim, H., Tor-Ngern, P., Palmroth, S., Oren, R., Marshall, J., Näsholm, T. (2015). Stem compression reversibly reduces phloem transport in *Pinus sylvestris* trees. *Tree Physiology* 35 (10), 1075-1085.

II Henriksson, N., Marshall, J. D., Tarvainen, L., Näsholm, T. Tree nitrogen acquisition in response to community level patterns in belowground C partitioning (manuscript).

III Henriksson, N., Marshall, J., Lundholm, J., Boily, Å., Boily, J-F., Näsholm, T. Improved in vivo measurement of alternative oxidase respiration in field-collected pine roots. (under revision for *Physiologia Plantarum*).

IV Henriksson, N., Marshall, J., Tarvainen, L., Näsholm, T. Shifting alternative oxidase partitioning in response to nitrogen addition has the potential to change carbon use efficiency (manuscript).

The contribution of Nils Henriksson to the papers included in this thesis was as follows:

I Performed the fieldwork together with H. Lim and P. Tor-Ngern. Prepared samples for analysis and analyzed the data. Wrote the manuscript with input from all authors.

II Designed the experiment together with professor Näsholm. Performed the fieldwork. Analyzed the data and wrote the manuscript with input from all authors.

III Developed the protocol and performed experimental treatments and incubations together with J. Lundholm. Provided input for J-F. Boily who developed the Matlab script. Analyzed the data and wrote the manuscript with input from all authors.

IV Performed experimental treatments and incubations together with J. Lundholm. Analyzed the data and wrote the manuscript with input from all authors.

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1 Introduction

Patterns in tree carbon (C) and nitrogen (N) assimilation and partitioning are active fields of research. The use and fluxes of both elements are measured at scales from cell to ecosystem, and are modeled to produce estimates and predictions of how changing environmental factors or management practices will affect growth, nutrition, and carbon sequestration of forests (Campioli et al., 2015; Cannell and Thornley, 2000; Fernández-Martínez et al., 2014; Giardina et al., 2003; Landsberg, 2003; Mäkelä and Valentine, 2001; Ryan, 1991; Tarvainen et al., 2016).

Although often studied separately, it is interesting to try visualizing both C and N flux within a tree simultaneously. The elements enter the tree at opposite ends (foliage and roots) and are transported along the stem in parallel tissues (phloem and xylem) to every part of the tree. Nitrogen is mainly used for protein synthesis and repair, and C is incorporated into structural biomass and metabolized for energy via respiration (De Vries et al., 1974). C is abundantly available as atmospheric CO_2 while N is frequently the limiting mineral nutrient in boreal forest ecosystems (Högberg et al., 2017; Tamm, 1991). Because N is necessary for producing the proteins involved in photosynthesis, it is important also for C assimilation, and this can be seen as the first link between tree C and N acquisition. On the other hand, roots that take up N from the soil rely on photosynthetic C to grow and maintain their mycorrhizal symbioses, so one would be equally justified in choosing to view this as the "first" link between tree C and N cycles within a tree. Because of these mutual dependencies, or complementary functions, a more complete picture is gained by trying to visualize the movement or influence of both elements at the same time.

Within this thesis, I have manipulated the belowground C flux in trees and studied the effect on N uptake, and used N-fertilization to investigate how respiratory energy efficiency responds to changes in N availability. The work

also included development and testing methods required to study these processes. The thesis consists of two method papers and two research papers. The methodological developments presented here allow the mutual influence of C and N cycles in trees to be studied in ways that have not been shown before.

2 Carbon transport: Phloem structure and physiology

Assimilated C is transported mainly as sugars to different parts of the tree via the phloem. The sites of photosynthesis are known as “source tissues” (for C) and the rest of the tree (stem, branches, roots) consists of “sink tissues” which must receive their C via phloem transport. The phloem is located between the sapwood (xylem) and the outer bark. In conifers, the phloem consists of sieve cells that are vertically linked to form continuous capillaries through which sap may flow from leaves and other photosynthetic tissues to every other part of the plant. The xylem (sapwood) conducts water and mineral nutrients upward from the soil to the foliage, and the phloem transports dissolved sugars in the opposite direction. Hormones and signaling compounds are also transported through the xylem and phloem, but this thesis will only consider sap flow as transporting assimilated C and N between belowground structures and tree foliage.

The main theory describing the mechanism driving phloem transport is known as the pressure flow hypothesis or the mass flow hypothesis, and was published by Ernst Münch in 1930 (Münch, 1930). This theory states that when compounds (mainly sugars) are loaded into the phloem at source tissues, such as leaves, an osmotic gradient is built up that draws water into the phloem from adjacent xylem cells, which increases the phloem’s turgor pressure near source tissues. At sink tissues, the opposite process takes place as sugars are unloaded from the phloem, causing its turgor pressure to drop. Because the lumen of the sieve cells are vertically connected to each other via sieve pores, while the length of interconnected sieve cells is separated from surrounding cells by a plasma membrane, the result is a structure where changes in sieve tube turgor pressure can be transmitted throughout the length of the phloem. The loading/unloading dynamic leads to high hydrostatic pressure near the source

tissues and low near sink tissues, and this causes the bulk of sap to flow towards sink tissues, such as roots (Gould et al., 2005).

Intact and functional phloem is notoriously difficult to study because it is pressurized. Puncturing it causes surging that clogs the breach with cellular debris and sap flow is re-routed to circumvent the blocked area. This makes it difficult to insert probes or other instruments for measuring flow rates or pressure, into functional phloem. However, the concentration of sugars and other solutes in the phloem sap can be easily measured from extracted samples.

The phloem's central role in plant growth and signaling combined with these methodological difficulties in observing its active function has afforded it a rather special place in plant physiology. Several papers have used unusually colorful vocabulary to describe it, such as "enigmatic", "mysterious", or "puzzling" (Knoblauch and Peters, 2010; Ryan and Asao, 2014; Turgeon, 2010).

This is because Münch's pressure flow hypothesis, in its original form, is not always compatible with reported observations. The theory holds up well for herbaceous plants, but several concerns have been raised where it comes to regulation of phloem transport in tall trees (Knoblauch and Oparka, 2012; Knoblauch and Peters, 2010; Turgeon, 2010). The reason for this is that the phloem of herbaceous plants has high turgor pressure and transport distances are relatively short, whereas trees reportedly have low phloem turgor pressure (Hammel, 1968; Sovonick-Dunford et al., 1981) and can require solutes to be transported over much longer distances (Turgeon, 2010).

It is possible that the methodological challenges of studying intact phloem have led to an underestimation of turgor pressure in the phloem of trees, or that future advancement in the understanding of capillaries with the phloem's biological characteristics could reconcile observations with theoretical calculations (Turgeon, 2010). Alternatively, the present theory may be incomplete and need to be be adjusted to accommodate the observed properties of phloem transport in tall trees.

The regulating mechanisms of phloem transport are not within the scope of this thesis. However, because I will be discussing C transport, the phloem still occupies a central role, most explicitly in the experiments where the method of stem compression was developed and used.

3 Belowground carbon partitioning

When C is unloaded from the phloem into a sink tissue, it can be stored, incorporated into new biomass, or respired to provide the chemical energy required for cellular growth and maintenance. In the case of root tips, the C can also be exported to mycorrhizal fungi in exchange for mineral nutrients such as N. This thesis mainly focuses on the role of C once it has been transported to a tissue, rather than specifically investigating the environmental or physiological cues that prompt certain patterns in tree C partitioning.

Phloem-transported soluble C is required for all energetic processes of roots, in addition to being the main structural component of biomass production, and 30-80 % of the total amount of C assimilated by photosynthesis is transported belowground (Davidson et al., 2002; Giardina et al., 2003; Giardina and Ryan, 2002; Raich and Nadelhoffer, 1989). Therefore, manipulating this C flux can be a powerful tool for investigating the downstream processes relying on this C as well as physiological responses to changes in the flux. Morever, blocking the phloem also enables studies of how photosynthesis or other up-stream processes respond to a build-up of C in the phloem (Asao and Ryan, 2015).

3.1 Blocking the belowground carbon flux

Several studies have been published where trees were girdled and various belowground processes (such as soil CO_2 efflux) subsequently measured. Because girdling entails stripping away the phloem around the stem, it effectively terminates phloem transport. However, eventually the roots will deplete their C reserves and the tree will die. This might not compromise the results of short-term experiments, but the trees will not be available for future studies. Moreover, the dying trees could bias future studies at the site.

Despite this long-term limitation, girdling studies have yielded much useful information on tree C partitioning and C-dependent processes. For instance, the importance of recently photosynthesized C for belowground processes was shown when girdling reduced soil CO_2 efflux by 52-65% in a boreal Norway spruce forest (Bhupinderpal-Singh et al., 2003; Högberg et al., 2001). Girdling studies have also been used to estimate how much of soil CO_2 efflux can be attributed to autotrophic, as opposed to heterotrophic sources (Binkley et al., 2006; Högberg et al., 2009).

Phloem chilling has been used on smaller plants and leaves (De Schepper et al., 2011), and one successful application to trees in the field has been published (Johnsen et al., 2007). This technique involves lowering the temperature of the phloem sap until its viscosity increases sufficiently to slow down sap flow. Chilling is not fatal to the plant, but for large trees in field conditions, it is expensive and presents significant engineering challenges.

3.2 Stem compression technique

Because of the adverse effects of girdling, I developed a different method for blocking belowground C flow (stem compression, or strangling) and tested it on Scots pine trees growing in the field. This new technique reduced C transport similarly to girdling, but most importantly the phloem recovered its functionality after the strangling equipment was removed.

The principle of the technique is to exert a radial pressure on the tree stem, squeezing shut the sieve tubes of the phloem to block the downward sap flow. This is similar to the wire-strangling (also termed *strapping* in horticultural literature) that is sometimes employed on fruit trees to improve fruit size and quality (Yakushiji and Nakatsuka, 2007; Yamanishi et al., 1993), which was also used on Scots pine (*Pinus sylvestris* L.) seedlings to study the dependence of mycorrhizal fungi upon tree-derived C (Björkman, 1944).

In my version of the technique, metal bands were fastened around the stems of Scots pine trees (figure 1) and tightened to a maximum pressure of 2.4 MPa, which is theoretically sufficient to counteract the phloem's turgor pressure (Hammel, 1968; Nikinmaa et al., 2014; Sovonick-Dunford et al., 1981). Applying this external pressure works because the phloem tissue itself is soft, and beneath it is the much harder sapwood. Thus tightening the bands resulted in pinching the phloem between the bands and the underlying sapwood.

I performed several tests to determine the effectiveness of the technique in

Figure 1. Stem compression. The tree stem (Pinus sylvestris, L.) is fitted with metal bands that are tightened to collapse the phloem tissue against the underlying sapwood, in order to block the flow of C through the phloem. Above and below the compression point are custom-made stem chambers containing infrared gas analyzers for measuring stem CO_2 efflux. This was one of the measurements performed to test the effectiveness of stem compression for terminating belowground C flux. Photo: M. Blackburn

blocking phloem transport (chapter I). Compressed trees were enclosed in whole-tree chambers of transparent plastic into which $^{13}CO_2$ was injected. The isotopically labeled CO_2 was assimilated by the needles, causing the sugars synthesized and transported through the phloem to become enriched with ^{13}C. By taking samples of the phloem below the stem compression bands, it was possible to detect (using isotope ratio mass spectrometry, IRMS) if any of the injected ^{13}C had been transported past the blockage. It wasn't. Figure 2 shows

that no ^{13}C was detected in the phloem sap below the compression point while the strangling equipment was in place. The pressure was then released, and the trees were left to recover. A second canopy labeling was performed at the start of the following growing season, at which time the phloem conducted the isotopically enriched C past the point of former compression. This clearly demonstrated that the phloem had recovered its functionality.

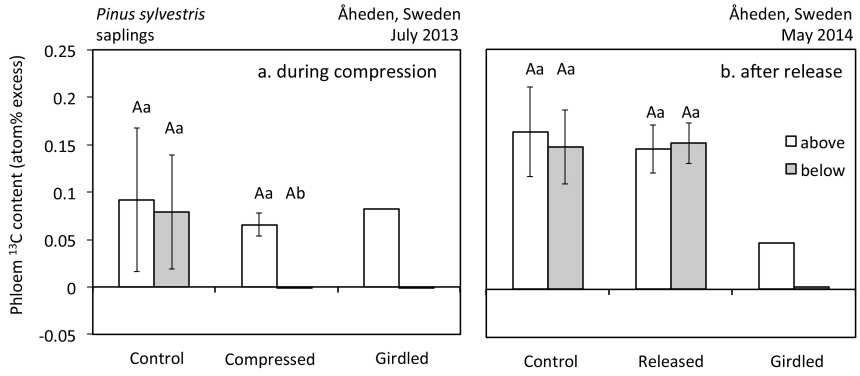

Figure 2. Phloem sap 13C content (atom% excess; mean +-SE) in samples collected above the blockage position (open bars) and below it (filled bars). The length of the control tree error bars is due to one of the trees having assimilated over 10 times more 13C than the other controls. This is attributed to weather conditions as well as greater amount of foliage. Different upper-case letters indicate differences between treatments (analysis of variance, $p < 0.05$). Different lower-case letters indicate a difference between stem positions within treatments (student's t-test). The left-hand panel (a) shows the 13C enrichment during the compression treatment and the right-hand panel (b) shows results from the following season, after the strangling pressure had been released.

Compared to controls, soil CO_2 efflux was reduced to ≈65% and ≈50% by compression and girdling, respectively. The fluxes on the control plots ranged between about 2-5 μmol m^{-2} s^{-1}. The lower efflux rates in control plots (somewhat above 2 μmol m^{-2} s^{-1}) were observed during the middle of the growing season and the higher rates of around 5 μmol m^{-2} s^{-1} were measured later in the summer (from early August), which is consistent with reports of tree belowground C partitioning increasing during this part of the growing season (Högberg et al., 2001). The relative reduction on the girdled and strangled plots compared to the controls actually resulted from an absence of this seasonal increase in soil CO_2 efflux, indicating that the phloem transport had ceased (chapter I, figure 7).

Stem compression may act to collapse the sieve tubes of the phloem, blocking the flow of assimilates, or it may damage the phloem tissue to the extent that conductivity is lost but the cambium remains functional (or is at least not beyond repair). In the first case, phloem recovery would be expected to occur soon after the pressure is released; in the second case, a new functional phloem would need to be differentiated from the cambium and a longer recovery time be required. Because the phloem function was not re-examined until the following spring (figure 2), neither of these possibilities can be conclusively rejected.

The effectiveness of stem compression was also tested by measurements of stem CO_2 efflux at two positions on the stems, above and below the compression bands. These measurements were conducted on a different set of trees than the ^{13}C canopy labeling. The labeled trees had to be small (2-3m tall, 3-7 cm diameter at breast height) in order to fit in the whole-tree chamber. Because we also wanted to measure how effective stem compression was in larger trees, I set up a parallel study using mature Scots pines (c. 15 m tall, 15-25 cm diameter at breast height), on which stem CO_2 efflux was recorded as a measure of cellular metabolic rate.

A custom-made stem chamber containing an infrared gas analyzer, IRGA (Vaisala CARBOCAP®, Vaisala, OUJ), was sealed to the stem (figure 1) and the rate of CO_2 buildup was recorded until the internal CO_2 concentration had risen to 40 ppm above ambient. Stem CO_2 efflux was measured on 10 days during the 66-day study period, focusing mostly on the period during and immediately after compression.

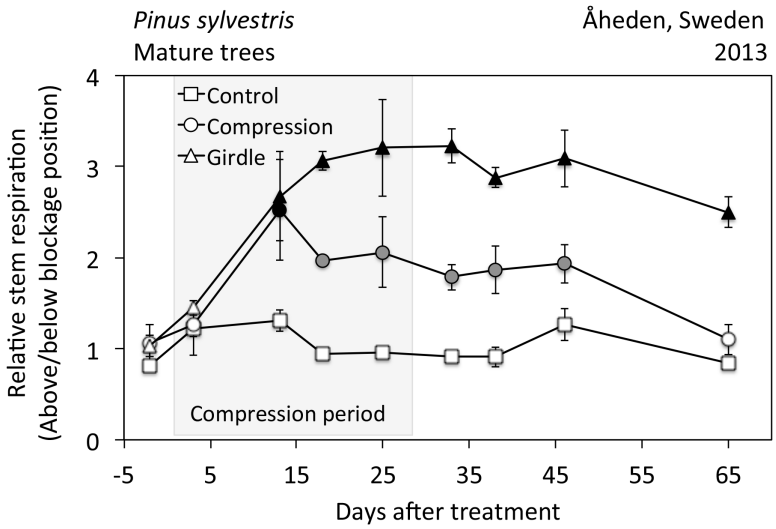

Figure 3. Stem CO_2 efflux, displayed as the ratio of above/below treatment height (mean +- SE). Markers of different colors (white, gray, or black) indicate that treatments were significantly different from each other on that measurement day (ANOVA, Tukey HSD, $p < 0.05$). Day 0 (July 19th, 2013) is the day when compression and girdling treatments began, and the compressed trees were released on day 27 (August 15th, 2013).

There have been indications that stem CO_2 efflux may to some extent contain CO_2 that was respired in roots or a different part of the stem and then dissolved and transported in xylem sap. There is an ongoing discussion as to how this may influence what inferences may be confidently made based on stem CO_2 efflux measurements (Tarvainen et al., 2014; Teskey et al., 2008; Ubierna et al., 2009). However, in the present context the two measurement points were only separated vertically by about 50 centimeters, and even if root-derived CO_2 composed part of the measured efflux, the difference between the two heights should be dominated by the treatment effect. Additionally, the obtained stem effluxes were analyzed relative to control trees at the same height.

3.3 N acquisition in relation to belowground C flux

Because tree growth is frequently limited by N availability in boreal forests, and because a large fraction of assimilated C is partitioned to belowground structures (roots and mycorrhiza), the connection between belowground C flux and tree N acquisition is a key question. N-fertilization has been shown to shift C partitioning to aboveground biomass (Lim et al., 2015; Vicca et al., 2012),

and it has been observed that elevated atmospheric CO_2 concentration causes increased N uptake (Finzi et al., 2006), at least initially.

Using stem compression, I performed an experiment to investigate how tree N uptake would be affected by direct reduction of belowground C flux. I also wanted to know if tree N-uptake was influenced by the belowground C-partitioning of trees growing nearby.

The context for this question was a series of studies leading up to a hypothesis about the regulating mechanism of mycorrhizal C-N exchange (Alberton et al., 2007; Franklin et al., 2014; Hasselquist et al., 2016; Näsholm et al., 2013). This hypothesis is based on stoichiometric constraints on fungal growth, and states that fungi prioritize their own growth and that the amount of N they can incorporate is dictated by their current C status. Because this C status is directly related to the host trees' belowground C partitioning, the hypothesis predicts that increased rates of tree belowground C flux should lead to reduced N uptake. Different methods have been employed to manipulate belowground C flux: Atmospheric CO_2 elevation (Alberton et al., 2007); canopy shading (Hasselquist et al., 2016); one study used N-fertilization to lower soil C:N ratio to alter stoichiometric conditions (Näsholm et al., 2013).

These studies inferred that greater belowground C partitioning would cause reduced N uptake by stimulating greater N-immobilization by mycorrhizal fungi under conditions of high C and low N availability. This conclusion is supported by observations that potted seedlings grown in N-limiting conditions maintain or increase C export to mycorrhiza despite receiving diminishing N-returns (Colpaert et al., 1996; Corrêa et al., 2008), and a model of mycorrhizal C-N trade was published that explains the results by calling upon multi-partner structures of ectomycorrhizal symbioses (Franklin et al., 2014).

Such a network structure, where multiple trees can be connected to the same fungi and vice versa (Beiler et al., 2015; Brownlee et al., 1983; Finlay and Read, 1986; Gorzelak et al., 2015), suggests that N-acquisition of one tree could be influenced not only by its own belowground C partitioning, but also by the belowground C flux of other trees growing nearby.

From these conclusions, two predictions can be made which could be tested using the stem compression technique:

1. That strangled trees (due to direct reduction of tree belowground C flux) would receive more N than untreated control trees.
2. If a subset of adjacent trees were strangled, then this N-benefit would not occur because N uptake is determined by the total belowground C flux of the tree community.

I therefore set up an experiment to investigate the coupling between belowground C partitioning and tree N acquisition. To achieve this, I combined stem compression and ^{15}N nitrate labelling (applied to the soil surface after the trees had been strangled for three weeks). The experiment comprised 23 plots containing strangled and/or control trees. The ^{15}N isotopic enrichment in the foliage of trees that received different treatments could then be compared to detect changes in uptake patterns related to belowground C flux (chapter II).

The specific aim was to measure how tree N uptake would respond to the decrease in belowground C flux resulting from stem compression. To address the question of interactions between the belowground C flux of different trees, the following treatments were allotted to the experimental plots: 1) All trees were strangled; 2) No trees were strangled; 3) Only one tree was strangled; or 4) A single control tree was surrounded by strangled trees. After the isotopically labeled N had been applied, needle samples were collected for analysis of their ^{15}N enrichment analyzed using isotope ratio mass spectrometry (IRMS).

Contrary to the predictions of the hypothesis outlined above, I found that needles from strangled trees contained one-third less ^{15}N than those from control trees. This also held true for trees within plots containing a single strangled tree (43% reduced N uptake in strangled trees (figure 4)).

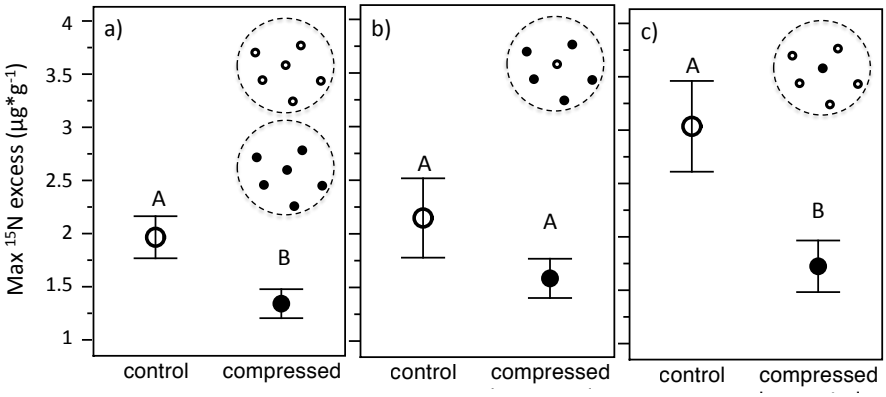

Figure 4. Nitrogen uptake in Scots pine trees, measured by ground application of ^{15}N nitrate and subsequent needle sampling and isotopic analysis. Shown is the highest needle ^{15}N contents observed for each treatment (µg ^{15}N g^{-1} needle dry weight, in excess of natural abundance; mean ± SE). Control trees are represented by open circles and strangled trees by filled circles. Different capital letters indicate a significant difference between treatments ($p < 0.05$; Student's t-test), and the dashed circles superimposed on the graphs depict the plot-scale treatments included in the analysis. The figure is modified from chapter II in this thesis.

However, in plots where the majority of trees were strangled (corresponding to 82% of plot basal area), there was no significant difference in N uptake between strangled trees and controls.

Thus the hypothesis may require adjustments to both accommodate my results and those from the previous studies reporting seemingly conflicting findings (Hasselquist et al., 2016; Näsholm et al., 2013). Perhaps the problem lies in the assumption that there is a linear relationship between belowground C-flux and tree N acquisition. I have shown via canopy ^{13}C labeling that the belowground C-flux was terminated by strangling (*figure 2*), whereas shading reduced photosynthetic C-gain by the tree to 60% (Hasselquist et al., 2016). Thus the two experiments using these methods may in fact not contradict each other after all. Rather it may be that maximum N-release from fungi to host trees actually occurs at some intermediate level of C-investment by the tree (figure 5). Presumably, zero C-export to mycorrhiza would eventually lead to C-starvation of the fungi and thus no N transfer to host trees. Analogously, if a tree exported sufficiently large amounts of C to its symbiotic fungi, these would become N-limited and thus not transfer any N to the host tree.

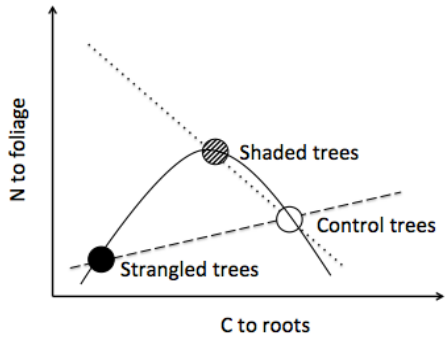

Figure 5. Conceptual representation of how export of C to roots and mycorrhiza could be related to the uptake of N into foliage. The white dot represents "natural" control conditions in an N-limited boreal forest stand, where rather large quantities of C are partitioned belowground, and a certain amount of N is acquired. The black dot represents the trees strangled in my experiment (based on ^{13}C canopy labelling data, this treatment completely shut off the flux of new C from the foliage) in which I found a decreased N uptake, as measured by ^{15}N enrichment of needles after a ground labelling with isotopically enriched nitrate. The shaded dot represents the reported effect of shading (Hasselquist, 2016), where they observed a marked increase in needle ^{15}N enrichment compared to controls, also after a labelling treatment.

3.3.1 Hypothesis and future studies

The results I present from this experiment could be said to describe ecological competition between trees, where belowground C transport facilitates N uptake. By restricting this flux, strangling lowers a tree's capacity to compete for soil N. However this interpretation would lead to the expectation that the competitive advantage of uncompressed trees should increase with the proportion of strangled competing trees. This was not the case. Within plots where the majority of trees had been strangled (82% of plot basal area), the advantage to the remaining uncompressed tree was no longer detectable (figure 4b). This observation suggests the presence of some additional interaction among trees, related to the combined belowground C transport of the tree community and the resulting N acquisition of individual trees. The net effect of this interaction seems to have been that excessively reducing combined tree belowground C flux, resulted in reduced advantage for uncompressed trees.

Thus the finding that N uptake is influenced by the belowground C flux of neighboring trees does indicate some connection between trees. Therefore I speculate that these observations, as well as the seemingly contradictory results of previous studies might be explained by a hypothesis based on trees being connected to a common network of fungi (Beiler et al., 2015; Brownlee et al., 1983; Finlay and Read, 1986; Gorzelak et al., 2015). If no individual tree is the sole C-source for its mycorrhizal partners (Beiler et al., 2015), then reduced C export by a single tree would have less impact on the fungi. This would reduce the tree's power to affect fungal N release by withholding C.

Hypothetically, if fungi could distinguish among host trees and preferentially exchange nutrients with hosts that provide more C, then a hypothesis could be formulated which accounts for previous findings: That mycorrhizal N-release depends on total C-flux (sum of all hosts) to a fungal network, with less N released at high fungal C status. Although an individual tree would not greatly influence fungal N-release, this hypothesis provides evolutionary incentive to maintain C-export despite diminishing N-returns by allowing competition for the mobilized N with other trees connected to the same fungi. This suggests that optimal N-mobilization should occur in response to intermediate levels of total belowground C flux, and that in the event of unequal export among trees, there is an N-benefit for the trees exporting more C belowground. The idea is related to the "market theory" of Franklin et al. (2014) and I suggest that further tests of this hypothesis are warranted.

3.3.2 Methodological considerations

From the above paragraphs, it can be seen that a handful of different methods have been used to manipulate tree belowground C flux. We also see that the result was not always the same, and thus we must consider the strengths and limitations of the techniques.

Canopy shading (Hasselquist et al., 2016) and manipulation of atmospheric CO_2 concentration (Alberton et al., 2007; Finzi et al., 2006) are two methods that directly affect photosynthetic C assimilation. This in turn affects belowground C flux. However, shading could also affect relative C partitioning among different parts of the tree, such that the change in belowground C flux is not always proportional to the change in photosynthetic rate (Poorter et al., 2012). Additionally, canopy shading may affect N distribution in tree foliage if shade-avoidance responses are induced (Niinemets, 2007; Trupkin et al., 2014). Soil temperature may also be affected, which may be significant in some cases.

N fertilization has also been used (Näsholm et al., 2013). The rationale here is that increasing soil N availability without actively altering belowground C flux, will lower soil C:N ratio. This is strictly not a manipulation of tree C partitioning, although fertilization has been reported to decrease belowground C partitioning in the long-term (Lim et al., 2015).

Girdling is very effective and because the phloem is completely removed, one can be quite certain that transport has ceased. Thus it is a direct manipulation of belowground C flux. However, the trees will eventually die, and soil decomposers will respond to this and potentially confound processes of interest. Stem compression (like girdling and stem chilling) is a treatment limited to individual trees, but does not permanently damage the phloem. If only a subset of trees in an area are to be treated, then only girdling and stem compression allow free choice of individual trees (shading and atmospheric CO_2 manipulations generally cover an area and chilling becomes difficult if the trees are widely spaced).

For the purposes of studying belowground processes, strangling seems similar to girdling, but without the listed drawbacks. However, with stem compression the phloem blockage may be less complete than with girdling, because it is difficult to know the pressure required to achieve blockage. Additionally, if a tree stem has an irregular shape, this can result in unaffected (or less affected) sections of phloem, where belowground C flux may still occur.

4 Carbon used for cellular respiration

In addition to its role in facilitating N uptake, photosynthetically fixed C provides energy for all energetic cellular processes. As a result, cellular respiration consumes a large portion of a plant's photosynthetically fixed C (Lambers and Ribas-Carbo, 2005; Ryan et al., 1997).

Respiration consists of a series of reactions that ultimately transfer energy stored in carbohydrates to ATP, in which form it can be used to drive energy-demanding cellular reactions. Respiration takes place in all the living cells of an organism, starting with glycolysis in the cytosol and finally ending in the terminal electron transport chain in the mitochondria.

In glycolysis, a series of reactions split glucose into pyruvate, which moves into the mitochondria where it is oxidized and decarboxylated. It then attaches itself to coenzyme A, to form acetyl coenzyme A – the substrate for the Krebs cycle. Next, the Krebs cycle oxidizes what is left of the pyruvate (the acetyl group of acetyl coenzyme A) to carbon dioxide (CO_2) and the electrons are transferred to the intermediate electron carriers NADH and $FADH_2$. Although some energy is conserved in glycolysis and the Krebs cycle (2 molecules of ATP each), most of the ATP is generated in the oxidative phosphorylation at the end of the electron transport chain.

The electron transport chain is a series of redox reactions that transfer electrons between electron carrying protein complexes, lowering their energy state with each step until the finally they are accepted by oxygen which combines with hydrogen to form water. At several of these steps, the energy released when an electron is transferred to a lower energy state is used to pump protons from the matrix to the mitochondrial inter-membrane space. The concentration gradient that is built up drives the transfer of protons back into the matrix via the protein ATP synthase. As protons pass through ATP synthase, they cause its component structures to shift across each other, bringing ADP and phosphate molecules together to synthesize ATP. This is the

main source of ATP production in respiration and it is driven by the proton gradient across the mitochondrial inner membrane. The amount of ATP that can be produced is thus proportional to the strength of the proton gradient.

4.1 About alternative oxidase respiration (AOX)

Plants have a branched terminal electron transport chain, and respiration can proceed along the complete pathway outlined above (known as the cytochrome pathway, COX), or an alternative pathway (called AOX – short for *alternative oxidase*) which bypasses most of the energy conserving steps and produces only a third of the ATP resulting from the complete pathway. The remainder of potential energy is simply lost as heat. Therefore, greater partitioning to AOX results in a drop in the energy efficiency of respiration.

Increased AOX activity has been associated with stress conditions such as low temperatures (Ribas-Carbo et al., 2000; Searle and Turnbull, 2011; Vanlerberghe, 2013) and nutrient deficiency (Sieger et al., 2005). Both conditions are commonly encountered in boreal forests, suggesting that AOX could be important in such systems. Furthermore, it has been indicated that AOX may contribute greatly to boreal soil CO_2 efflux, and less in warmer climates (Angert et al., 2003, 2012), which suggests that neglecting AOX in physiological growth models could cause the largest potential errors in boreal systems. Because high AOX activity leads to less ATP being synthesized per carbon respired, relative AOX/COX partitioning directly affects biosynthetic efficiency (biomass produced divided by biomass produced plus carbon spent to produce the required energy for synthesis) in grams C per grams C (Ryan, 1991).

Despite these suggestions that AOX could be a potentially important route of C flow through forest ecosystems, it is rarely mentioned in this context (but see Angert et al., 2003, 2012; Ryan, 1991). The majority of studies on forest C dynamics or tree C use efficiency do not include AOX in any significant sense (review in DeLucia et al., 2007). This is likely due, at least in part, to methodological difficulties in measuring AOX partitioning *in vivo*. If AOX partitioning of plants and soil microorganisms were to shift in response to changing climate conditions, then it could impact C sequestration in both biomass and soil organic matter. If current levels of AOX partitioning are indeed dictated by low temperatures or low N availability, then predicted effects of global change and pollution (rising temperatures, N deposition, extended growing seasons etc.) could potentially cause AOX partitioning of plant respiration to shift in the future.

4.1.1 Measuring AOX (the oxygen isotope discrimination technique)

Electron partitioning between the two paths can be measured using the oxygen isotope discrimination technique (Guy et al., 1989). This technique makes use of the fact that the two terminal oxidases (COX and AOX) discriminate differently against the heavier isotope oxygen-18 (^{18}O). AOX is slightly less likely to react with ^{18}O than COX is, meaning that if respiring in a sealed container – COX would deplete the air's ^{18}O at a faster rate than AOX would. This discrimination rate is calculated as the slope of change in $^{18}O/^{16}O$ proportion of air as O_2 is being consumed by respiration. In practice, this means placing a respiring tissue into an air-tight container and monitoring the gradual change in isotopic composition ($^{18}O/^{16}O$) of the residual air as oxygen is being depleted by respiration (figure 6).

In plant tissues, both pathways are to some extent active simultaneously such that the measured ^{18}O discrimination of respiration is the result of an unknown mix of both COX and AOX. To quantify the partitioning between the to pathways, one must be deactivated so that only one reaction is consuming oxygen in the incubation volume. This has been done by applying pathway-specific respiratory inhibitors to the tissue sample. Cyanide (CN) is used to inhibit COX and salicylhydroxamic acid (SHAM) has been used to deactivate AOX. Theoretically this will provide the discriminations of the isolated respiratory pathways, and can then be used as endpoints in a linear regression model. The location of non-inhibited respiration along this regression line will then reveal the relative contributions of the two pathways to respiration.

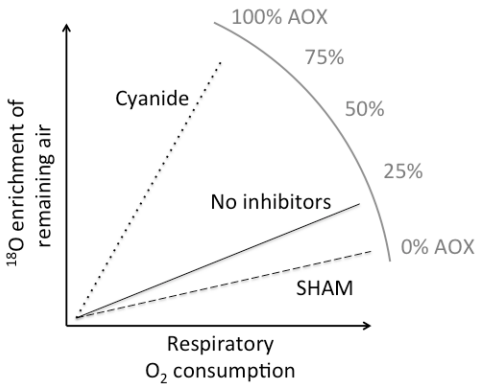

Figure 6. Theory of isotopic discrimination technique for measuring electron partitioning between the AOX and COX pathways of respiration. The sample is treated with cyanide to inhibit COX (dotted line) or SHAM to inhibit AOX (dashed line) and incubated in a sealed container from which gas samples are taken. The gas samples are analysed for $^{18}O/^{16}O$ ratio as well as total O_2 concentration, using isotope ratio mass spectrometry (IRMS). When non-inhibited samples are incubated (solid line), they have an isotopic discrimination against ^{18}O that falls somewhere between those obtained from incubation of the inhibited samples, and this can be translated into the relative contributions of COX and AOX to oxygen consumption.

In the literature, this difference between COX and AOX has been estimated to be around 8.6 ±2‰, with the discrimination by COX being placed at 18.8 ±1.8‰ and that by AOX at 27.2 ±2.6‰ (Gonzàlez-Meler et al., 2001; Guy et al., 1989; Guy and Vanlerberghe, 2005; Kornfeld et al., 2012; Macfarlane et al., 2009; Millar et al., 1998; Nagel et al., 2001; Rachmilevitch et al., 2007; Robinson et al., 1995; Searle et al., 2011). Leaf tissue from castor bean (*Phaseolus vulgaris*) has been used in several studies and inhibitions with SHAM and CN have yielded isolated pathway discriminations against ^{18}O of 19.0-21.6‰ for COX respiration and 25.8-30.3‰ for AOX (Gonzàlez-Meler et al., 2001; Kornfeld et al., 2012; Searle et al., 2011). For *Arabidopsis thaliana* leaves, discrimination by COX has been reported as 16.2-20.9‰, and for AOX respiration the published values are 23.7-31.2‰ (Armstrong et al., 2008; Florez-Sarasa et al., 2011, 2007).

However, the inhibitors are not always straightforward to apply. It can be difficult to ensure that full inhibition has been achieved (Searle et al., 2011), because application of both inhibitors together doesn't completely block respiration. Additionally, as seen above, the strength of either discrimination may possibly vary between species. Thus there is some variation among reported discrimination values that could result from differences in inhibition success, incubated plant species and tissue types, or combinations of several sources of variation.

Because the specific discriminations of the two pathways are found to differ by only 8.6‰ on average, accurate estimation of these discrimination values is important. Small changes in the measured discrimination values used as endpoints in the regression can strongly influence the result when observations are translated into AOX/COX partitioning. Additionally, tree species are underrepresented in the literature and there have been no reports on conifers using the isotope discrimination technique. If the apparent between-species variation that can be inferred from a literature survey is indeed real, then measurements on less represented species would provide important information.

As a result it is not known to what extent the isotopic discriminations of the two respiratory pathways differ between species or groups. Should AOX and COX of gymnosperms and angiosperms be expected to have the same discriminations? What about other plants and fungi? In order to approach such questions, it is necessary to identify any sources of variation that can be controlled or corrected for, so that experiments can be confidently compared to each other.

4.1.2 AOX activity in *Pinus sylvestris* roots

We developed a protocol using the oxygen isotope discrimination technique to measure AOX partitioning in roots of Scots pine (*Pinus sylvestris, L.*) collected from a nearby pair of experimental forest stands (Rosinedalsheden, 64°10'N, 19°45'E, 145m a.s.l.). This is the focus in chapter III of this thesis. The protocol can be described as consisting of 5 steps: 1) Roots were collected from the field, placed in moist paper, transported to the lab and stored overnight in a +4°C refrigerator; 2) The roots were weighed into incubation vials (22ml glass septum-capped vials) containing a CO_2 trap (KOH pellet) to prevent build-up of CO_2 from limiting respiration. The air in the vials was evacuated and replaced with fresh outside air, to ensure identical starting conditions; 3) Incubation with periodical gas-sampling by an autosampler connected to an isotope ratio mass spectrometer (IRMS); 4) The raw data was cleaned using a Matab-script to remove inappropriate sections of the incubation data (more about this in the next paragraph); 5) the final step was to correct for root moisture content, which I found to have significant influence on ^{18}O discrimination.

Moisture content ((fresh weight-dry weight)/fresh weight) of incubated roots significantly affected their isotopic discrimination (figure 7). Presumably this was due to increasing diffusion barriers across liquid water, but the mechanism was not investigated. This could be an important problem when comparing discrimination values from different studies, because the sample moisture content is not generally stated. Further, the AOX inhibitor SHAM must be applied as a solution in water. Sample is submerged or placed between wet tissues for some period of time before incubation. This could easily cause differences in moisture content between inhibited samples from different studies. Even more worrying is the fact that both successful inhibition of AOX and the moisture-artifact cause respiratory discrimination to decrease, making it difficult to separate the two effects. In my experiments I focused on the relative contributions of COX and AOX, and not on the actual isotopic discrimination of the two enzymes, therefore I used the residuals of an analysis of covariance (ANCOVA) to adjust all our discrimination values to the reference moisture content of 65%. The reference moisture was chosen because it was close to the moisture content of many of our control roots. Further tests indicated that the choice of reference moisture did not affect the calculated AOX contribution to respiration (in % of total O_2 consumption).

We incubated fine roots (<2mm, and mostly <1mm) from two *P. sylvestris* stands, separated by about 2 kilometers. Both were the same size (13 ha), monocultures and approximately 100 years old, one had been annually fertilized with ammonium nitrate for nine consecutive years (100kg $ha^{-1}yr^{-1}$ for

seven years and 50 kg $ha^{-1}yr^{-1}$ for another two years), and the other stand was an untreated control.

Inhibiting the roots with CN and SHAM yielded isotopic discriminations of 14.6±0.2‰ for COX and 23.8±0.4 for AOX. These are low values compared to those found in the literature. The lowest I have found is 15.7±0.4‰ (Nagel et al., 2001) and 23.7±1.1‰ (Armstrong et al., 2008) for COX and AOX respectively. However, the distance between our measured discriminations was 9.2‰, which is very similar to most previous reports. However, there are no published discrimination values for *P. sylvestris* or any other conifer species, so it is not possible to determine whether the low values are due to some artifact or actual biological characteristics of the species. The current study focused on the relative electron partitioning between COX and AOX, and therefore the obtained discrimination values were sufficient for our purposes.

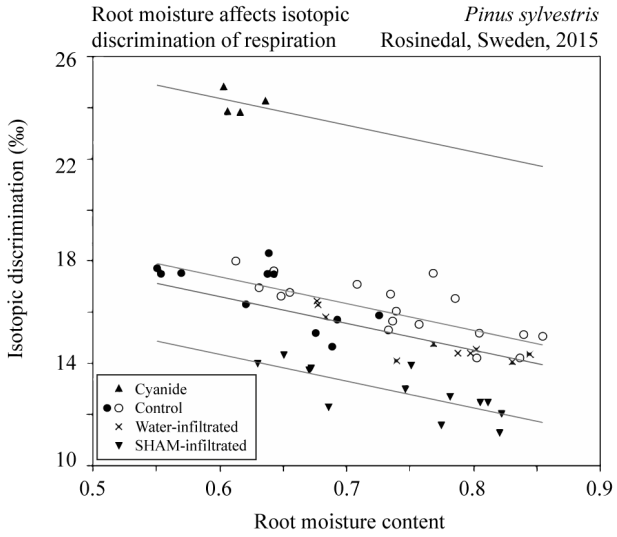

Figure 7. Effect of sample moisture content on respiratory isotopic discrimination against ^{18}O. The effect was found in untreated roots (circles; filled = field-collected roots; open = roots from potted seedlings, used to fill out the moisture-range for analysis), as well as those treated with specific respiratory inhibitors (triangles; upward=cyanide-inhibition of COX, downward=SHAM-inhibition of AOX), and roots infiltrated with tap water in place of SHAM solution (crosses). Root moisture content significantly affected discrimination against ^{18}O during respiratory O_2 consumption. Analysis of covariance predicted lower isotopic discrimination in wetter roots ($p < 0.0001$), as well as differences among untreated, CN-treated, SHAM-infiltrated, and water-infiltrated roots ($p < 0.032$). The adjusted R^2 of the entire ANCOVA model was 0.94.

We found that there was indeed a difference in how roots from the two stands used the two respiratory pathways (figure 8). The roots were incubated at two temperatures (6°C and 20°C), and AOX partitioning did not differ at

6°C and roots from both stands displayed a discrimination of 16.3-16.6‰ (corresponding to 18-21% AOX), but fertilized roots used only half as much AOX at 20°C (12.2%, compared to 21.6% in the control stand). This could suggest that AOX had a role to play at the lower temperature irrespective of root N status.

Although not explicitly included in this thesis, fungi and bacteria also have AOX (Campos et al., 2015; McDonald, 2008; McDonald et al., 2009). Because the incubated fine roots were mycorrhizal, some degree of fungal respiration was inherently included in our study. In this context it is notable that ectomycorrhizal sporocarp abundance in the fertilized stand was essentially zero, while the non-fertilized stand contained significantly more: 8 kg ha^{-1}, according to Hasselquist et al (2012). Thus it is possible that the degree of mycorrhizal infection of incubated fine roots could differ significantly between stands. Boreal forest soils have been associated with surprisingly high (>50% of total soil CO_2 efflux) AOX partitioning (Angert et al., 2012), and based on our observation that tree fine roots displayed less extreme ^{18}O discrimination, it is possible that other soil organisms have significantly greater AOX activity. However, AOX/COX dynamics remain uninvestigated in many species found in boreal forests. Such investigations could yield new information about the ecophysiology of respiration in boreal forests.

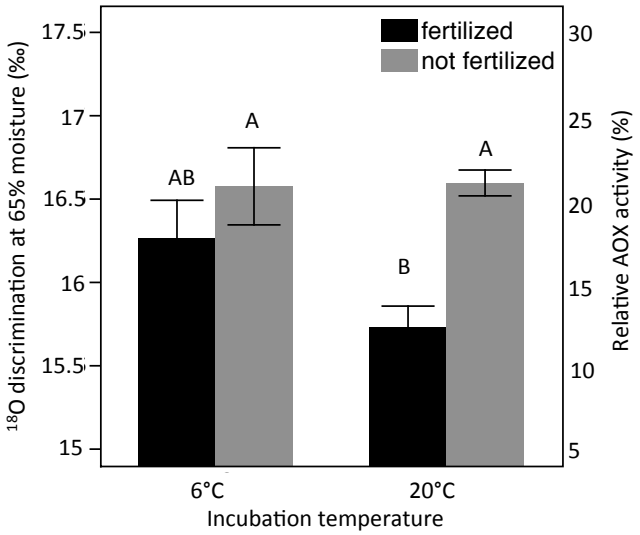

Figure 8 Isotopic discrimination (mean ± SE) of roots from the two stands when incubated at different temperatures (blue bars: 6°C, red bars: 20°C). Different letters indicate significant differences detected by a two-way analysis of variance. The right-hand Y-axis provides the calculated AOX contribution to the total respiratory flux.

5 Summary

5.1 Methodological advances

Strangling is a tree-scale treatment that can be applied to single or multiple trees for field studies on a range of topics related to C partitioning or phloem functioning. It can be used without introducing biases such as long-term root death and decomposition (girdling) or altered temperature or light and precipitation levels at the forest floor (shading). When it comes to upstream parts of the tree (upper stem, branches and foliage) strangling is comparable to girdling in terms of sugar build-up and possible feedback regulation of photosynthesis. Shading will not induce such responses, but shade avoidance responses of trees could result in altered nutrient and growth distribution within the crown, which may need to be considered when sampling foliage. Phloem chilling seems to have a similar effect on C transport as girdling and strangling, but only one successful report in large trees has been reported (Johnsen et al., 2007), possibly due to non-trivial engineering challenges.

The isotope discrimination technique for measuring AOX/COX partitioning is not an experimental treatment like strangling, but a measurement technique. It uses field-collected tissues, but is otherwise a laboratory protocol for quantifying the relative activities of a pair of specific cellular reactions. As mentioned above, several workers have focused on studying the role of AOX respiration of plant tissues and isolated mitochondria and cell cultures under various growth conditions (Dahal et al., 2014; Guy and Vanlerberghe, 2005; Sieger et al., 2005), and some efforts have been directed toward *in vivo* measurement under field conditions (Kornfeld et al., 2012; Searle et al., 2011). The isotope discrimination technique is currently the most reliable method for measuring AOX/COX partitioning, but it is sensitive to the determined values of the endpoints (isotopic discrimination of the individual pathways). These must be measured using inhibitors and the potentially greatest source of error

in this technique stems from the possibility of incomplete inhibition, which leads to inaccurately defined endpoints for the regression. This can have a significant impact on the inferred AOX/COX partitioning in non-inhibited samples. As there is currently no alternative method for verifying the discrimination of the pathways, it is important to identify and minimize effects of other factors that can influence isotopic discrimination. I found that moisture content of the sample had a significant effect on discrimination, with wetter samples displaying a lower discrimination. Because comparisons with previous reports is currently the closest thing to an actual validation of inhibited discrimination rates, it is important that significant covariates be identified and reported in the literature.

5.2 Conclusions

I found that reduced belowground C transport impaired N uptake (chapter II), and roots growing in soil with poor N availability displayed reduced respiratory efficiency, which leads to lower biosynthetic efficiency (chapter IV). This could be seen as a kind of "downward spiral" where low N leads to less photosynthesis which feeds back to less N uptake.

In N-fertilized stands, soil CO_2 efflux has been reported to decrease (Giardina and Ryan, 2002; Haynes and Gower, 1995), although at least one study has reported that soil CO_2 efflux was not affected by fertilization (Hasselquist et al., 2012). In relative terms, a reduction in tree belowground C partitioning has also been reported to occur with fertilization (Haynes and Gower, 1995; Lim et al., 2015). If fertilization also shifts AOX/COX partitioning to improve the energy-yield of root respiration, then less C is required to synthesize the same amount of ATP and thus fuel a given amount of belowground biomass production. This could hypothetically lead to a reversal of the negative feedbacks described in the previous paragraph. This further suggests that changes in respiration and production need not be proportional to each other, because if AOX becomes less active, then less C need be partitioned belowground to achieve the same yield of chemical energy or root biomass. If fertilization improves the biosynthetic efficiency of roots (and possibly fungi and other soil organisms), then this could explain some portion of the reduced soil CO_2 efflux observed after fertilization of forests.

Termination of belowground flux of recently fixed C negatively affected tree N-gain as measured by ^{15}N soil labeling and subsequent needle sampling. Strangled trees received 32% less ^{15}N, on average, than control trees. Additionally, my results suggest that tree N uptake is further affected by the

belowground C partitioning of other nearby trees. This causes a situation that is similar to competition between trees for soil N, but breaks down if an excessive proportion of trees cease their belowground C flux. In this case, there was no statistically detectable advantage to non-strangled trees.

Increased foliar N is coupled to higher photosynthetic capacity under N limited conditions (Evans, 1989). Thereby increased N uptake facilitated by competitive belowground C partitioning could result in greater belowground C flux and thus further improving the tree's ability to compete for soil/mycorrhizal N.

In conclusion, the results presented in this thesis indicate that soil N availability can affect root C use efficiency (via shifted AOX partitioning), while belowground C partitioning mediates N uptake from the soil and mycorrhiza – N that can then be transported to the foliage and increase photosynthetic C assimilation. Taken together, the results from this thesis point toward two systems of feedbacks where C and N acquisition can act to reinforce each other: One mechanism where belowground C partitioning mediates uptake of soil/mycorrhizal N; and another where high soil N availability can trigger a shift in AOX partitioning, leading to increased biosynthetic efficiency of roots, potentially allowing more efficient use of the belowground C flux.

5.3 Future perspectives

The indicated interactions of belowground C transport by trees and their N acquisition led to a hypothesized mechanism of C-N exchange between trees and mycorrhizal fungi. However, at present this hypothesis must remain speculative, as no measurements of mycorrhizal C-status or tests of connectivity via common mycorrhizal networks were conducted. The hypothesis is based upon the results from the field experiment presented in this thesis, and also draws upon the conclusions of previous studies that specifically included mycorrhizal N-retention in their experimental setups. My results are at first glance contrary to these studies, which indicate that blocking belowground C flux of trees should have yielded increased N uptake due to greater N-mobilization by mycorrhiza with insufficient C to utilize it for their own biomass production. The suggested hypothesis could potentially reconcile these findings with my own. I have planned an experiment to test this hypothesis in a more controlled environment next year.

There is also need of additional studies on the AOX partitioning of a range of boreal plant species. This thesis includes measurements of fine root AOX partitioning in *Pinus sylvestris* (L.) trees. However, in order to integrate AOX

into C budgets and growth models, its contribution to all respiratory fluxes should be quantified. Thus, AOX partitioning of needle respiration and stem respiration, as well as of respiration by fungi, understory species, and mosses are called for.

Although the protocol presented in this thesis includes improvements for *in vivo* measurement of AOX partitioning, several methodological issues remain to be addressed. Perhaps the most urgent is the question of how to deal with tissues that are difficult to inhibit with KCN or SHAM.

Personal experience with conifer needles, which responded to KCN treatment but not to SHAM or m-CLAM (a different inhibitor of AOX), points to a serious limitation in the method's applicability to boreal species. It may be significant to note that KCN is taken up as a gas, and SHAM is applied by soaking the tissue in a solution. Of course, roots naturally absorb liquids efficiently, whereas needles are constructed for gas exchange. Thus perhaps we should expect SHAM-inhibition of needles to be more challenging than roots.

Nonetheless, these are issues that must be addressed if AOX is to be meaningfully included in ecosystem-scale C studies. Perhaps research aimed at characterizing AOX molecular structure in different tissue types within a species could point to a solution? If it were found that AOX expressed in roots is sufficiently similar to AOX expressed in needles, then it may be defensible to apply the inhibitors only to the tissue that most readily absorbs it. Surveying the literature, one can see that inhibition of roots often yields lower discrimination than the corresponding inhibition of leaves. This could either be caused by a biological difference in AOX and COX expressed in different tissues, or it could be caused by some methodological artefact. Whichever is the case, the answer should lead to improved understanding of AOX partitioning in plants and how it can affect C balances at different scales.

References

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.

Angert, A., Barkan, E., Barnett, B., Brugnoli, E., Davidson, E.A., Fessenden, J., Maneepong, S., Panapitukkul, N., Randerson, J.T., Savage, K., Yakir, D., Luz, B., 2003. Contribution of soil respiration in tropical, temperate, and boreal forests to the ^{18}O enrichment of atmospheric O_2. *Glob. Biogeochem. Cycles* 17, 1089.

Angert, A., Rodeghiero, M., Griffin, K., 2012. High alternative oxidase activity in cold soils and its implication to the Dole Effect. Geophys. Res. Lett. 39, L16710.

Armstrong, A.F., Badger, M.R., Day, D.A., Barthet, M.M., Smith, P.M.C., Millar, A.H., Whelan, J., Atkin, O.K., 2008. Dynamic changes in the mitochondrial electron transport chain underpinning cold acclimation of leaf respiration. *Plant Cell Environ.* 31, 1156–1169.

Asao, S., Ryan, M.G., 2015. Carbohydrate regulation of photosynthesis and respiration from branch girdling in four species of wet tropical rain forest trees. *Tree Physiol.* 35, 608–620.

Beiler, K.J., Simard, S.W., Durall, D.M., 2015. Topology of tree-mycorrhizal fungus interaction networks in xeric and mesic Douglas-fir forests. *J. Ecol.* 103, 616–628.

Bhupinderpal-Singh, Nordgren, A., Ottosson-Löfvenius, M., Högberg, M.N., Mellander, P.-E., Högberg, P., 2003. Tree root and soil heterotrophic respiration as revealed by girdling

of boreal Scots pine forest: extending observations beyond the first year. *Plant Cell Environ.* 26, 1287–1296.
Binkley, D., Stape, J.L., Takahashi, E.N., Ryan, M.G., 2006. Tree-girdling to separate root and heterotrophic respiration in two Eucalyptus stands in Brazil. *Oecologia* 148, 447–454.
Björkman, E., 1944. Strangling effect on mycorrhiza establishment in pine. *Sven. Bot. Tidsskr.* 38, 1–14.
Brownlee, C., Duddridge, J.A., Malibari, A., Read, D.J., 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant Soil* 71, 433–443.
Campioli, M., Vicca, S., Luyssaert, S., Bilcke, J., Ceschia, E., Chapin III, F.S., Ciais, P., Fernández-Martínez, M., Malhi, Y., Obersteiner, M., Olefeldt, D., Papale, D., Piao, S.L., Peñuelas, J., Sullivan, P.F., Wang, X., Zenone, T., Janssens, I.A., 2015. Biomass production efficiency controlled by management in temperate and boreal ecosystems. *Nat. Geosci.* 8, 843–846.
Campos, C., Cardoso, H., Nogales, A., Svensson, J., Lopez-Ráez, J.A., Pozo, M.J., Nobre, T., Schneider, C., Arnholdt-Schmitt, B., 2015. Intra and Inter-Spore Variability in Rhizophagus irregularis AOX Gene. *PLOS ONE* 10, e0142339.
Cannell, M.G.R., Thornley, J.H.M., 2000. Modelling the components of plant respiration: some guiding principles. *Ann. Bot.* 85, 45–54.
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., 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.
Dahal, K., Wang, J., Martyn, G.D., Rahimy, F., Vanlerberghe, G.C., 2014. Mitochondrial Alternative Oxidase Maintains Respiration and Preserves Photosynthetic Capacity during Moderate Drought in Nicotiana tabacum. *Plant Physiol*. 166, 1560–1574.
Davidson, E.A., Savage, K., Bolstad, P., Clark, D.A., Curtis, P.S., Ellsworth, D.S., Hanson, P.J., Law, B.E., Luo, Y., Pregitzer,

K.S., others, 2002. Belowground carbon allocation in forests estimated from litterfall and IRGA-based soil respiration measurements. *Agric. For. Meteorol.* 113, 39–51.
De Schepper, V., Vanhaecke, L., Steppe, K., 2011. Localized stem chilling alters carbon processes in the adjacent stem and in source leaves. *Tree Physiol.* 31, 1194–1203.
De Vries, F.P., Brunsting, A.H.M., Van Laar, H.H., 1974. Products, requirements and efficiency of biosynthesis a quantitative approach. *J. Theor. Biol.* 45, 339–377.
DeLucia, E.H., Drake, J.E., Thomas, R.B., Gonzalez-Meler, M., 2007. Forest carbon use efficiency: is respiration a constant fraction of gross primary production? *Glob. Change Biol.* 13, 1157–1167.
Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78, 9–19.
Fernández-Martínez, M., Vicca, S., Janssens, I.A., Sardans, J., Luyssaert, S., Campioli, M., Chapin III, F.S., Ciais, P., Malhi, Y., Obersteiner, M., Papale, D., Piao, S.L., Reichstein, M., Rodà, F., Peñuelas, J., 2014. Nutrient availability as the key regulator of global forest carbon balance. *Nat. Clim. Change* 4, 471–476.
Finlay, R.D., Read, D.J., 1986. The structure and function of vegetative mycelium of ectomycorrhizal plants: I. translocation of 14C-labelled carbon between plants interconnected by a common mycelium. *New Phytol.* 103, 143–156.
Finzi, A.C., Moore, D.J., DeLucia, E.H., Lichter, J., Hofmockel, K.S., Jackson, R.B., Kim, H.-S., Matamala, R., McCarthy, H.R., Oren, R., others, 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. *Ecology* 87, 15–25.
Florez-Sarasa, I., Flexas, J., Rasmusson, A.G., Umbach, A.L., Siedow, J.N., Ribas-Carbo, M., 2011. In vivo cytochrome and alternative pathway respiration in leaves of Arabidopsis thaliana plants with altered alternative oxidase under different light conditions: In vivo regulation of leaf respiration. *Plant Cell Environ.* 34, 1373–1383.
Florez-Sarasa, I.D., Bouma, T.J., Medrano, H., Azcon-Bieto, J., Ribas-Carbo, M., 2007. Contribution of the cytochrome and

alternative pathways to growth respiration and maintenance respiration in Arabidopsis thaliana. *Physiol. Plant.* 129, 143–151.

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.

Giardina, C.P., Ryan, M.G., 2002. Total Belowground Carbon Allocation in a Fast-growing Eucalyptus Plantation Estimated Using a Carbon Balance Approach. *Ecosystems* 5, 487–499.

Giardina, C.P., Ryan, M.G., Binkley, D., Fownes, J.H., 2003. Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. *Glob. Change Biol.* 9, 1438–1450.

Gonzàlez-Meler, M.A., Giles, L., Thomas, R.B., Siedow, J.N., 2001. Metabolic regulation of leaf respiration and alternative pathway activity in response to phosphate supply. *Plant Cell Environ.* 24, 205–215.

Gorzelak, M.A., Asay, A.K., Pickles, B.J., Simard, S.W., 2015. Inter-plant communication through mycorrhizal networks mediates complex adaptive behaviour in plant communities. *AoB Plants* 7, plv050.

Gould, N., Thorpe, M., Koroleva, O., Minchin, E.H., 2005. Phloem hydrostatic pressure relates to solute loading rate: a direct test of the Münch hypothesis. Funct. Plant Biol. 32, 1019–1026.

Guy, R.D., Berry, J.A., Fogel, M.L., Hoering, T.C., 1989. Differential fractionation of oxygen isotopes by cyanide-resistant and cyanide-sensitive respiration in plants. *Planta* 177, 483–491.

Guy, R.D., Vanlerberghe, G.C., 2005. Partitioning of respiratory electrons in the dark in leaves of transgenic tobacco with modified levels of alternative oxidase. *Physiol. Plant.* 125, 171–180.

Hammel, H.T., 1968. Measurement of turgor pressure and its gradient in the phloem of oak. *Plant Physiol.* 43, 1042–1048.

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

Hasselquist, N.J., Metcalfe, D.B., Högberg, P., 2012. Contrasting effects of low and high nitrogen additions on soil CO_2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Glob. Change Biol.* 18, 3596–3605.

Haynes, B.E., Gower, S.T., 1995. Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiol.* 15, 317–325.

Högberg, P., Bhupinderpal-Singh, Löfvenius, M.O., Nordgren, A., 2009. Partitioning of soil respiration into its autotrophic and heterotrophic components by means of tree-girdling in old boreal spruce forest. *For. Ecol. Manag.* 257, 1764–1767.

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. Manag.* 403, 161–185. d

Högberg, P., Nordgren, A., buchmann, N., taylor, A., F.S., Ekblad, A., Högberg, M., N., Nyberg, G., Ottosson-Löfvenius, M., Read, D., J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411, 789–792.

Johnsen, K., Maier, C., Sanchez, F., Anderson, P., Butnor, J., Waring, R., Linder, S., 2007. Physiological girdling of pine trees via phloem chilling: proof of concept. *Plant Cell Environ.* 30, 128–134.

Knoblauch, M., Oparka, K., 2012. The structure of the phloem - still more questions than answers: Sieve Tube Structure and Function. *Plant J.* 70, 147–156.

Knoblauch, M., Peters, W.S., 2010. Münch, morphology, microfluidics - our structural problem with the phloem: Münch, morphology, microfluidics. Plant Cell Environ. 33, 1439–1452.

Kornfeld, A., Horton, T.W., Yakir, D., Searle, S.Y., Griffin, K.L., Atkin, O.K., Subke, J.-A., Turnbull, M.H., 2012. A field-compatible method for measuring alternative respiratory pathway activities in vivo using stable O_2 isotopes: Measuring alternative respiration in the field. *Plant Cell Environ.* 35, 1518–1532.

Lambers, H., Ribas-Carbo, M., 2005. *Plant Respiration: From Cell to Ecosystem*. Springer, Dordrecht, The Netherlands.

Landsberg, J., 2003. Modelling forest ecosystems: state of the art, challenges, and future directions. *Can. J. For. Res.* 33, 385–397.

Lim, H., Oren, R., Palmroth, S., Tor-ngern, P., Mörling, T., Näsholm, T., Lundmark, T., Helmisaari, H.-S., Leppälammi-Kujansuu, J., Linder, S., 2015. Inter-annual variability of precipitation constrains the production response of boreal Pinus sylvestris to nitrogen fertilization. *For. Ecol. Manag.* 348, 31–45.

Macfarlane, C., Hansen, L.D., Florez-Sarasa, I., Ribas-Carbo, M., 2009. Plant mitochondria electron partitioning is independent of short-term temperature changes. *Plant Cell Environ.* 32, 585–591.

Mäkelä, A., Valentine, H.T., 2001. The ratio of NPP to GPP: evidence of change over the course of stand development. *Tree Physiol.* 21, 1015–1030.

McDonald, A.E., 2008. Alternative oxidase: an inter-kingdom perspective on the function and regulation of this broadly distributed "cyanide-resistant" terminal oxidase. *Funct. Plant Biol.* 35, 535.

McDonald, A.E., Vanlerberghe, G.C., Staples, J.F., 2009. Alternative oxidase in animals: unique characteristics and taxonomic distribution. *J. Exp. Biol.* 212, 2627–2634.

Millar, A.H., Atkin, O.K., Menz, R.I., Henry, B., Farquhar, G., Day, D.A., 1998. Analysis of respiratory chain regulation in roots of soybean seedlings. *Plant Physiol.* 117, 1083–1093.

Münch, E., 1930. *Die stoffbewegungen in der pflanze.* Gustav Fischer, Jena, Gerrmany.

Nagel, O.W., Waldron, S., Jones, H.G., 2001. An Off-Line Implementation of the Stable Isotope Technique for Measurements of Alternative Respiratory Pathway Activities. *Plant Physiol.* 127, 1279–1286.

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

Niinemets, üLo, 2007. Photosynthesis and resource distribution through plant canopies. Plant Cell Environ. 30, 1052–1071.

Nikinmaa, E., Sievanen, R., Höltta, T., 2014. Dynamics of leaf gas exchange, xylem and phloem transport, water potential and carbohydrate concentration in a realistic 3-D model tree crown. *Ann. Bot.* 114, 653–666.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control: Tansley review. *New Phytol.* 193, 30–50.

Rachmilevitch, S., Xu, Y., Gonzalez-Meler, M.A., Huang, B., Lambers, H., 2007. Cytochrome and alternative pathway activity in roots of thermal and non-thermal Agrostis species in response to high soil temperature. *Physiol. Plant.* 129, 163–174.

Raich, J.W., Nadelhoffer, K.J., 1989. Belowground Carbon Allocation in Forest Ecosystems: *Global Trends. Ecology* 70, 1346.

Ribas-Carbo, M., Aroca, R., Gonzàlez-Meler, M.A., Irigoyen, J.J., Sánchez-Díaz, M., 2000. The electron partitioning between the cytochrome and alternative respiratory pathways during chilling recovery in two cultivars of maize differing in chilling sensitivity. *Plant Physiol.* 122, 199–204.

Robinson, S.A., Ribas-Carbo, M., Yakir, D., Giles, L., Reuveni, Y., Berry, J.A., 1995. Beyond SHAM and cyanide: opportunities for studying the alternative oxidase in plant respiration using oxygen isotope discrimination. *Funct. Plant Biol*. 22, 487–496.

Ryan, M.G., 1991. Effects of Climate Change on Plant Respiration. *Ecol. Appl.* 1, 157–167.

Ryan, M.G., Asao, S., 2014. Phloem transport in trees. *Tree Physiol.* 34, 1–4.

Ryan, M.G., Lavigne, M.B., Gower, S.T., 1997. Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. *J. Geophys. Res.* 102, 28871–28883.

Searle, S.Y., Thomas, S., Griffin, K.L., Horton, T., Kornfeld, A., Yakir, D., Hurry, V., Turnbull, M.H., 2011. Leaf respiration and alternative oxidase in field-grown alpine grasses respond to natural changes in temperature and light. *New Phytol*. 189, 1027–1039.

Searle, S.Y., Turnbull, M.H., 2011. Seasonal variation of leaf respiration and the alternative pathway in field-grown Populus×canadensis. *Physiol. Plant.* 141, 332–342.
Sieger, S.M., Kristensen, B.K., Robson, C.A., Amirsadeghi, S., Eng, E.W.Y., Sbdel-Mesih, A., Moller, I.M., Vanlerbrghe, G.C., 2005. The role of alternative oxidase in modulating carbon use efficiency and growth during macronutrient stress in tobacco cells. *J. Exp. Bot.* 56, 1499–1515.
Sovonick-Dunford, S., Lee, D.R., Zimmermann, M.H., 1981. Direct and indirect measurements of phloem turgor pressure in white ash. Plant Physiol. 68, 121–126.
Tamm, C.O., 1991. *Nitrogen in terrestrial ecosystems: Questions of productivity, vegetational changes, and ecosystem stability*. Springer Verlag, Berlin.
Tarvainen, L., Lutz, M., Räntfors, M., Näsholm, T., Wallin, G., 2016. Increased Needle Nitrogen Contents Did Not Improve Shoot Photosynthetic Performance of Mature Nitrogen-Poor Scots Pine Trees. *Front. Plant Sci.* 7.
Tarvainen, L., Rantfors, M., Wallin, G., 2014. Vertical gradients and seasonal variation in stem CO2 efflux within a Norway spruce stand. *Tree Physiol.* 34, 488–502.
Teskey, R.O., Saveyn, A., Steppe, K., McGuire, M.A., 2008. Origin, fate and significance of CO2 in tree stems. *New Phytol.* 177, 17–32.
Trupkin, S.A., Legris, M., Buchovsky, A.S., Tolava Rivero, M.B., Casal, J.J., 2014. Phytochrome B Nuclear Bodies Respond to the Low Red to Far-Red Ratio and to the Reduced Irradiance of Canopy Shade in Arabidopsis. *Plant Physiol.* 165, 1698–1708.
Turgeon, R., 2010. The puzzle of phloem pressure. *Plant Physiol.* 154, 578–581.
Ubierna, N., Kumar, A.S., Cernusak, L.A., Pangle, R.E., Gag, P.J., Marshall, J.D., 2009. Storage and transpiration have negligible effects on 13C of stem CO2 efflux in large conifer trees. *Tree Physiol.* 29, 1563–1574.
Vanlerberghe, G., 2013. Alternative Oxidase: A Mitochondrial Respiratory Pathway to Maintain Metabolic and Signaling Homeostasis during Abiotic and Biotic Stress in Plants. Int. J. Mol. Sci. 14, 6805–6847. doi:10.3390/ijms14046805

Vicca, S., Luyssaert, S., Peñuelas, J., Campioli, M., Chapin, F.S., Ciais, P., Heinemeyer, A., Högberg, P., Kutsch, W.L., Law, B.E., Malhi, Y., Papale, D., Piao, S.L., Reichstein, M., Schulze, E.D., Janssens, I.A., 2012. Fertile forests produce biomass more efficiently: Forests' biomass production efficiency. *Ecol. Lett.* 15, 520–526.

Yakushiji, H., Nakatsuka, A., 2007. Recent persimmon research in Japan. *Jpn. J. Plant Sci.* 1, 42–62.

Yamanishi, O.K., Nakajima, Y., Hasegawa, K., 1993. Effect of late season trunk strangulation on fruit quality, return bloom, and fruiting in Pummelo trees. *J. Jpn. Soc. Hortic. Sci.* 62, 337–343.

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As a master's student I had the opportunity to work as a field assistant during summer break. A researcher who didn't have a Swedish driver's licence needed to get to her field site every day, and I was hired to drive the car (and to generally help out during the days in the field). This was my first encounter with scientific work. She taught me about field work and to be cheery in spite of the obstacles that constantly present themselves but that somehow still manage to be unexpected every time (unhelpful weather, broken equipment, forgotten equipment, running out of duct tape, sugar-lows, etc.). I was delighted to learn that scientific research could include this kind of work, with its practical problem-solving and beautiful surroundings. Apparently some scientists got to spend all summer out in the forest!

It turned out that field-work is also a good way to make friends because 1) solving interesting problems together is fun; and 2) solving tedious problems together is… at least satisfying in retrospect. Actually that's not only true for field-work. During my time as a PhD student, I have met a lot of people who gladly offered advice, shared their experience and basically were friendly and helpful, and if I tried to include everybody here who deserves to be acknowledged, then the list would be long and I might accidentally forget someone. So I won't do that. I will make a really short list, and hope that those of you who are not on it know that you are included in this paragraph, and don't feel overlooked or unappreciated (you're not).

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I was going to add a quote from T. H. White's *The Goshawk*, but now I think it would be a bit much…

(first paragraphs in chapter 4), and I recommend the book!

/Nils