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Tamm Review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests

Peter Högberg^a, Torgny Näsholm^a, Oskar Franklin^{a,b}, Mona N. Högberg^{a,*}

^a Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden

^b International Institute for Applied Systems Analysis (IIASA), A-2361, Laxenburg, Austria

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ABSTRACT

The supply of nitrogen commonly limits plant production in boreal forests and also affects species composition and ecosystem functions other than plant growth. These interrelations vary across the landscapes, with the highest N availability, plant growth and plant species richness in ground-water discharge areas (GDAs), typically in toe-slope positions, which receive solutes leaching from the much larger groundwater recharge areas (GRAs) uphill. Plant N sources include not only inorganic N, but, as heightened more recently, also organic N species. In general, also the ratio inorganic N over organic N sources increase down hillslopes. Here, we review recent evidence about the nature of the N limitation and its variations in Fennoscandian boreal forests and discuss its implications for forest ecology and management.

The rate of litter decomposition has traditionally been seen as the determinant of the rate of N supply. However, while N-rich litter decomposes faster than N-poor litter initially, N-rich litter then decomposes more slowly, which means that the relation between N % of litter and its decomposability is complex. Moreover, in the lower part of the mor-layer, where the most superficial mycorrhizal roots first appear, and N availability matters for plants, the ratio of microbial N over total soil N is remarkably constant over the wide range in litter and soil C/N ratios of between 15 and 40 for N-rich and N-poor sites, respectively. Nitrogen-rich and -poor sites thus differ in the sizes of the total N pool and the microbial N pool, but not in the ratio between them. A more important difference is that the soil microbial N pool turns over faster in N-rich systems because the microbes are more limited by C, while microbes in N-poor systems are a stronger sink for available N.

Furthermore, litter decomposition in the most superficial soil horizon (as studied by the so-called litter-bag method) is associated with a dominance of saprotrophic fungi, and absence of mycorrhizal fungi. The focal zone in the context of plant N supply in N-limited forests is further down the soil profile, where ectomycorrhizal (ECM) roots become abundant. Molecular evidence and stable isotope data indicate that in the typical N-poor boreal forests, nitrogen is retained in saprotrophic fungi, likely until they run out of energy (available C-compounds). Then, as heightened by recent research, ECM fungi, which are supplied by photosynthate from the trees, become the superior competitors for N.

In N-poor boreal soils strong N retention by microorganisms keeps levels of available N very low. This is exacerbated by an increase in tree C allocation to mycorrhizal fungi (TCAM) relative to net primary production (NPP) with decreasing soil N supply, which causes ECM fungi to retain much of the available soil N for their own growth and transfer little to their tree hosts. The transfer of N through the ECM fungi, and not the rate of litter decomposition, is likely limiting the rate of tree N supply under such conditions. All but a few stress-tolerant less N-demanding plant species, like the ECM trees themselves and ericaceous dwarf shrubs, are excluded.

With increasing N supply, a weakening of ECM symbiosis caused by the relative decline in TCAM contributes to shifts in soil microbial community composition from fungal dominance to bacterial dominance. Thus, bacteria, which are less C-demanding, but more likely to release N than fungi, take over. This, and the relatively high pH in GDA, allow autotrophic nitrifying bacteria to compete successfully for the NH_4^+ released by C-limited organisms and causes the N cycle to open up with leaching of nitrate (NO_3^-) and gaseous N losses through denitrification. These N-rich conditions allow species-rich communities of N-demanding plant species. Meanwhile, ECM fungi have a smaller biomass, are supplied with N in excess of their demand and will export more N to their host trees. Hence, the gradient from low to high

* Corresponding author.

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N supply is characterized by profound variations in plant and soil microbial physiologies, especially their relations to the C-to-N supply ratio. We propose how interactions among functional groups can be understood and modelled (the plant-microbe carbon-nitrogen model).

With regard to forest management these perspectives explain why the creation of larger tree-free gaps favors the regeneration of tree seedlings under N-limited conditions through reduced belowground competition for N, and why such gaps are less important under high N supply (but when light might be limiting). We also discuss perspectives on the relations between N supply, biodiversity, and eutrophication of boreal forests from N deposition or forest fertilization.

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1. Introduction

Boreal forests cover vast areas in the northern circumpolar region (Chapin et al., 2011), which is characterized by long cold winters and short summers. Another important feature is that plant growth is commonly constrained by a low supply of N, i.e. plant growth is enhanced by additions of N (e.g., Tamm, 1991). Tree growth varies with hill-slope position (e.g., Hägglund and Lundmark, 1977), likely because of effects of hill-slope hydrology on the availability of N. Variations in N supply also affects the species composition and other functions of boreal forests.

People dependent on products from the forests and the possibility to clear them for the purpose of agriculture (permanently or temporarily as swidden agriculture) probably recognized already centuries ago the existence of relations between certain plant species and soil fertility. A first more systematic description linking the common forest types (based on descriptions of the field-layer plants) to forest productivity in Finland was given by Cajander (1909, 1926) and Cajander and Ilvessalo (1922). According to these schemes, and followers from Sweden (Arnborg, 1990) and Norway

(Kielland-Lund, 1982), ericaceous dwarf shrub communities cover the poorer soils, short herbs are common at intermediate soil fertilities and tall herbs dominate richer soils. The exact causes of these differences in species composition and productivity were not clarified and there is still no commonly accepted explanation of their nature.

This review serves the purpose of describing these patterns and their likely causes in the perspective of recent advances in our understanding of the interactions among soils, soil microorganisms, and plants. We will focus on boreal forests on mineral soils (i.e. make little reference to forests on peat) in Fennoscandia. However, the patterns and processes discussed may be important also in other N-limited forests, e.g., other boreal and temperate forests. For example, forest growth on drained boreal peatlands can likewise be predicted based on the composition of the original field-layer plant community (Hånell, 1988). Moreover, an understanding of these patterns and processes and their underlying causes is essential not only for ecologists interested in the structure and function of these ecosystems, but also pertinent to aspects on forest management (e.g., Hynynen et al., 2005; Mäkinen et al., 2006).

2. Background: Previous observations of and hypotheses about variations in N cycling in Fennoscandian boreal and other similar forests

2.1. Early observations

In the early 20th century, it was well established that the supply of N often limited the production of agricultural crops. The first straightforward N addition experiment in Swedish forests made by Hesselman in the early 1920s indicated that this limitation may also prevail in forests (Hesselman, 1926). This has subsequently received massive support by N fertilizer trials across Fennoscandia (e.g., Nilsen, 2001; Nohrstedt, 2001; Saarsalmi and Mälkönen, 2001). Moreover, already in 1917 Hesselman reported the occurrence of nitrate in leaves of herbs in lush tall herb vegetation found in “lunddälder” (Hesselman, 1917). “Lund” refers to vegetation dominated by deciduous trees and herbs (and sometimes broad-leaved grasses) and “dälder” is plural for small valleys, i.e. sites likely to receive groundwater discharge, at least temporarily. Hesselman assumed that the NO_3^- in the leaves was not produced by the plants (which indeed is very rare, and is only known to occur in a few N_2 -fixing legume species, see Hipkin et al., 2004), but must have been taken up from the soil. He also noted that NO_3^- occurred in leaves of some plant species in clear-fellings. Corroborating observations were later made using an *in vivo* method for measuring leaf nitrate reductase activity (NRA), which revealed increasing NRA along a gradient from ericaceous dwarf shrub type, through a short herb type to a tall herb type (Högberg et al., 1990) and elevated NRA in clear-fellings (Högbom et al., 2002).

The early observations of spatial variations in forest plant growth stimulated soil studies aiming at unravelling the underlying causes. Ambitious surveys and studies were conducted in Finland by Viro (1951, 1955), who found a correlation between exchangeable Ca^{2+} and forest productivity. After re-examining this material, Dahl et al. (1961) concluded that the correlation was not unequivocally supporting the notion that Ca was limiting forest growth. They made a detailed regional survey in Hedmark County, Norway, of relations between soil conditions and plant community composition (Dahl et al., 1967). The survey showed very strong correlations among forest vegetation types (classification based on field-layer species), forest growth and soil variables like base saturation (which is dominated by the contribution of exchangeable Ca^{2+}), but also N concentration. The latter was, of course, particularly interesting given the then well-established N limitation to forest growth.

Subsequently, Lahti and Väisänen (1987) made use of vegetation and soils data from previous surveys in Finnish southern boreal forests. They found striking correlations among forest vegetation types and soil pH, exchangeable Ca^{2+} and % N, i.e. variables known to correlate with forest growth. Remarkable variations in such relations between soils and vegetation can be found in short distances, as demonstrated by Giesler et al. (1998) in a study of a 90-m-long transect from a groundwater recharge area (GRA) to a discharge area (GDA), along which forest production varied by a factor three. Furthermore, survey data from Finland (Lahti and Väisänen, 1987) and Norway (Dahl et al., 1967), and the sharp local boreal Swedish gradient (Giesler et al., 1998) fits almost the same regression between % N and exchangeable Ca^{2+} (Fig. 1a). With comparable forest types in the three countries, they could be ordered from poor to rich forest types (i.e. from dwarf shrub types to tall herb types) along this positive relation (Giesler et al., 1998, see Fig. 1b). Similarly, there is a very strong negative correlation between the soil C/N ratio (which, of course, is very strongly negatively correlated with % N in the organic mor-layer) and base saturation, BS, as shown by data from the Swedish Forest Soil Survey (Fig. 2).

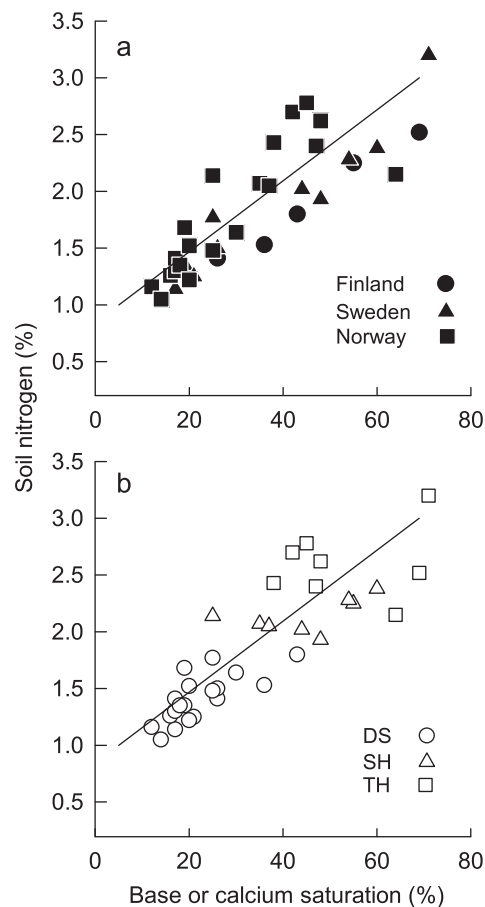


Fig. 1. The relation between N concentration and base saturation in mor-layers in (a) Finland (Lahti and Väisänen, 1987), Hedmark county, Norway (Dahl et al., 1967), and along the Betsele transect in Sweden (Giesler et al., 1998). (b) The relationship between % N and % base saturation (BS) in the mor layer in dwarf shrub (DS), short herb (SH), and tall herb (TH) forest types in Finland, Sweden, and Norway. Data from Finland (Lahti and Väisänen, 1987) represent 921 forest stands. Base saturation data from Giesler et al. (1998) and Dahl et al. (1967) is determined in unbuffered 1.0 mol/L NH_4NO_3 and $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$, respectively, whereas the Finnish material represents Ca saturation (extraction method unknown). The difference should be minor because of the dominant role of Ca^{2+} among base cations.

2.2. N cycling – classical perspectives

So, how do the N cycles and N release patterns differ among forest types? A classical paradigm assumes there is a positive relationship between litter decomposability (loss of litter mass) and plant N availability and focuses on effects of litter quality (Bosatta and Ågren, 1991; Ågren and Bosatta, 1996), i.e. primarily the C/N ratio, and on abiotic constraints (e.g., temperature, moisture), on the rate of litter decomposition (e.g., Swift et al., 1979; Berg and McLaugherty, 2008).

According to studies of decomposition of litter enclosed in so called litter bags, a widely used method, the rate of decomposition is initially (for the first few years) higher in N-rich litter, but then becomes slower than in N-poor litter (Berg and McLaugherty, 2008). Hence, the relation between the release of N in plant available forms and the C/N ratio of the litter is not straightforward. In addition to the abiotic constraints, moisture and temperature, the amount of plant available N is also dependent on the amount of litter deposited, and on the strength of competing biotic and abiotic sinks for available N. A crucial question to be discussed further below (see Section 3.2.) is if a change of plant species composition, and hence litter characteristics, can cause a substantial change in the nature of the N cycle.

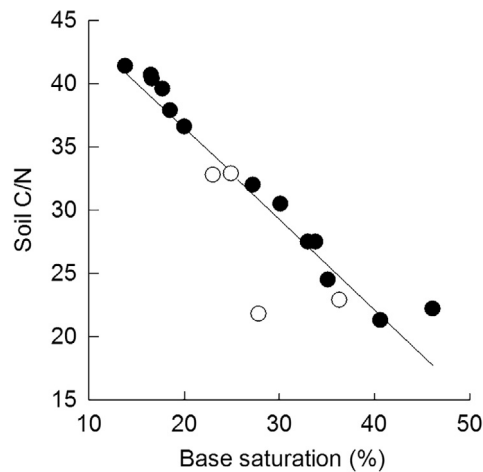


Fig. 2. The relation between mor-layer C/N ratio and base saturation for forests in Norrland, Sweden. Data are from the Forest Soil Survey 1993–2002 and are means for different vegetation types. The black circles are contributed by 2587 samples, which represent 79% of the total number of samples ($n = 3264$ from an area of 12.45 million ha of forests). An additional 18% were contributed by forest types dominated by forest types characterized by grasses and sedges (white unfilled circles), which indicates that they may have been used for crops or as pastures. We have excluded rare forest types, with less than 10 samples each. The DS, SH and TH forest types (filled circles) contribute 79%, 12% and 9%, respectively, to the data (means). The regression line is based on all data points in the graph ($R^2_{adj} = 0.85$, $n = 17$, $p < 0.001$).

One line of reasoning has focused on the possibility that much of the initial plant litter N deposited is not released by decomposers, but perhaps ultimately forms the more stable N compounds deeper down in the soil. To this, depolymerization-recondensation pathways or various other chemical immobilization or selective preservation pathways could contribute in making soil organic N less available to decomposers (Stevenson, 1994; Knicker, 2011). There is an array of organic N forms that could be formed through reactions between inorganic or organic N sources and lignin compounds or other phenolic compounds. The traditional view on causes to the stability of soil organic matter C and N focused on the chemical properties of molecules *per se*, and stable organic matter was thus said to be formed through the formation of humic substances or through selective preservation of resistant components (Stevenson, 1994).

Over time, the view has changed to a continuum of many different organic compounds at various stages of decomposition (Bosatta and Ågren, 1991) and the emerging understanding is that most old soil organic matter can be further decomposed (Knicker, 2011; Schmidt et al., 2011; Lehmann and Kleber, 2015). It has also been proposed that this material, while of plant origin initially, consists of material proximally derived from microorganisms (Gleixner, 2013). However, with time smaller and less energy-rich compounds remain (Bosatta and Ågren, 1991) and the supply of available C to the decomposer microorganisms diminishes. Recent research shows that much of the older soil organic N consists of peptide-like compounds, which (unlike the original litter N) have the potential to be selectively preserved during further decomposition and to dominate the soil organic N pool (Knicker and Kögel-Knabner, 1998; Knicker, 2011).

Studying plant available N is complex, because N occurs in many forms in the soil and most of the N occurs in compounds turning over very slowly, whereas the active pools are very small and difficult to measure (Binkley and Hart, 1989). For example, in a boreal forest mor-layer, extractable inorganic N (NH_4^+ and NO_3^-) and extractable (using KCl as extractant) amino acids and microbial N contributed 1%, 3% and 10%, respectively, of the total

N (Näsholm et al., 1998). These small pools turn over quickly, days for inorganic N and weeks for microbial N (Högberg et al., 2006), as a result of rapid biological mobilization and immobilization processes, lysis of microorganisms and grazing on them by soil animals.

For long, the major methods to assess plant available N have been biological (so called bio-assays) and based on incubation of samples in the field or in the laboratory, under conditions that should promote N mineralization from organic sources (e.g., Binkley and Hart, 1989). Chemical extraction (usually with a solution of KCl or K_2SO_4 as extractant) at intervals after soil sampling is followed by analysis of inorganic N in the filtered extracts. These widely used methods are based on the assumptions that the same biological processes that cause release of plant available N under undisturbed conditions in the field are also responsible for the production of inorganic N in the laboratory procedures (Bundy and Meisinger, 1994 and references therein) and that plant available N is restricted to inorganic N forms. In agricultural soils, for which these biological methods to estimate net N mineralization was first developed, there is usually a rapid net release of inorganic N (Jansson, 1958).

2.3. Towards a more diverse perspective on N availability

In contrast to the situation in arable soils, there is often no net N mineralization or net nitrification during the first months of incubation in most boreal and other high-latitude soils (e.g., Priha and Smolander, 1999; Persson et al., 2000; Stark et al., 2003). However, trees and other plants apparently do take up N also under these circumstances. Discrepancies between net N mineralization and plant N uptake stimulated research on uptake of organic N sources (Chapin et al., 1993; Kielland, 1994) and measurements of gross (actual) N mineralization (see below) and gross nitrification rates (e.g., Davidson et al., 1991, 1992; Binkley et al., 1992a; Hart et al., 1994; Stark and Hart, 1997; Fisk and Fahey, 2001; Merilä et al., 2002; Stark et al., 2003; Högberg et al., 2006). Such studies indeed showed measurable mineralization rates also in the shorter term (see below); supporting the idea that inorganic N could be a source, albeit not the single source of plant N in boreal forests.

Which could the N sources be if the supply of inorganic N from decomposition of organic matter seems too low? Trees of *Alnus* forms symbiosis with N_2 -fixing actinobacteria, from which they gain N (Johnsrud, 1978; Huss-Danell, 1997), but other boreal tree species lack this ability. This means that the “missing” sources of N for the other boreal tree species could be organic N forms, or perhaps inorganic N is still important, but release and uptake are so tightly coupled that free inorganic N cannot be observed. The latter is possible if the microbial community remains a strong N sink even under long laboratory soil incubations (Hart et al., 1994). This condition changes dramatically when the microorganisms exploiting a specific ageing substrate over time become C-limited, with increased gross N and even net N mineralization as a result (Hart et al., 1994) in line with our current understanding of microbial physiology (Franklin et al., 2011; Robertson and Groffman, 2015).

In C-limited soil many soil microorganisms are found close to roots as a result of exudation of highly degradable C substrates (Nazir et al., 2010, and references therein); the high C supply can support a microbial N sink strength and, hence, contribute to a low N availability for plants. Gadgil and Gadgil (1971, 1975) showed in a study on New Zealand that decomposition could become greater on trenched plots excluding the influence of active ECM roots. They ascribed the higher rate of decomposition to a higher availability of N. Subsequent studies of the so called “Gadgil effect” have, however, been inconsistent (Fernandez and Kennedy, 2016).

Alternatively, as proposed in other studies, the higher C supply and microbial biomass in the rhizosphere can stimulate N mineralization of available organic N sources and N immobilization processes (e.g., Norton and Firestone, 1991, 1996; Norton et al., 1990), and can accelerate the decomposition of soil organic matter for additional N mineralization, so called real priming (Kuzyakov and Xu, 2013). Similarly, Clarholm (1985) stressed the importance of a microbial loop, in which bacterial activity was stimulated by root exudation of C, which in turn fueled bacterial breakdown of complex N sources and plant assimilation of the N released after amoebae grazing on the bacteria. If this is the case, nitrogen mineralization should be lower in soil samples without active plant roots.

The rapid turnover of small pools of N in the soil can be studied by the ^{15}N pool dilution method (Kirkham and Bartholomew, 1954; Jansson, 1958; Davidson et al., 1991, 1992). This method uses measurements of the change in pool size and labelling of the N pool studied. In studies of gross N mineralization, a trace of NH_4^+ highly enriched in ^{15}N is injected into the soil, whereas the NO_3^- pool is labelled if gross nitrification is studied. In both cases the assumption is that the endogenous N source, from which the N in the studied inorganic pool is produced, is unlabeled, i.e. has an atom % ^{15}N at natural abundance. The method was introduced in agricultural settings in the 1950s (Kirkham and Bartholomew, 1954; Jansson, 1958), but was first used in forests in the late 1980s (e.g., Davidson et al., 1991, 1992). There, a common problem is the much smaller sizes of the inorganic pools of N. Interestingly, however, the method revealed rapid turnover of the pools of NH_4^+ and sometimes also NO_3^- (Hart et al., 1994; Stark and Hart, 1997; Högberg et al., 2006) in forest soils, where these pools were small and where soil incubations for the monitoring of net release of these ions found very low rates of release if any release at all (Davidson et al., 1992; Högberg et al., 2006).

Most ecologists interested in N uptake by plants assumed for long that the plants take up N only in inorganic forms, despite the fact that plant physiologists working in parallel in laboratories observed plant uptake and growth on amino acid N (Hutchinson and Miller, 1912; Brigham, 1917; Virtanen and Linkola, 1946). Moreover, uptake of ^{15}N in glutamic acid by a mycorrhizal fungus (grown under otherwise sterile conditions) transferring the ^{15}N to its plant host *Pinus sylvestris* was reported in a laboratory study already in the early 1950s (Melin and Nilsson, 1953). However, it remained until the 1990s before plant growth on amino acids was revisited (Chapin et al., 1993) and uptake of an intact amino acid was demonstrated directly in the field in a boreal forest (Näsholm et al., 1998).

Before that, the idea of organic N as an important N source for boreal forest plants, in particular, was explored in laboratory studies by Read (1983, 1986, 1991) and his co-workers (Abuzinadah et al., 1986; Abuzinadah and Read, 1986). Read made reference to the observations that ectomycorrhizal (ECM) trees and ericaceous dwarf shrubs with ericoid mycorrhizas (ERM) were the dominant plants of boreal forests in America, Europe and Asia, and that this dominance was associated with N-poor conditions and formation of acid organic mor-layers on top of the mineral soil. Furthermore, based on the high capacity of many ECM and ERM fungi to take up organic N sources, Read and co-workers proposed that the dominances of the host plant species was linked to this capacity (Abuzinadah and Read, 1986; Abuzinadah et al., 1986). However, a screening including AM, ERM, ECM and non-mycorrhizal boreal forest plants did not show any difference in uptake rates of organic N in the form of amino acids (Persson and Näsholm, 2001). Moreover, detailed studies of the model plant *Arabidopsis thaliana* indicates that even this ruderal, non-mycorrhizal plant has a well-developed capacity for organic N nutrition (Forsum et al., 2008). A widespread capacity to take up organic N is also

expected from an organism nutrition perspective because of the lower C cost and associated higher N-use efficiency of growth compared to uptake of inorganic N (Franklin et al., 2017).

In Read's model he also included that more southerly forests and steppe vegetation is associated with a more complete decomposition of organic matter, a greater supply of N, but less of P, and a greater prominence (nemoral forests) or total dominance of plants (steppe) with arbuscular mycorrhiza (AM) (Read, 1991; Read and Perez-Moreno, 2003). Such variations from dominance of ECM and ERM associations to dominance of AM associations among field-layer plants can occur locally in the context of short (90-m-long) transects from recharge areas to discharge areas in boreal forests (Högberg et al., 1990; Giesler et al., 1998; Nilsson et al., 2005). The release of available N by mycorrhizal fungi from complex N sources represents another microbial loop, in which some of the N goes directly to the plant, some remains in the mycelium, but can become more freely available after grazing by *Collembola* and *Acari* on the mycorrhizal mycelium.

With this as background, we would like to turn to greater detail of what we now believe we know about the nature of the N limitation to plant growth that prevails in most boreal forests. Attention will be drawn to what we consider critical new insights and emerging reformulations of old questions in the contexts of forest ecology and management in Fennoscandia.

3. Nitrogen supply to and within boreal forests

3.1. Which are the inputs and how large are they?

Minerals very rarely contain significant amounts of N, which can become available for plants as a result of weathering. Exceptions are minerals with NH_4^+ fixed in crystal lattices, in which case studies in N. America have shown that weathering may even release enough NH_4^+ to support high rates of nitrification and soil acidification (Dahlgren, 1994; Morford et al., 2016). Bedrocks of the Caledonian overthrust forming the Scandes Mts. contain traces of N (Dixon et al., 2012), but estimates of natural weathering are uncertain as well as of contributions of this source of N to the plants. As far as we know, there are no reports of release of N from the forested Fennoscandian bedrock shield, which is dominated by granites and gneisses. This leaves atmospheric N deposition and biological N_2 fixation as major known external sources of N to soil organisms and plants.

Sampling of the ice sheet of Greenland has revealed that there was some deposition of NO_3^- even before the impact of industrialization (Mayewski et al., 1986), when the deposition of this compound doubled. In Europe the increase was larger, but is now slowly declining (Lajtha and Jones, 2013). There has probably also always been a minor background contribution of N in dust from desert storms and aerosols of marine origin. As regards northern Fennoscandian boreal forests the total ambient N deposition ranges between 1 and 3 kg N ha $^{-1}$ yr $^{-1}$ (Sponseller et al., 2016) and decreases from the SW to the NE.

Dinitrogen fixation is carried out by several genera of prokaryotic organisms functioning along a continuum from those living freely in soil to more intimate associations with other microorganisms and plants. In highly developed symbioses, the plant hosts supply the N_2 -fixing bacteria with the energy sources (C-compounds) needed to break the strong triple bonds of N_2 and reduce it to NH_3 to support growth of the bacteria and the plant. Highly developed symbioses are in boreal forests represented by actinobacteria, *Frankia*, in root nodule symbiosis with woody species like *Alnus incana* and *Myrica gale*, and by lichens forming tripartite symbioses between fungi, green algae, and N_2 -fixing cyanobacteria; typical examples of which are the lichens

Stereocaulon paschale, *Peltigera aptosa* and *Nephroma arcticum*. Grey alder (*A. incana*) occurs in a narrow zone of some tens of meters on recently exposed ground (due to iso-static rebound after the last glaciation) along the seashore of the Gulf of Bothnia, banks of rivers, in ditches along roads and occasionally as clones in boreal conifer forests. Across a primary chronosequence studied by Blaško et al. (2015) a build-up rate of soil N of $\sim 20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ occurred in the narrow zone of alder forests, which may possibly fix more N_2 (e.g., Johnsrud, 1978; Binkley et al., 1992b; Huss-Danell et al., 1992). Grey alder may also occur locally after clear-felling, and was used in swidden agriculture in Finland (Mikola et al., 1983). Leguminous plants are rare in boreal forests, but may occur after fire; we are unaware of estimates of their N_2 -fixation under such conditions.

Rates of N_2 fixation in lichen symbioses are lower than in actinorhizal symbiosis; in a field study of a boreal pine forest Huss-Danell (1977) reported, based on acetylene reduction activity (ARA), a contribution by *Stereocaulon paschale* of $< 2 \text{ kg N ha}^{-1} \text{ year}^{-1}$. She also found that ARA in the lichen was very sensitive to dry conditions (Huss-Danell, 1977). With regard to N_2 -fixing bacteria more loosely associated with plants, most interest has recently been on cyanobacteria associated with mosses (e.g., Basilier, 1979; DeLuca et al., 2002). The contribution by the moss-associated N_2 fixation in boreal forest is estimated to $1\text{--}3 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (DeLuca et al., 2002; Lindo et al., 2013; Stuiver et al., 2015). Heterotrophic free living N_2 -fixing bacteria in soil are able to fix at a rate of $\sim 1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Nohrstedt, 1985).

Recent studies suggest endophytic bacteria may also contribute to tree N acquisition. Dinitrogen-fixing bacteria have been identified in tissues such as roots, needles and stems in some tree species (Moyes et al., 2016, and references therein), but the extent to which these endophytes contribute to the N nutrition of their host trees is unknown. In a ^{13}N labelling study it was shown that endophytes of *Pinus* needles fixed N at rates $< 0.14 \text{ g N ha}^{-1} \text{ day}^{-1}$ (Moyes et al., 2016), i.e. $28 \text{ g N ha}^{-1} \text{ yr}^{-1}$ if the active season is assumed to be 200 days, which suggest minor contributions to plant N supply. The genera of *Rhizobium* and *Bradyrhizobium* have been observed in association with ECM fungi in mycorrhizas as well as fungal sporocarps (Barbieri et al., 2005; Nguyen and Bruns, 2015), and were found in soils of natural N rich as well as N-fertilized boreal forests (Högberg et al., 2014a). Dinitrogen fixation (by bacteria) has also been detected in *Suillus tomentosus* tuberculate ECM, i.e. densely packed clusters of mycorrhizas (Paul et al., 2007). Again, it is not fully known to what extent these more recent findings of plant microbial associations enhance plant N supply.

The summed ecosystem N input and also output fluxes (Sponseller et al., 2016; Table 1) are throughout smaller than the internal cycling of N annually in boreal forests, with large losses in forest fires as exceptions. Gross N mineralization estimates which correct for microbially assimilated inorganic N, commonly show rates between 10 and $100 \text{ mg N m}^{-2} \text{ day}^{-1}$, although rates above $400 \text{ mg N m}^{-2} \text{ day}^{-1}$ have been observed at exceptionally productive sites (Högberg et al., 2006; Blaško et al., 2015; Table 2).

Table 1

Pools and fluxes of N in typical boreal forests (Sponseller et al. (2016), except for denitrification flux in which case data are from Klemetsson et al. (2005)).

Stocks of N	kg ha ⁻¹ of N	Internal turnover of N	kg ha ⁻¹ yr ⁻¹ of N
Trees	100–500	Plant uptake	15–50
Mor-layer soil	300–500	Litter-fall	5–25
Mineral soil	800–4000	Net mineralization	–5–15
Total	1200–5000	Net nitrification	–1–1
Inputs of N	kg ha ⁻¹ yr ⁻¹ of N	Outputs of N	kg ha ⁻¹ yr ⁻¹ of N
N deposition	1–3	Leaching	0.5–1.5
Biological N_2 -fixation	1–3	Denitrification	0.1–1

Table 2

Chemical and biological characteristics of mor-layer soil (F + H horizons) along the 90-m-long N supply gradient from a dwarf shrub type through a short herb type to a tall herb type near Betsse, N. Sweden (data are from Giesler et al., 1998[†], Nordin et al., 2001[†], Högberg et al., 2003[†], 2006[#], 2007a[§]). Microbial biomass was estimated by the FE (fumigation extraction) method. Percentage organic N is water extractable organic N out of the sum of organic and inorganic N. Data are means (1.0 SE). Seasonal means (N = 3–4) except for soil pH and C/N (n = 9), total C and N (n = 24), and CEC_e (n = 4–5).

Parameter	Dwarf shrub type	Short herb type	Tall herb type
pH _(H2O) [§]	4.0 (0.1)	4.6 (0.1)	5.3 (0.1)
C/N [§]	38.1 (2.4)	22.9 (1.1)	14.9 (0.3)
Total C (kg ha ⁻¹) [#]	12,754 (435)	12,679 (510)	26,515 (1695)
Total N (kg ha ⁻¹) [#]	312 (10)	529 (10)	1472 (94)
Organic N (%) [#]	68 (6)	44 (7)	16 (1)
NH ₄ ⁺ -N (salt extractable, kg ha ⁻¹) [#]	0.294 (0.035)	0.604 (0.134)	1.962 (0.250)
NO ₃ ⁻ -N (salt extractable, kg ha ⁻¹) [#]	0.132 (0.020)	0.274 (0.061)	1.122 (0.230)
CEC _e (mmolc kg ⁻¹ organic matter) [*]	316 (44)	657 (144)	831 (41)
Retention of ¹⁵ NH ₄ ⁺ (%) [#]	79.0 (6.2)	35.0 (2.5)	23.0 (1.8)
Gross N mineralization rate (kg ha ⁻¹ day ⁻¹) [#]	0.3 (0.1)	1.1 (0.3)	4.3 (1.1)
Net N mineralization rate (kg ha ⁻¹ 50 days ⁻¹)	0.0 (0.0)	0.1 (0.0)	0.6 (0.1)
Ratio fungi/bacteria [†]	0.44 (0.10)	0.18 (0.02)	0.02 (0.00)
Microbial C/N [†]	11.7 (2.0)	6.9 (1.6)	4.8 (1.3)
Microbial biomass C (kg ha ⁻¹) [†]	284.3 (57.1)	223.3 (79.9)	494.3 (156.7)
Microbial biomass N (kg ha ⁻¹) [†]	24.3 (3.1)	31.7 (5.9)	102.3 (5.0)
Microbial C out of total soil C (%) [†]	1.42 (0.06)	1.45 (0.29)	1.45 (0.18)
Microbial N out of total soil N (%) [†]	7.6 (1.0)	6.9 (1.3)	7.3 (0.4)

Assuming 200 days of effective N_2 -fixation and N mineralization per year, the internal N cycling may reach 20–200 kg N ha⁻¹ year⁻¹, which is one-two orders of magnitude greater rates than all input fluxes of N combined. Similarly, such rates of N turnover, although they do not include the turnover of organic N compounds like amino acids, by far outcompete the N output fluxes (hydrological and gaseous pathways). Runoff of N is usually < 2 kg N ha⁻¹ year⁻¹ (Kortelainen et al., 1997, 2006; Schelker et al., 2016) and consist to 70–90% of organic N species (Stepanauskas et al., 2000; Sponseller et al., 2014). As regards denitrification, Klemmedtsson et al. (2005) estimated losses to be of the order 0.05–0.8 kg N ha⁻¹ year⁻¹, i.e. comparatively small.

In conclusion, the internal turnover of N is much larger than the inputs and outputs. In fact, in most boreal forests the N cycle is relatively closed, and therefore constrained by rates and feedbacks within the local plant-soil ecosystem.

3.2. How important are plant litter characteristics?

Evidently plant species produce foliage and litter of highly variable food qualities for grazers and decomposing organisms; for which qualities differ between plant species and within species depending on site conditions (Berg and McClaugherty, 2008). The large site variations in soil N availability in boreal forests are associated with considerable variations in litter composition. But are these differences in litter quality indicators of variations in soil edaphic conditions rather than drivers of the site variability themselves?

Decomposition studies using litter bags show that N-rich litters decompose faster initially (typically for the first 0–2 years), but then slower than N-poor litters (Berg and McClaugherty, 2008). Litter bag studies can cover up to around 5 years, after which the litter is so fragmented that loss through the mesh of the bags and ingrowth of mosses and lichens limit their use (Berg and McClaugherty, 2008). Such studies allow observations of processes in the litter physically above the mycorrhizosphere, but not in this zone where N availability to plants is expressed (see Section 3.5). Bomb ¹⁴C dating of the horizons of the mor-layer in *Pinus sylvestris* forest showed 5 ± 0.4 (mean ± 1.0 SE) years for the uppermost layer, the S-layer, 14 ± 1.0 years for the F-layer, where mycorrhizal tree roots and their fungi first appear, and 42 ± 0.5 years for the H-layer, the lowest horizon of the organic mor-layer (Franklin et al., 2003, see also Lindahl et al., 2007 and Section 3.5.). So how relevant are observations of the initial stages of litter decomposition to our discussions about the nature of the N limitation in boreal forests?

If we accept the emerging view that virtually all soil organic matter can be degraded (Schmidt et al., 2011; Gleixner, 2013; Lehmann and Kleber, 2015), it seems logical to assume that also most of the N may ultimately be released, and it matters less if the annual release can be attributed to a slow or a fast process from several or fewer cohorts (age-classes) of litter, respectively. Conversely, if the litter quality is crucially important in this context, the system may not remain in steady-state provided the species composition and the quality of litter is changing.

We would like to stress the fact that in N-limited boreal forests, the microbial biomass in the F + H-layer contains around 6–9% of total N found in these layers and have a C/N ratio of around 8–12 as compared to around 34–41 for the OM (Table 2; Martikainen and Palojärvi, 1990; Giesler et al., 1998; Näsholm et al., 1998; Högberg et al., 2003; Blaško et al., 2015; see also Bauhus and Khanna (1999) for data from other regions). Thus, the microbial biomass itself may be an important sink for N released in bioavailable forms. In fact, the constancy of the ratio microbial N/total N across a wide range of soil C/N ratios (Table 2) suggests that it is the size and the turnover of the microbial N pool in the F + H hori-

zon that is the major bottleneck in terms of the N flow between soils and plants, rather than variations in initial C/N of the litter. According to Table 2, the microbial N pool in the N-rich tall herb, TH, type is 4 times larger than in the N-poor dwarf shrub, DS, forest type, while the rate of gross N mineralization rate is 14 times higher, despite the nearly constant ratio of microbial N over total N. Note also that there is no net N mineralization in the DS type.

Hence, variations in litter C/N ratio (e.g., Berg and McClaugherty, 2008) or in potential influences of plant secondary compounds (e.g., Smolander et al., 2012; Adamczyk et al., 2016), do not appear to greatly influence the relative size of the total soil N pool that is microbial N in the horizon where litter-derived N first becomes a substrate for mycorrhizal plant roots. This, and the facts that litter decomposition studies have not been able to trace the N in litter in detail from litter-fall to plant N uptake and that the effect of % N of litter on decomposition rate shifts over time (Berg and McClaugherty, 2008) have convinced us to leave out from this review the very rich literature on plant litter decomposition, and focus directly on processes in the vicinity of plant roots. Before we do so, we must stress that the often strong correlations among the C/N ratios of fresh plant litter, presence of plant secondary compounds, and the availability of N do not necessarily imply that the N supply to plant roots is proximally controlled by these factors. An alternative view, which we will explore, is that soil N turnover in the root zone is physiological and controlled by the relations between the relative rates of C and N supplied in available forms to the soil organisms.

In systems rich in N, e.g., the TH forest vegetation type at Betsele (Table 2), the C/N ratio of microorganisms in the F + H layer is around 5, which indicates a total dominance of bacteria over fungi. There is also a much lower N sink strength (see data on immediate retention of ¹⁵NH₄⁺ in Table 2) and a faster turnover of N than in the poorer forest types (see data on net and gross N mineralization in Table 2). Microorganisms in boreal forest soils, especially below the S-horizon, are likely C-limited (Ekblad and Nordgren, 2002; Demoling et al., 2008, see also Nazir et al. (2010) for a general discussion on the rhizosphere) and this limitation should be strongest in the TH forest types as indicated by their microbial C/N ratios (Table 2). Under such conditions, the rate of decomposition of litter and associated release of N may well limit the rate of N cycling in the soil-plant system. In contrast, in systems with a higher ratio of fungi/bacteria, a much higher C/N ratio and slower turnover of microorganisms, the microbial N retention is much higher (Table 2).

Thus, we propose that the rate of litter decomposition may not always limit the rate of N-cycling and plant N uptake in boreal ecosystems. It likely does in the richer systems, where microbial immobilization in the root zone appears much less important (Table 2), but in the more N-limited systems recent findings suggest that the ECM fungi play a crucial role by sequestering much available N (Näsholm et al., 2013; Franklin et al., 2014; Hasselquist et al., 2016), which they do not transfer to their tree hosts; this hypothesis will be elaborated more in detail in Section 5.

3.3. How is N released in available forms and which forms of N are used by microbes and plants?

Ever since Sprengel formulated his mineral theory (Sprengel, 1826; von Liebig, 1840, 1855), plants have been considered the critical interphase between the inorganic and organic realms. This view prevailed, and still prevails in some quarters in spite of experimental evidence that plants may utilize organic N (Hutchinson and Miller, 1912; Brigham, 1917; Paungfoo-Lonhienne et al., 2012). Strong correlations between plant productivity and the occurrence of inorganic N, in particular NO₃⁻, as well as rapid

growth responses of plants following supply of inorganic N formed a logical underpinning to this classic view. Occurrence of organic N in soil solution (e.g. Ivarson and Sowden, 1969) was not considered relevant for plant N nutrition, since it was assumed that microbes would always be superior competitors for N because their much larger surface to area ratio would effectively hinder any organic N from reaching plant roots (Schimel and Bennett, 2004; Kuzyakov and Xu, 2013).

Recent studies have shown that N can be taken up by plants in a variety of forms, including peptides, amino acids, NH_4^+ and NO_3^- (Näsholm et al., 2009). There is even evidence suggesting non-mycorrhizal plant roots may acquire N through uptake and digestion of microbes (Paungfoo-Lonhienne et al., 2010). This wide-ranging capacity of plants, with different types of mycorrhizas, suggest plant N nutrition to be constrained by access to N substrates, rather than by inherent plant competences to take up different forms of N.

The release of available forms of N can occur by a number of processes. Many organisms, notably microorganisms, exude exoenzymes with the capacity to cleave polymers like proteins and peptides (Schimel and Bennett, 2004), but also plants exude proteases enabling for them to use complex N sources (Adamczyk et al., 2009). Release of N also occurs when microorganisms and plant roots die, which is followed by lysis, or when they experience a decreasing supply of C. Phage attacks have been known to drive turnover of microbial biomass in aquatic systems, but the ecological function of the highly abundant and diverse soil viruses is mainly unknown (Fierer et al., 2007; Srinivasiah et al., 2008). Co-evolution of soil bacteria and soil viruses suggests bacteria-phage interactions may also be pertinent to N turnover in soils (Gómez and Buckling, 2011). Grazing on bacteria by protozoa (Clarholm, 1985), and on fungi by *Collembola* (Högberg et al., 2010) and *Acari* (Remén et al., 2008) accelerates the turnover of microbial N as it is followed by excretion of NH_3 by the soil animals.

The view that plant N nutrition also includes organic N sources rests on three fundamental observations: (i) all plants studied until now have been shown capable of absorbing and utilizing simple organic N forms such as amino acids, (ii) transporters mediating amino acid uptake have been identified both in mycorrhizal fungi and in plants, and expression of these transporters has been shown to respond to the presence of amino acids in the root media (see below), and (iii) dissolved organic N often constitutes a significant share of soil solution and runoff (Sponseller et al., 2014). Thus, on the one hand we have firm evidence indicating both the occurrence of the organic N substrates (e.g., amino acids) and the active and regulated uptake of them by plants from the soil. On the other hand, strong criticism against a significant role of organic N for plant N nutrition has been presented, arguing that plants are inferior competitors to soil microbes for organic N (Kuzyakov and Xu, 2013). The ECM fungi are particularly interesting in this context, given that they ensheath the vast majority of tree fine root tips, and although they are microorganisms they are conventionally ascribed the role of extensions of the tree root systems. We will elaborate on this notion below (3.5).

Studies using dual labelled organic N compounds were believed to offer a possibility to assess plant organic N uptake in the field (Schimel and Chapin, 1996; Näsholm et al., 1998, 2001). This approach was later criticized for being non-conclusive and for favouring plants rather than microbes by adding unrealistically high concentrations of labelled substrates (Jones et al., 2005; Rasmussen et al., 2010). However, although the initial studies encompassing use of dual labelled tracers relied solely on bulk-analyses of stable isotopes in plants by Isotope Ratio Mass Spectrometry, later studies that also used Gas Chromatography Mass Spectrometry provided proof for plant uptake of intact organic N

from soil (Näsholm et al., 2001; Öhlund and Näsholm, 2001; Nordin et al., 2004).

Organic N nutrition of boreal forest plants is, based on the results discussed above, a reality. The quantitative importance of organic N for plants in this, or in any ecosystem is, however, still unknown. New insights into this field may come from studies of the genetic underpinnings of plant N uptake and assimilation (Näsholm et al., 2009).

3.4. Methodological limitations and developments

Transporters mediating root uptake of various forms of N, including both NH_4^+ , NO_3^- , amino acids and peptides, are present in plants (for overview see Näsholm et al., 2009; Nacry et al., 2013). Not surprisingly, therefore, plants have been shown capable of absorbing and utilizing a wide range of N compounds. This potentially ubiquitous capacity of plants to acquire both organic and inorganic N sources suggest soil supply of N to be an important determinant of both the amounts and the chemical composition of plant N sources. To this end, recent developments of techniques to monitor soil N fluxes points to a much larger importance of organic N than previously recognized (Inselsbacher and Näsholm, 2012).

As discussed above, soils are dynamic systems, exhibiting rapid turnover rates of many compounds, not least N compounds. The same is true for the supply of plant photosynthate C, which fuels soil organisms with energy (Högberg and Read, 2006), which affects their N metabolism. Such rapid conversions are also characteristic, and maybe more intuitively recognized for endogenous compounds of an animal. The classical observer effect, i.e. the fact that any attempt to study a system also is at risk of affecting the system, was early documented within the field of neuroscience. Hence, methods enabling low-invasive monitoring of neurotransmitters were developed, and a method later termed microdialysis was presented (Delgado et al., 1972). The method relies on a process termed induced diffusion and may best be explained as a form of reverse dialysis. The standard dialysis setup aims at removing substances from a solution while in the microdialysis procedure, the aim is to capture small molecules into a stream of pure water. The small size of the probe (a standard setup uses a probe with a $0.5 \text{ mm} \times 10 \text{ mm}$ (diameter \times length) membrane), means disturbance of the study system can be kept to a minimum. Recovering N compounds through induced diffusion will also likely give more relevant information on soil N availability compared to measurements of soil solution concentrations. This is because flux rates rather than concentrations determine plant N capture. A full account of the use of microdialysis to study soil N dynamics is given by Inselsbacher et al. (2011).

Several investigations using the microdialysis technique to study soil N fluxes in boreal forests have recently been published (Inselsbacher and Näsholm, 2012; Inselsbacher et al., 2014; Oyewole et al., 2016). These studies collectively show that compared to soil extraction with either H_2O or a salt solution, microdialysis sampling yields much lower fractions of inorganic N, in particular NH_4^+ , and substantially larger fractions of amino acids. Thus, while NH_4^+ strongly dominated in conventional extracts, in N poor soils accounting for 80 % of the pool of inorganic N and amino acids, amino acids dominated the diffusive fluxes captured with microdialysis, accounting for 80 % of the N flux (e.g. Inselsbacher and Näsholm, 2012). A method comparison suggests this discrepancy results from breakdown of organic N and/or release of NH_4^+ after soil sampling and during the subsequent sieving and extraction procedures (Inselsbacher, 2014).

Sampling, sieving and storage of soil samples also affects the supplies of C and N from organic substrates to soil microorganisms, and in particular the supply of photosynthate from plants to their mycorrhizal fungi and other organisms in the mycorrhizosphere

(Högberg et al., 2003; Högberg, 2004; Högberg and Read, 2006). Severing of fungal mycelium disrupts the cytoplasmic transport and may cause death and lysis of cells. The termination of the supply of C to ECM fungi weakens their strength as sinks for nutrients like N. Thus, there is a need to develop non-intrusive methods for studies of microbial physiology *in situ*. This insight has stimulated us to make larger-scale stable isotope labelling directly in the field (e.g., Högberg et al., 2010; Näsholm et al., 2013).

The modern approaches as well as the now more traditional all have their inherent weaknesses. Progress has undoubtedly been made, but one should always be cautious and regard any numbers derived by these methods as proxies of actual quantities or process rates. We find that the greatest values of these numbers are found when they are used for comparison, such as in studies of vastly different systems like the forest types at Betsale (Table 2).

3.5. Where in the soil profile is N supplied to trees and other plants?

We would like to repeat that the root zone and its extension, the mycorrhizosphere including the extramatrical mycorrhizal hyphae with its associated microbes, is the focal site of expressions of the balance between the supply of N and plant N uptake. In boreal forests mycorrhizal plant roots are not present in the uppermost part, the S-layer of the organic mor-layer (where most of the litter decomposition takes place), but become abundant first in the lower part of the mor, the F- and H-layers. Mor-layers are described as consisting of a superficial (S) layer of mosses, or lichens and plant litter, followed by the F-horizon (a Swedish word for decomposition was historically incorrectly translated to fermentation, hence F) in which plant remains can still be identified as such, and finally a “humus” (H) horizon in which the organic material is amorphous (Romell and Heiberg, 1931). Please observe that the internationally more common description of the uppermost layer as a litter layer (L-layer) is rarely appropriate in the open boreal forests on infertile soils, which commonly allow considerable penetration of light through tree canopies and thus a vital and continuous cover of mosses or lichens on the ground. These grow through and become mixed with above-ground litter, which complicates studies of decomposition using the litter-bag method.

An observer gently removing the S-layer with its mosses or lichens and litter will observe the underlying F-horizon with an abundance of roots and dense fungal mycelial networks. Recent molecular studies have shown that saprotrophic fungi dominate in the litter in the S-horizon, which seems to lack ECM fungi (Lindahl et al., 2007; Clemmensen et al., 2013). The ECM fungi attain an almost total dominance in the F + H-horizons and further downwards (Lindahl et al., 2007; Clemmensen et al., 2013). We propose that this shift in dominance is associated with a diminishing availability of C in easily degradable forms to saprotrophic fungi as the litter they decompose is degraded and moved down the profile when new litter is added on the top, and as mosses and lichens grow upwards through the fragmented litter. Furthermore, while saprotrophs experience a diminishing supply of easily degradable C-compounds, they become poorer competitors for N in relation to the ECM fungi, which receive sugars, high-quality C substrates, directly from their tree hosts. The appearance of prolific growth of fungal mycelia in the F-horizon occurs in the context of the shift in dominance from saprotrophs to ECM fungi observed in a study of decomposing needle litter (Lindahl et al., 2007).

Here, we would like to make an analogue with the competition for phosphorus between saprotrophic fungi and ECM fungi as studied in laboratory mesocosms by Lindahl et al. (2001). In this, and similar studies, ECM fungi receiving photosynthate from pine seedlings were competing with saprotrophs supplied by C from wood blocks for P supplied as radioactive ^{32}P to either the saprotroph

or the ECM fungus when they came in close contact. The subsequent movement of the tracer towards the mycorrhizal mycelium and its symbiotic plant or to the saprotroph was monitored by autoradiography. By manipulating the C supply rate to the saprotroph by varying the size of the wood block Lindahl et al. could demonstrate that the saprotroph became a stronger competitor for P when its C supply was larger.

Competition for N should follow the same principle, i.e. the sink with the greatest C supply should be the stronger sink. However, this competition will be more difficult to study, because radioactive N isotopes are extremely short-lived (most long-lived is ^{13}N with a half-life of c. 10 min). Such studies would need to be based on the stable isotope ^{15}N and destructive sampling.

Competition for N between microbes and plants is a classic theme in soil science with agronomic perspective, but also in soil ecology in general (e.g., Kaye and Hart, 1997). The role of mycorrhizal fungi in this context is particularly difficult to establish. Are they mere extensions of the plant root systems or is their origin as saprotrophic microbes still expressed to some extent? A recent analysis (Lindahl and Tunlid, 2015) concluded that while ECM fungi evolved from saprotrophs their saprotrophic capacities are in general not much expressed. Some ECM species can produce important exo-enzymes, and hence oxidize organic matter by so called ‘brown-rot’ Fenton chemistry or through the action of ‘white-rot’ peroxidases. Lindahl and Tunlid (2015) suggested that ECM fungi thus improved their access to N from organic matter, but that the associated release of C compounds would likely be less as important C sources for these fungi, but more important to saprotrophs.

The distribution of the stable isotopes of N (here expressed as per mil deviations, $\delta^{15}\text{N}$, from natural abundance of ^{15}N in relation to that in the standard, atmospheric N_2) in soil profiles provide interesting evidence about N processes and spatial separation of the processes without any disturbance of the studied system (Högberg et al., 1996; Högberg, 1997; Lindahl et al., 2007; Hobbie and Högberg, 2012). Typically, in boreal forests the tree foliage is depleted in ^{15}N relative to the deeper soil horizons. This difference, ϵ , is often up to around 10‰ (e.g., Högberg et al., 1996; Sah et al., 2006; Blaško et al., 2015) and is created by N isotope fractionation as N is taken up by ECM fungi which become enriched in ^{15}N , but pass isotopically lighter N to their host plants (Högberg et al., 1999; Hobbie and Högberg, 2012). It should be stressed that the change in isotopic composition with increasing soil depth reflects a change in the isotopic mass balance of soil N. The difference ϵ is influenced by the extent to which ECM fungi are active in tree N uptake; if the role of ECM is diminished, e.g., by decreasing the C supply to the roots and ECM fungi by high N additions (see further below), ϵ decreases, but can increase again if the N load is terminated and the functional role of ECM fungi in N-limited forest is restored (Högberg et al., 2011).

Interestingly, when Lindahl et al. (2007) studied fungi on degrading pine litter, they observed no change in the $\delta^{15}\text{N}$ of the litter as long as it was colonized by saprotrophic fungi only, i.e. during the first 3 years, which shows there was no change in the isotopic mass balance. A rapid increase in $\delta^{15}\text{N}$ started after 10 years when the ECM fungi had become dominant. The high enrichment in ^{15}N of deeper soil horizons thus suggests that N in ECM fungi may be an important precursor for more slowly turning over soil N pools (Högberg et al., 1996; Clemmensen et al., 2013).

In line with the revised view of so called recalcitrance (Schmidt et al., 2011), the observed ^{15}N enrichment might be reinforced by recycling of ECM N by ECM fungal mycelium and associated microorganisms (Gleixner, 2013). If saprotrophic fungi were the proximal cause of the change in $\delta^{15}\text{N}$, by supplying ^{15}N -depleted N to the trees, and leaving ^{15}N -enriched N in the soil, the very high $\delta^{15}\text{N}$ of ECM fungi in relation to both plant and saprotroph N

(Taylor et al., 2003) would remain a mystery. Clearly, the isotopic and molecular evidence show that decomposition by saprotrophic fungi and uptake of N by ECM fungi are spatially separated (Lindahl et al., 2007).

In summary, multiple lines of evidence imply that the main proximal source of soil N for trees in the boreal forest is N transferred through ECM fungi, which extract inorganic and organic N forms from organic horizons below the litter. At the same time ECM fungi also contribute to N immobilization and subsequently, buildup of more slowly turning over soil N. We will return to the role of ECM fungi in Sections 4.2, 5.1 and 5.2.

4. Variations in N supply in boreal landscapes

4.1. Causes of variations in plant N supply in relation to hill-slope hydrology: abiotic perspectives.

Statistical analysis by Hägglund and Lundmark (1977) of field survey data from the National Forest Inventory of Sweden confirmed the common observation that forest growth correlates with hill-slope position with greatest growth found in toe-slope positions (Holmgren, 1994), as indicated by a lush and diverse field-layer dominated by tall herbs (see also Giesler et al., 1998; Zinko et al., 2005). Hägglund and Lundmark did not attempt to test if this correlation had anything to do with variations in N supply, although the common N limitation to tree growth was well established. However, there are several reasons why N supply should increase down slopes (Tamm, 1991; Giesler et al., 1998; Högberg, 2001). Some of these are of an abiotic nature (others which are biotic to their nature are considered in Sections 4.2 and 4.3):

- Nitrogen occurs in many soluble forms, which are components of the water transport downhill slopes. The loss of N through leaching from GRAs is usually small, but this flux of N has the potential to become concentrated in GDAs. Rodhe (1987) estimated that such areas, which are typically found in toe-slope positions, comprise ca. 10% of the area in Swedish forests. Thus, there could in theory be a tenfold higher N-flux in such areas, provided no soluble N is lost through abiotic or biotic immobilization or denitrification along the path from GRAs to GDAs (Högberg, 2001). Data for boreal forests from the Swedish Forest Soil Survey confirm the strong link between N and base cations, which is expected as they are components of the same water flux; in Fig. 2 we show data on C/N ratios vs. BS (we choose C/N rather than N % because the former is insensitive to mixing with the mineral soil, which influences the latter, and may occur as an effect of forest operations or as a result of variations in the delineation between the mor and the mineral soil among soil surveyors).
- Nitrogen losses through fires should be larger uphill because fires usually climb uphill and less likely start under the moister conditions downhill. Hence, the potential total amounts of N that can become available should be higher downhill (see e.g., data on the forest types in Table 2). Furthermore, nitrogen lost in runoff after fires in GRAs is exported to GDAs. This, and the previous mechanism represent external inputs to GDAs. Other mechanisms described below and in coming paragraphs describe mechanisms of greater internal turnover of N.
- Microbial turnover of N is affected by soil moisture. Unless the soil is saturated and anoxic conditions prevail, the effect of soil moisture should be positive, which means that N turnover should be greater in toe-slope positions.
- Nitrogen supply to plant roots is likewise affected by moisture. Traditionally, this has been considered only as the product of the amount of water moving towards the root surfaces as mass flow (driven by plant transpiration) times its concentrations of

N in available forms. However, mass flow of water may also affect the concentration gradient from the root (or hyphal) surface out in the soil, thereby enhancing diffusion-mediated N supply.

Recently, the above-mentioned microdialysis technique was further developed to allow for simultaneous measurements of diffusion and mass flow of N compounds in soils (Oyewole et al., 2014). In this study, mass flow was shown to significantly enhance total N fluxes in soil, but the increase in N flux was only to a limited extent a result of the product of water flux and soil solution concentration. In fact, mass flow enhanced diffusion of N was the main cause of the increase in soil solution N flux. Hence, this study points to a greater importance of transpiration driven mass flow in tree N nutrition than previously recognized. The possibility of a mass flow enhancement of diffusion was discussed by Nye and Marriott (1969), and thus results from microdialysis sampling in the absence and presence of mass flow corroborate their predictions. The magnitude of enhancement of N fluxes due to mass flow under field conditions is not yet known. An enhancement should amplify the variability in site fertility.

We assume that water supply *per se* is not limiting forest growth. First and foremost, the very few experiments examining the effects of water additions to Fennoscandian boreal forests show small, if any response, and second, an effect of water on tree growth could also be because water increases soil microbial activity and mass flow of nutrients towards roots and mycorrhizal hyphae, as discussed above. Watering a boreal spruce forest at Flakaliden through the summers over a period of 10 years did not result in greater stem growth, nor did trees on plots sprinkled with a nutrient solution grow better than on plots receiving solid fertilizers (Bergh et al., 1999).

Tamm et al. (1999) claimed evidence of water limitation in an experiment in pine forest at Norrliden, in which water was sprinkled in response to dry conditions. However, based on the plot data published by Aronsson et al. (1999) it is not clear that the positive effect of around 14% on stem growth reported is really significant. Further, there is no evidence based on foliar or soil organic matter $\delta^{13}\text{C}$ that trees in GRAs are more water-stressed; along the Betsele gradient $\delta^{13}\text{C}$ increases towards the wetter GDA, which actually contradicts this notion (Högberg et al., 2005). The higher $\delta^{13}\text{C}$ can in this context possibly be linked to a higher rate of photosynthesis in tree foliage with a higher N concentration, and in which variations between wet and dry years can be larger (Betson et al., 2007). In a long-term N-fertilization trial, between-year variation in aboveground growth was to a significant extent explained by differences in precipitation during the growing season in fertilized, but not in control plots (Lim et al., 2015). These observations point at a potential interaction between N and water availabilities as determinants of forest production.

We should also ask if low temperature is a major cause of the low N supply in boreal forests. Of course, this is difficult to test. However, the large variations in N supply and plant growth along the Betsele gradient from a GRA to a GDA (Table 2) are not associated with any differences in soil temperature (Högberg, 2004). Higher turnover of soil organic matter and thus of N is expected in slopes with an aspect to the south as compared to the north (in the northern hemisphere), but we are not aware that any substantial differences in release of available N have been reported from Fennoscandian boreal forests. In the spring, the air rapidly warms up to temperatures that would make photosynthesis by conifers possible, but the frozen soils restricts the availability of water (Bergh et al., 2003). Thus, earlier warming of the soil in recent years may explain why boreal trees start photosynthesis earlier and earlier (see Section 6.4).

A direct soil warming experiment showed a doubling of tree growth after 6 years (Strömberg and Linder, 2002), which occurred along with a 50% increase in tree N uptake (Strömberg, 2001). We do not know if such a large response will be sustained; it may partly be a transient result of accelerated turnover of a limited pool of labile N. Moreover, large increases, e.g. up to fourfold increases in tree stem growth have been observed in boreal forests after fertilization (Bergh et al., 1999), i.e. after a treatment which may even slightly decrease soil temperature as a result of increased foliar biomass and shading of the soil. Thus, it does not appear that the cold soil temperature *per se* is the major restriction, but the N supply rate, which is affected by temperature.

4.2. How important are soil pH and N supply for microbial release of N, and which is the role of mycorrhizal fungi (biotic perspectives)?

A classical paradigm about soil microorganisms states that soil fungi and bacteria are favored at low pH and high pH, respectively (e.g., Alexander, 1984). This is corroborated by observations in boreal forests (e.g., Yarwood et al., 2009; Högberg et al., 2014a; see also Table 2), as well as at larger continental scales (Fierer and Jackson, 2006; Lauber et al., 2009). So how large are the variations in soil pH and what is the evidence that they are decisive for microbial community composition and the release of N?

Mor-layers in boreal forests typically have a pH of ca. 3.8–4.2, but mor-layers in GDAs are commonly much less acid (Giesler et al., 1998, 2002). This is because of transport through deeper and more neutral mineral soils or bedrock before the discharge of the water at the soil surface. An example of the resulting variations in pH is given in Table 2. We have stressed that N and base cations are components of the same flow of water down-hill slopes. But is it also possible that the supply of Ca is directly influencing the supply of N, i.e., can additions of Ca (liming) increase the supply of N and hence tree growth on the low-N low-Ca soils found in a majority of boreal forests of the dwarf shrub forest type (Figs. 1 and 2). The answer appears to be no; most liming experiments across Fennoscandia commonly show no or negative effects on forest growth (Binkley and Högberg, 1997, 2016; Nilsen, 2001; Nohrstedt, 2001; Saarsalmi and Mälikönen, 2001). Additions of wood ash are frequently also negative for tree growth (Jacobsson et al., 2014) although exceptions may occur (Saarsalmi et al., 2004, 2010, 2012). Wood-ash contributes bases, but also a mix of other nutrients. Liming may stimulate nitrification on more N-rich soils (Kreutzer, 1995), but the evidence from typical N-poor acid mor-layers from boreal and similar forests is that N mineralization is not enhanced (Persson et al., 1990; Smolander et al., 1995, 1998).

Thus, additions of lime to N-limited forests do not appear to enhance tree growth on N-poor soils as do additions of N, despite the strong positive correlations among forest growth and % N on the one hand and base saturation on the other hand in boreal forest mor-layers (Fig. 3). Nor do experiments with additions of acids or acidifying high N additions cause a decline in forest growth (Tamm, 1989; Binkley and Högberg, 1997) as predicted in some quarters during the forest decline debate in the early 1990s (e.g., Sverdrup et al., 1993).

The effects of soil N on microbial communities have received considerable less attention. The strong concomitant shifts in pH and N in natural boreal forest ecosystems indicate that other factors than pH *per se* may determine the large variations in fungal/bacterial contributions and microbial species composition (Högberg et al., 2003, 2007a). In fact, inorganic N (\log_{10} for the sum of NH_4^+ and NO_3^-) is strongly positively correlated with bacterial PLFA biomarkers but negatively correlated with fungal biomarkers (Högberg et al., 2007a).

Indications of causes to variations in microbial community composition other than direct effects of soil pH and N supply came

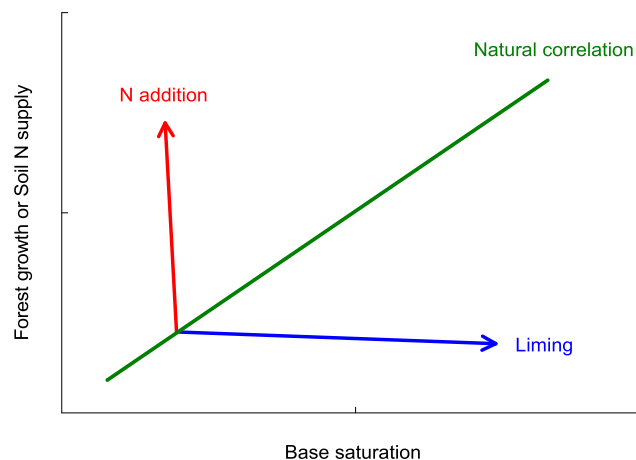


Fig. 3. Effects of additions of N or lime (or wood ash) to N-limited boreal forests (Högberg, 2001 and references in Section 4.2). Effects of N additions on tree growth on richer soil soils, i.e. soils with a higher natural N supply and base saturation, are much less dramatic (e.g., Sikström et al., 1998). The natural correlations among soil N % and forest growth on the y-axis and base saturation on the x-axis is shown in green (cf. Fig. 1).

from a large scale tree girdling experiment in N-poor pine forest, in which tree below-ground C allocation (TBCA) and hence the transport of C to the ectomycorrhizosphere, was terminated (Högberg et al., 2001), and from long-term N addition experiments also known to decrease tree C allocation to mycorrhiza (TCAM). Bacterial community composition correlated with soil pH in natural forest ecosystems, but where soil pH was virtually unaffected in a long-term N addition experiment, nitrogen supply affected composition (Högberg et al., 2014a). After tree girdling, which terminates TCAM, *acidobacteria* and *alphaproteobacteria* decreased (Yarwood et al., 2009). This was unlikely to be caused by the increase in soil pH by 0.2 pH units, but more likely by the reduction in production of low molecular weight organic acids substrates driven by the TCAM (Giesler et al., 2007). Saprotrophic fungi increased significantly after tree girdling (Yarwood et al., 2009), but under undisturbed conditions fungal composition was largely determined by the abundance of ECM fungi; these were, as expected, eliminated after tree girdling (Högberg et al., 2001; Högberg and Högberg, 2002; Yarwood et al., 2009) and declined drastically under higher N concentrations along a natural N supply gradient (Table 2) and in response to N fertilization at Norrleden (Högberg et al., 2007a, 2014a).

There is good evidence that, in forest ecosystems TBCA (of which TCAM is a part), expressed as a fraction of NPP (relative TBCA; TBCA_R), decreases upon N fertilization and is higher on poor compared to on rich sites (Giardina et al., 2003; Litton et al., 2007; Mäkelä et al., 2008, 2016). Obviously, a decrease in TBCA_R , can be caused by a decrease in belowground C allocation, an increase in aboveground C allocation or both. In the context of this review, and in particular to explore the interaction between C allocation and N cycling of forest ecosystems, distinguishing between a relative change in belowground allocation caused by an increase in aboveground allocation and an absolute change in belowground allocation is important. Unfortunately, direct monitoring of belowground C allocation is difficult, especially for forest ecosystems and over extended periods of time.

Olsson et al. (2005) measured soil respiration in combination with interruption of phloem transport (girdling) of *Picea abies* trees and found that the fraction of respired C accounted for by belowground C transport is significantly smaller in fertilized compared to non-fertilized trees. Several studies have also shown that fertilization and N-deposition decreases growth of fine roots (Ahlström

et al., 1988), mycorrhiza, and mycorrhizal extramatrical mycelia, i.e. mycelia growing outside the roots (Haynes and Gower, 1995; Wallenda and Kottke, 1998). Collectively, these studies suggest fertilization reduces TBCA. Some studies, however, suggest fertilization promotes a shift in C allocation between fine roots and coarse roots rather than, or in addition to, a shift in TBCA. Thus, Linder and Axelsson (see Cannell, 1989) showed that, in a long-term fertilization trial of *Pinus sylvestris* forest, a decrease in growth of fine roots (< 2 mm) occurred concomitantly with an increase in growth of coarse roots (> 2 mm). As and additional example, Majdi (2001) investigated *Picea abies* root growth at the Flakaliden nutrient optimization trial and found that fertilization increased both fine- and coarse root production, while the fraction of mycorrhizal root tips decreased. Helmisaari et al. (2007) studied *Picea abies* and *Pinus sylvestris* forests from south to north Finland, and found that both fine root biomass and the C/N ratio of the mor-layer increased towards the nutrient-poorer north. Moreover, the ratio of foliage biomass over fine root biomass decreased in the same direction.

We may thus speculate that a decreased allocation of C to fine roots and mycorrhiza as well as to the extramatrical mycelium of ectomycorrhizal fungi in response to N additions may result either from a decreased belowground allocation of C or from an altered allocation of C between different root classes. To that end, detailed studies of the non-mycorrhizal plant *Arabidopsis thaliana* have identified two pathways of regulation of initiation of lateral roots. On one hand, direct contact between a root meristem and an enriched source of nitrate leads to initiation of root branching. On the other hand, the early growth of the lateral root is sensitive to, and inhibited by, nitrate absorbed by the plant (Zhang and Forde, 2000). This systemic response leads to a lowered branching of the root system. Assuming the same, or a similar mechanism is active in forest trees, N additions to boreal forests would be expected to lead to decreased root branching and hence also to lowered abundance of mycorrhizal fungi and C allocated belowground preferentially used for growth and maintenance of coarse, rather than fine roots. In this scenario, the end result, i.e. a lowered abundance of mycorrhizal root tips and a decreased biomass of the extramatrical mycelia of ectomycorrhizal fungi, is the same, but the result of an altered allocation of C between different root classes rather than an altered allocation of C between above and belowground structures of trees. As discussed above, N additions often results in a lowered soil autotrophic respiration. A constant belowground allocation, but a decreased soil autotrophic respiration, requires that the ratio of maintenance respiration to growth (construction) respiration of the root system is shifted (Ryan et al., 1996; Jia et al., 2011), i.e. that the C-use efficiency of belowground production increases as a result of N addition.

Whether N addition results in lowered TBCA or an altered distribution of C between different classes of roots (or both), the consequence is a lowered allocation to fine roots and mycorrhiza. We argue that focusing on the TCAM rather than on TBCA is likely of greater functional relevance for understanding C-N interactions in boreal forests.

Noteworthy is that along the Betsele gradient the highest number of ECM fungal species were found under intermediate N supply, i.e., in the short herb forest type, possibly indicating that the N poor dwarf shrub forest type was too limited by N to allow a high diversity of ECM fungi (Högberg et al., 2014a). Assuming that a higher TCAM could support a wider range of ECM fungal life styles or genotypes, this observation of higher richness may corroborate a model prediction of maximum TCAM at intermediate and not low N supply (Franklin et al., 2014). Taken together, these observations heighten the potential importance of the TCAM in shaping the microbial community structure and potential interactions between

plant N supply and TCAM (Högberg et al., 2003, 2007a, 2007b, 2010, 2014a; Näsholm et al., 2013; Franklin et al., 2014).

How large can changes in TBCA be? Tree below-ground C allocation is difficult to measure directly. We have girdled all trees on larger plots to partition the directly TBCA-driven “autotrophic” soil respiratory component from heterotrophic decomposer activity. Such studies have suggested that the autotrophic component accounts for 50–65% of the total soil respiratory activity during the vegetation period in boreal forests (Högberg et al., 2001, 2009; Olsson et al., 2005). These data are from N-limited forests; in case of heavily N-fertilized plots, the autotrophic component, as identified by girdling of the trees, was reduced to c. 40% of total soil respiratory activity (Olsson et al., 2005).

Along the Betsele gradient, where forest production increases 300%, total soil respiratory activity increases around 30% only (Högberg et al., 2003). This small increase is associated with a dramatic decrease in the ratio fungi/bacteria indicating a considerable decline in ECM fungal biomass (Table 2). Furthermore, litter-fall must also increase significantly along with plant growth, which means that the very modest increase in soil respiration can only be explained by a much lower TBCA_R in the GDA and also a lower heterotrophic activity relative to the higher litter-fall in direction of the GDA (Högberg et al., 2003). This also tallies with the results of girdling of N fertilized and control plots (see above). The difference between GRA and GDA in the rates of accumulation of soil C are considerable (Högberg et al., 2006; Table 2), and may reflect a retarding effect of N on the decomposition of SOM (Berg and McLaugherty, 2008) in addition to a greater litter production in the GDA.

Hence, there are considerable spatial variations in the ratio fungi/bacteria, which appear to be linked to TCAM. We have attempted two methods to estimate the biomass of ECM fungi, which dominate over saprotrophs in the F+H horizon (Lindahl et al., 2007; Clemmensen et al., 2013). The first was based on the loss of microbial cytoplasm C after tree-girdling; in this case our estimate was that ECM fungi accounted for ca. one-third of total microbial biomass (Högberg and Högberg, 2002). This estimate is likely conservative, because the dying ECM mycelium will become a substrate for other microbes. The second estimate is based on ¹³C-labelling via the TCAM pathway, and represents a non-intrusive method. In this case the estimate for ECM biomass was ca. 40% (Högberg et al., 2010). Both these estimates were from N-limited boreal pine forests; in N-fertilized plots TBCA and TCAM became lower (Högberg et al., 2010).

We would like to once again stress that in N-poor forests the ECM fraction of the microbial biomass is most likely a much stronger sink for available N than saprotrophic fungi and bacteria in the F+H horizon. The ECM fungi receive fresh C-substrates, but the major organic matter substrates for heterotrophs have at this stage been exploited by heterotrophs for > 5–10 years, and thus lost readily available C during several years. Along the N supply gradient at Betsele, the shifts in microbial community composition and microbial C/N ratios are striking (Table 2). The N-poor DS ecosystem richer in fungi has a microbial C/N ratio of 12, which compares with 7 in the short herb, SH, system and 5 in the TH system. It is commonly assumed that bacteria cycle N at higher rates than fungi under C limited conditions, and that this is associated with lower C demand relative to that of N (low C/N ratios) and the lower C use efficiency of bacteria (Keiblinger et al., 2010; Franklin et al., 2011).

We would also like to emphasize that the comparatively large changes in microbial community composition and C/N ratios along the Betsele gradient are associated with modest differences in microbial biomass and soil basal respiration per m², but large concomitant changes in N retention and gross N mineralization (Table 2). In line with this, we found in a study of a chronose-

quence of boreal forests developed in the context of isostatic rebound at the coast of the Gulf of Bothnia, that the ratio of microbial N over total N increased moderately (while microbial C over total C did not increase) with ecosystem age in the coniferous forest, while forest productivity, C inputs to the soil and foliar N % declined (Blaško et al., 2015). This may reflect changes to variations in TCAM, we will discuss their potential implications in Section 5.2.

4.3. May other elements than N limit plant growth?

A high supply of N may cause limitation of plant growth by another nutrient element (Tamm, 1991) and such a limitation may lead to excess of available N. Experiments with high additions of N have shown that boron (B) is the second most limiting nutrient element in Swedish and Finnish boreal forests (Aronsson, 1983; Möller, 1983; Lehto and Mälkönen, 1994; Möttönen et al., 2003). In Norway, with a larger influence of sea-spray, an important carrier of marine B, boron deficiency may be less widespread in boreal forests on mineral soils.

Another candidate as limiting nutrient is P, which is often limiting in old soils in the tropics, where phosphates become strongly bound to oxides of Fe and Al (Walker and Syers, 1976; Brady and Weil, 2002). A shortage of phosphorus in the sense that additions of P increases tree growth has been demonstrated in trials on deep boreal peat soils (Moilanen et al., 1996; Silverberg and Moilanen, 2008), presumably because of the limited contact between tree roots and P-sources in the mineral soil.

It has been speculated that boreal forests should develop a P deficiency also on mineral soils, like the tropical forests, if they are allowed to develop for a long time without major disturbances (Wardle et al., 2004). This idea is based on observations on small islands with up to one meter thick mor-layers, which have developed in the absence of fires (Hesselman, 1926); clearly a unique situation. More commonly, boreal forests are subject to rather frequent fires (Zackrisson, 1977; Niklasson and Granström, 2000), which consume partly or wholly the mor-layer, and maintain a situation in which tree roots have access to mineral soil P sources. Moreover, their exposure to chemical weathering has been < 10 000 years as compared to millions of years for old tropical soils.

However, in GDAs, there can be a substantial discharge of Fe^{2+} , which becomes oxidized to Fe^{3+} , with a considerable capacity to bind phosphate (Giesler et al., 1998, 2002). Accordingly, very low levels of soil phosphate and low plant P/N ratios indicating a possible P-limitation have been found in some GDAs (Giesler et al., 1998, 2002, Table 3). We propose, in line with plant allocation theory, and the fact that discharge areas have low fungi/bacteria ratios as revealed by PLFA analysis, DNA sequencing, and production of ECM mycelium (Table 2; Högborg et al., 2003, 2007, 2014a; Nilsson et al., 2005), that ECM fungi play a very small role at such sites. The absence of this potentially large sink for N, and the limited P supply, result in a surplus of N and an open N cycle, e.g., in the TH forest type at Betsele, where microbial P did not vary along the gradient (Table 3), despite a threefold higher forest production driven by the remarkably higher N supply at the rich TH end. For example, the immediate retention of added ^{15}N is very low in the TH forest type (Högborg et al., 2006, Table 2). A high supply of NH_4^+ , the absence of strong biotic competition for N, but a strong C limitation, and the high pH, make such GDA sites hot spots for nitrification and denitrification (Högborg et al., 2006), and support species-rich plant communities (Giesler et al., 1998).

Can we be sure that P is limiting under these conditions? Our data for the TH forest type at Betsele show lower P/N ratios for plant foliage and microbial cytoplasm, much higher P uptake for the root bioassay (which is expected if P is limiting) and that microbial cytoplasm P is a much lower fraction of total P than in the DS and SH forest types (Table 3); although a P/N ratio of 0.14

Table 3

Phosphorus and P/N relations of mor-layer soil (F + H horizons), plants, and microbial cytoplasm along the 90-m-long N supply gradient from a dwarf shrub type through a short herb type to a tall herb type near Betsele, N. Sweden (data from Giesler et al., 1998*, Högborg et al., 2003*). Soil and plant P data are means (1.0 SE), N = 4–5 unless otherwise stated. Microbial cytoplasm P (unpublished) and N were estimated by the FE (fumigation extraction) method. Means of three seasons (N = 3, 1.0 SE), n = 5 per season. Microbial cytoplasm P is not adjusted for the efficiency of fumigation and extraction in these organic soils and are hence not converted to microbial biomass P data.

Parameter	Dwarf shrub type	Short herb type	Tall herb type
Soil total P (kg P ha ⁻¹) ^a	34.1 (3.7)	52.3 (2.1)	131.3 (N=1)
Plant P/N ^b	0.11 (0.01)	0.12 (0.01)	0.08 (0.01)
Root P bioassay (cpm mg ⁻¹ (f.w.) 15 min ⁻¹) ^c	183 (31)	199 (61)	712 (14)
Microbial cytoplasm P (kg P ha ⁻¹)	6.2 (0.9)	8.3 (1.2)	6.1 (2.2)
Microbial cytoplasm P out of total soil P (%)	18.1 (2.6)	15.8 (2.3)	4.7 (1.7)
Microbial cytoplasm P/N ^d	0.63 (0.02)	0.66 (0.03)	0.14 (0.05)

is not considered low for bacteria (Cleveland and Liptzin, 2007). Giesler et al. (2002) also conducted a plant growth bioassay using mor-layer soil from the site and additions of N, P or N + P. Additions of N did not increase plant growth, unlike additions of P and N + P. However, these indications do not prove that trees in the field experience a P limitation. It may occur very locally, and be experienced by microbes and smaller plants, but large trees may have roots extending up-hill into soils richer in P; the only unequivocal test would be to apply P at the field scale.

5. Interactions among trees, microbes and soil: The Plant-Microbe Carbon-Nitrogen model

5.1. On the C for N exchange rates in mycorrhizal symbiosis

Many of the more recent findings discussed above call for a new physiological model describing interactions among plants, microbes and soil, and especially the role of these components in linking the cycles of C and N. Such a model should encompass the large variations in N supply and plant growth encountered in the Fennoscandian boreal landscapes. We have discussed above why the classic idea of variations in litter decomposition rates cannot be the sole basis for such a model. It does not describe the situation in the soil horizon, where mycorrhizal roots access the N released from litter.

Another classic problem is the theoretical and methodological separation of the functions of mycorrhizal fungi from those of other soil microbes (Högborg, 2004; Lindahl and Tunlid, 2015). This separation now appears even more critical, given the high microbial N sink strength capacity in N-limited boreal forests (Table 2; Fig. 4; see also Section 5.2.). Based on tracer studies following ^{13}C from canopy photosynthesis to fungal PLFA in the F/H horizon, and to ECM sporocarps, we have attributed this sink to ECM fungi (Högborg et al., 2010; Näsholm et al., 2013).

Many ecologists have assumed that the exchange rate between C from plant photosynthesis for the N from soil through mycorrhizal fungi is always favorable for the plant. However, model experiments with mycorrhizal pine seedlings supplied with N at very low rates (Alberton et al., 2007; Corrêa et al., 2008), and our own field experiments linking ^{13}C from tree photosynthesis to ^{15}N supplied to the soil (Näsholm et al., 2013) show that mycorrhizal symbiosis does not lead to greater N supply to the tree foliage under conditions of low N supply. Clearly, greater transport of tracer ^{15}N from the soil to tree canopies occurs after large N additions (Näsholm et al., 2013), in which the large additions of

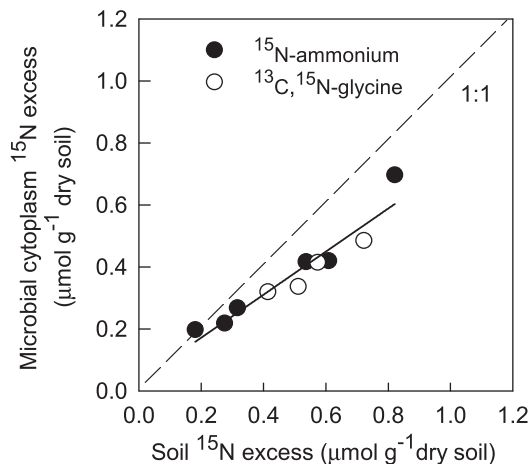


Fig. 4. Relation between short-term (~6 h) microbial immobilization and total soil immobilization in a dwarf shrub forest type (M. N. Högborg's previously unpublished data from the study by Näsholm et al. (1998), which reported data for 6 h up to 7 days after labelling). N-15 labelled NH_4^+ (solid circles) or glycine (open circles) was injected into the F + H-horizons of the mor-layer. Microbial cytoplasm was determined using the chloroform-fumigation method (Näsholm et al., 1998). The slope of the regression line suggests that ~70% of the total N retention was microbial. The regression line ($y = 0.034 + 0.694x$) is based on all data points in the graph ($R_{\text{adj}}^2 = 0.90$, $n = 10$, $p < 0.001$).

un-labelled N would be assumed to dilute the tracer and not to concentrate it.

Furthermore, when 15-yr-old *Pinus sylvestris* trees were studied in a factorial experiment, in which the effects of shading (a 60% reduction in photosynthetic C gain) and additions of N were examined, the ratio of ^{15}N (tracer N initially added to the soil) in mycorrhizas over that in needles was higher in control plots as compared to N-treated and shaded plots (Hasselquist et al., 2016). The results of this experiment, thus, also supported the contention that mycorrhizal fungi immobilize more N when their supply of C is greater.

These observed interactions of N and C fluxes may suggest that trees and ECM fungi individually strive to maximize their own benefits in exchanging N and C with the symbiotic partners (Näsholm et al., 2013; Franklin et al., 2014). An ECM fungus will deliver only the N it does not need for its own growth to the trees – exporting more would reduce its growth and exporting less would make it a

less preferred recipient of tree C export in competition with other fungal partners (see below). At the same time, the proportion of assimilated C that trees allocate below ground always declines when N uptake per C expended increases due to higher soil N availability (e.g. Franklin et al., 2012).

Consequently, when N availability in the soil increases, trees do not increase their C allocation to the ECM fungi in proportion to the increasing N uptake by the fungi, which makes the ECM fungi export an increasing proportion of their N uptake to the trees, i.e. the N in excess of their own demand (Fig. 5). Conversely, declining soil N availability causes an increase in TCAM_R , which increases the fungal N immobilization more than the N export to the trees (Fig. 5). While higher C allocation to ECM fungi may enable higher N uptake and growth of trees in less N poor systems (Terrer et al., 2016), it likely contributes to maintaining or even aggravating plant N limitation in N poor boreal forests (Näsholm et al., 2013; Franklin et al., 2014).

From an evolutionary perspective, it may not be obvious why the trees keep exporting C to the ECM fungi even if this aggravates their N limitation. Similarly, under increasing N availability, why do the fungi keep increasing N transfer to the trees if this eventually reduces their supply of C from the trees? We propose that the answer to both these questions lies in multiple partner structure of the symbiosis, referred to as mycorrhizal network (Simard et al., 1997), which supports a competitive C-N market trade mechanism. Competition for C among fungal partners of the same tree drives each fungus to increase N delivery to the tree per C received compared to its competitors, and *vice versa* for multiple trees delivering C to the same fungus (Franklin et al., 2014). Consequently, resource competition on both sides of the mycorrhizal symbiosis interact to stabilize the symbiosis, regardless of the secondary consequences at the ecosystem level, such as aggravation of plant N limitation. These mechanisms may play a key role for the observed vegetation and N cycling dynamics of N poor boreal forest, as described below.

5.2. Plant-microbe interactions – implications for N cycling

In our studies of soil gross N mineralization, i.e. actual N mineralization, we found that c. 80% of the added ^{15}N tracer was immobilized immediately in the DS forest type, which means it was removed from the labelled pool already at the immediate, time-zero t_0 , extraction < 1 min after addition (Table 2; see also Fig. 4).

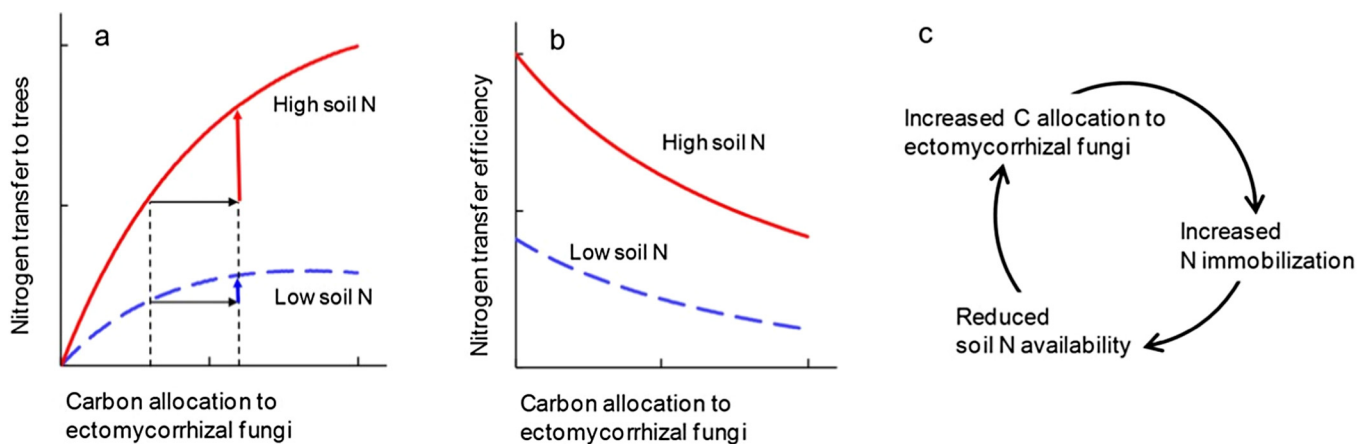


Fig. 5. On the relations between N transfer from the soil to the trees through ectomycorrhizal (ECM) fungi as a function of the C allocation to the ECM fungi. Based on a simple model (see text and Näsholm et al. (2013), by permission of John Wiley & Sons), (a) shows how increased C allocation to ECM fungi (horizontal arrows), e.g., under rising atmospheric $[\text{CO}_2]$ increases N transfer to trees (vertical arrows) more at high than at low soil N availability (red solid vs. dashed blue lines differ in N availability by a factor 2). (b) Nitrogen transfer to host trees per C allocated to ECM fungi (transfer efficiency) increases with soil N availability (red vs. blue lines in b), but decreases with increasing C allocation to ECM fungi. (c) Increasing C allocation to ECM fungi may increase N immobilization, which reduces soil N availability for plants, which further increases C allocation to ECM fungi by the host trees –potentially driving a positive feedback loop.

In the richest plant community, the TH type, with almost no ECM fungal contribution to the soil microbial community (extremely low fungi/bacteria ratio), only 20% was immobilized.

Similarly, high gross N mineralization rates but low N immobilization capacities were found in the N-rich alder zone of a boreal chronosequence (Blaško et al., 2015) and in experimental plots subject to high levels of N additions, but where the fungi/bacteria ratio based on phospholipid fatty acids (PLFAs) did not always shift (Blaško et al., 2013). However, it should be noted that the high correlation between the PLFA 18:2 ω 6,9 and ECM fungi (Högberg et al., 2011) may be confounded in ecosystems showing exceptionally high contribution of saprotrophic fungi to the microbial community, which indeed could be expected in the alder zone and in high N addition plots with high litter production (Blaško et al., 2013; Högberg et al., 2014b). A surprisingly strong negative correlation was found between gross N mineralization and soil fungi/bacteria ratios along the natural N gradient at Betsele and in the long-term N fertilization experiment at Norrliden (Högberg et al., 2007b). Soil metagenomics sequencing efforts could clarify these questions regarding linkages between fungal and bacterial contributions and N supply rates.

Across the natural N supply gradient at Betsele the large differences in N retention could not be attributed to cation exchange capacity, CEC (Table 2), but was correlated with the microbial community composition, notably the fungi/bacteria ratio and variations in the C/N ratio of microorganisms. This observation is important because the result is unexpected, i.e. when adding tracer $^{15}\text{NH}_4^+$ to the NH_4^+ pool, one expects most of it to be replaced by the release of unlabeled N from decomposition, not that the reverse flow of labelled N to the microbes is much greater, as observed in the N-poor dwarf shrub community at Betsele (Högberg et al., 2006) and elsewhere (Blaško et al., 2015), i.e. the forest type most typical of boreal forests.

Can we be sure that abiotic sinks for the ^{15}N are not involved? More research is needed here, but the close correlation with the microbial community structure mentioned above and the observation by Näsholm et al. (1998) that around 70–80% of the variation in retention of tracer ^{15}N added as NH_4^+ or glycine could be explained by immobilization in microbial cytoplasm (as determined by chloroform-fumigation followed by extraction) suggests that microbial immobilization is critically important in boreal forest soils (Fig. 4). The differences in microbial N sink strength observed along the natural N supply gradient at Betsele (Högberg et al., 2006) tally with the variations in TCAM_R in response to variations in soil N availability (Högberg et al., 2010; Näsholm et al., 2013; Hasselquist et al., 2016). These observations support our proposition that variations in N supply to plants are amplified by the TCAM feed-back (Fig. 5).

Here, we would like to add further data supporting arguments presented in paragraph 3.5. on inferences on ECM fungal mycelium as immobilizer of N based on the natural distribution of $\delta^{15}\text{N}$ in the soil-plant system. As said there, and reported elsewhere (e.g., Högberg et al., 1996), a large difference in $\delta^{15}\text{N}$ between trees and soils, ϵ , suggests that the mycelium is a strong retainer of N. We have shown that tree foliage $\delta^{15}\text{N}$, the more dynamic of the two (plant and soil $\delta^{15}\text{N}$), correlates with the PLFA 18:2 ω 6,9 in the F + H-horizon, in which this PLFA correlates with ECM DNA (Högberg et al., 2011, 2014a). Here, in Fig. 6, we add data on the relation between tracer ^{15}N retention (as described above) and ϵ from two major N-loading experiments, Stråsan (Blaško et al., 2013) and Norrliden (Högberg et al., 2014b), and from two natural gradients, the N-supply gradient Betsele (Högberg et al., 2006) and the chronosequence Bjuren (Blaško et al., 2015). Moreover, in another chronosequence study spanning millennia, Wallander et al. (2009) pointed at an increasing fungal biomass as driver of soil ^{15}N -enrichment. As shown in Fig. 6 there is a significant corre-

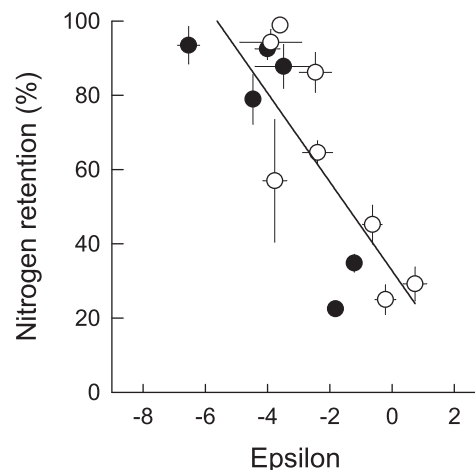


Fig. 6. The relation between N retention (here the immediate retention of tracer ^{15}N injected into mor-layer soil (F + H horizon)) and epsilon. The regression line is based on all data points in the graph ($R^2_{\text{adj}} = 0.62$, $n = 14$, $p < 0.001$). Epsilon, ϵ , the enrichment factor ($\delta^{15}\text{N}$ needles - $\delta^{15}\text{N}$ soil). Data (means ± 1.0 SE) from natural N supply gradients (filled circles) and N-loading experiments (unfilled circles) (Blaško et al., 2013, 2015; Högberg et al., 2006, 2014b).

lation between N retention and ϵ , further supporting a strong role for ECM fungi in closing the N cycle and in part so by immobilizing N in N-poor systems, i.e. the DS systems in the upper left corner of Fig. 6.

Accordingly, in the poorest communities, i.e. dwarf shrub types, the high TCAM_R results in a high biomass and N sink strength of ECM fungi and associated microorganisms (Table 2, Fig. 7). We expect that any available N becomes quickly immobilized in the presence of the large biological sinks, notably by ECM fungi. This explains why levels of inorganic N are low in intact forests, but increase after clear-felling, which weakens the microbial N sink by removing the C supply to ECM fungi and associated microorganisms like bacteria. After clear-felling or other disturbances, more N-demanding plant species, like, e.g., *Epilobium angustifolium*, may enter the scene; under undisturbed conditions only stress-tolerant species like the ECM tree species and ericaceous dwarf shrubs (with ericoid mycorrhiza) are able to close their life cycles. A stress-tolerant competitor like *Avenella flexuosa* (L.) Drejer (cf. *Deschampsia flexuosa* (L.) Trin.), may prevail in the closed forest, but does not flower unless the forest is fertilized with N or disturbed by, e.g., by clear-felling (Nordin et al., 2006).

At the other end of the spectrum, we have rich tall herb forests. In these systems, which are typically found in GDAs, the ratio of fungi/bacteria is low (Table 2), which indicates a low TCAM and a C-limited microbial community (Fig. 7). This microbial community is dominated by organisms with a cytoplasmic C/N ratio of around 5, and with little capacity to immobilize more N (Table 2). Thus, nitrogen in available forms is present at levels exceeding the demand of the microbial community. Gross N mineralization is rapid, and nitrifiers can compete more successfully for NH_4^+ and they are also favored by the high pH in the GDA. This results in a very open N cycle, with substantial inputs in discharge water, but also large losses through leaching of NO_3^- and denitrification (Högberg et al., 2006). The high N supply favors fast-growing N-demanding plant species with tall herbs dominating the field-layer.

Between those two extremes are the short herb communities, which we postulate to be labile and relatively more sensitive to disturbance (e.g., in response to clear-felling) than the dwarf shrub type. In the short herb type, ECM fungi are still important because of a large biomass, but they are better supplied with N and transfer more N to their tree hosts. It is interesting that these communities

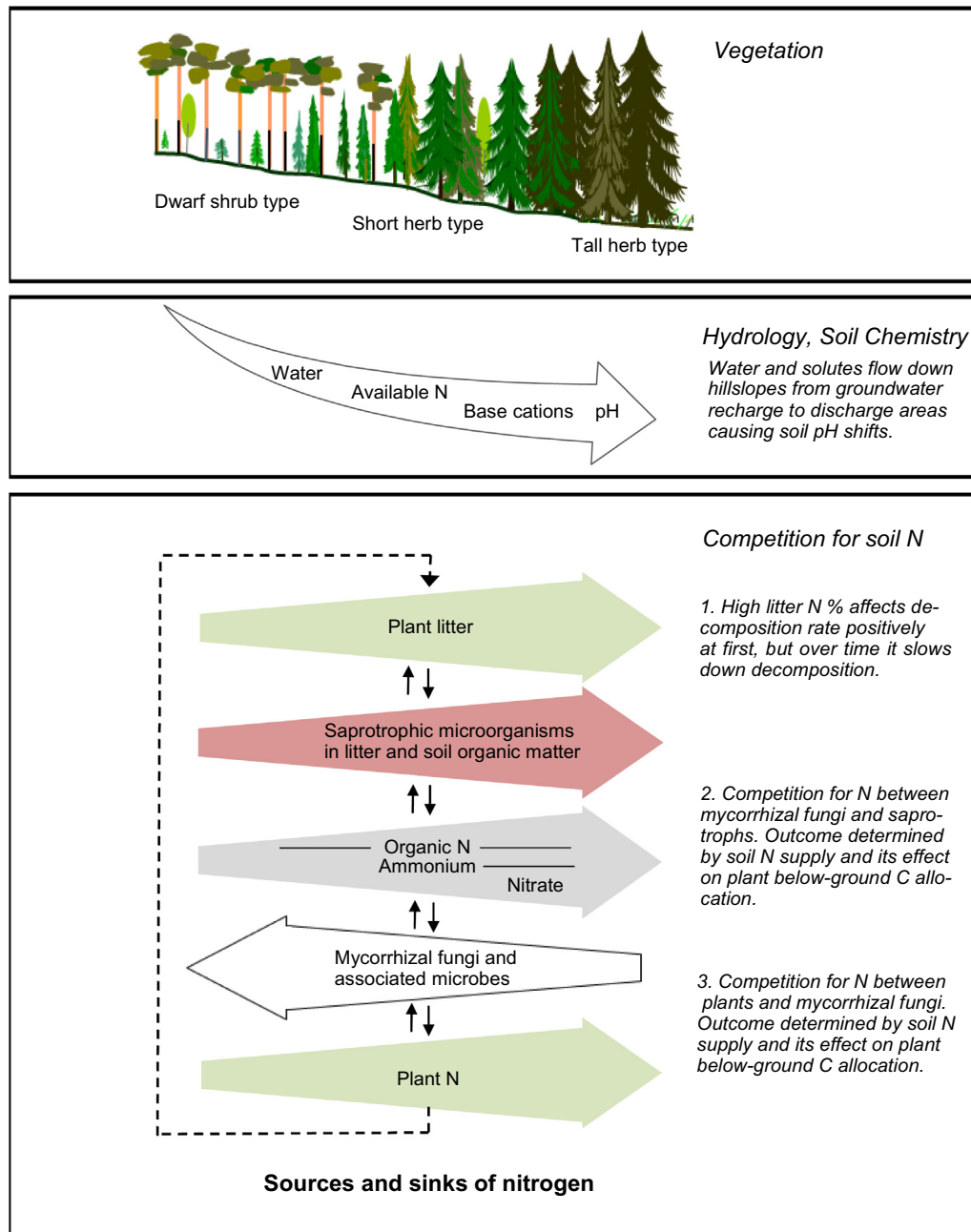


Fig. 7. The plant-microbe carbon-nitrogen (PM-CN) interactions model depicting interactions among vegetation, hill-slope hydrology and soil chemistry, and the outcomes of competition for soil N as determined by plant and microbial physiologies. For a reference with field data see [Table 2](#).

have the highest species richness of ECM fungi (Högberg et al., 2014a).

Along the gradient from N-poor to N-rich, the available N goes from a low supply rate totally dominated by organic N forms to a higher supply and especially an increasing supply of inorganic N (Table 2, Fig. 7). This reflects the increasing C-limitation of soil microbes. Thus, the presence of inorganic N can be viewed as an indicator of N supply in excess of the biological demand, which is dependent on the C supply to the organisms.

Our studies have thus indicated that TCAM plays a key role in shaping the forest N cycle as a major amplifier of the variations in N availability (Table 2) and plant community composition and growth (Högberg et al., 2003, 2006, 2007a, 2010, 2013, 2014a; Nilsson et al. 2005; Näsholm et al., 2013; Franklin et al., 2014; Blaško et al., 2015; see also Figs. 5 and 7).

6. Implications for forest ecology and management

6.1. Should all forests be regenerated by the same method (clear-felling vs. continuous-cover forestry)?

If the supply of nutrients is low, as is common in boreal forest, regeneration of tree seedlings is often poor in the vicinity of competing larger trees (e.g., Aaltonen, 1919; Ruuska et al., 2008; Axelsson et al., 2014). Mycorrhizal theory suggest that an ecological market mechanism may aggravate this competition effect (Franklin et al., 2014). In competition with large trees the seedlings may not be able to provide as much C per N to their common ECM network, which means that the ECM fungal network will preferentially deliver N to the established trees (Franklin et al., 2014). The observation of poor establishment of seedlings in N-limited boreal

forests follows the general patterns outlined by Coomes and Grubb (2000), according to which below-ground competition limits regeneration by seedlings where nutrients and water are in poor supply, while light supply limits regeneration where the supplies of nutrients and water are high.

This importance of belowground competition was nicely illustrated in trenching experiments made by Hesselman (see Romell and Malmström, 1945) in boreal pine forests. In these, plots of tens of m² between trees were trenched to exclude the influence of active tree roots. This led to loss of sporocarp production by ECM fungi and prolific regeneration of tree seedlings in trenched plots under intact tree canopies, i.e. without any manipulation of the light conditions. Thus, there were more and taller seedlings and their content of chlorophyll was greater, indicating a higher N supply (Romell and Malmström, 1945). Romell used the term the “assart effect” to describe the effect on plant growth of this nutrient release. This research and practical demonstrations by practitioners like Joel Wretling paved the way for the accelerated use of clear-felling and planting as method of forest regeneration (Kuuluvainen et al., 2012; Lundmark et al., 2013).

Similarly, there was loss of ECM fungal sporocarp production but prolific seedling regeneration in plots with girdled trees in another N-poor pine forest (Axelsson et al., 2014) in an experiment comprising 900 m² plots in which all trees were girdled or left nongirdled as controls (Högberg et al., 2001). The massive regeneration from seeds started before the pine trees became defoliated three years after the girdling treatment, and was thus not initially a result of changing light conditions, but rather the removal of competition from active ECM tree roots. In fact, an analysis of understory ericaceous plants showed increases in foliar $\delta^{15}\text{N}$ alongside increases in % N, which suggested that ECM N had been released already before the trees were defoliated (Bhupinderpalsingh et al., 2003).

We strongly advocate that discussions about clear-felling vs. continuous-cover forestry should consider that the outcomes of these practices will be very much dependent on site fertility. Foresters should always ask whether competition for nutrients or for light limits the regeneration of seedlings.

6.2. A few remarks on leakage of N from boreal forests

In the late 1960s Likens et al. (1970) demonstrated that massive leaching of NO_3^- occurred after clear-felling of forest in New England. Similar watershed experiments in boreal Sweden showed much less dramatic responses (e.g., Wiklander, 1981; Rosén et al., 1996), likely because of the overall low availability of N in these forests. However, one would logically assume that forest landscapes used intensely, including clear-felling followed by replanting as major method of regeneration, would leach more N than forests left as they are. This may not be the case; Vitousek and Reiners (1975) demonstrated, also in New England, that older forests leached more N than younger forests.

Studies of runoff water from large boreal watersheds, in which clear-felling is commonly practised, now show declines in runoff of NO_3^- and total inorganic N over the last three decades (Lucas et al., 2013, 2016). It should be stressed that the study by Lucas et al. (2016) focused on northerly boreal forests in Sweden, in which case the absolute decline in N deposition over the last decades has been very minor. The overall decline in runoff of inorganic N suggest it is likely that the elevated leaching losses in the young clear-fellings are more than compensated for by higher N uptake by the young and fast growing forests (cf. Johnson, 1992). Their soil N sink strength may be amplified by the TCAM feed-back. In fact, studies of coniferous forests in southern Sweden indicate that the largest production of extramatrical ECM fungal biomass occurs in relatively young forests (Wallander et al., 2010), and that leach-

ing of inorganic N correlates negatively with the production of ECM fungi (Bahr et al., 2013) and the ratio fungi/bacteria (Högberg et al., 2013, Fig. 8).

6.3. Can a self-sustaining greater N cycle be induced by boost additions of N?

This idea was proposed by the plant physiologist Ingestad (1987). He hypothesized that additions of N and other nutrient elements would speed up the biological cycles of these elements considerably, and that these higher rates would be sustained. In the case of N, there is now ample evidence against this hypothesis from N fertilizer trials. The increase in stem production after common forest fertilization (i.e. with around 150 kg N ha^{-1}) lasts around 10 years (e.g., Nohrstedt, 2001). A few additional fertilizations may have the same response, but growth does not continue at the higher level (Nohrstedt, 2001).

However, there can be carry-over effects on the young forest established after clear-felling of the old fertilized forest, especially after repeated fertilization, i.e. with doses double the common 150 kg N ha^{-1} (From et al., 2015). Even experimentally N-saturated forest can return to N limitation. The outstanding example is the experiment E55 at Norrlieden, N. Sweden, where a *Pinus sylvestris* forest was fertilized annually at three rates, and where the highest N dose, N3, was terminated after 20 years of treatment, when $1960 \text{ kg N ha}^{-1}$ in total had been added (Högberg et al., 2011, 2014b). During the subsequent 20 years the foliage N concentration as well as several other indicators of high N availability declined (Högberg et al., 2014b). In several cases the N3 plots were no longer different from the control plots, e.g., in the case of gross

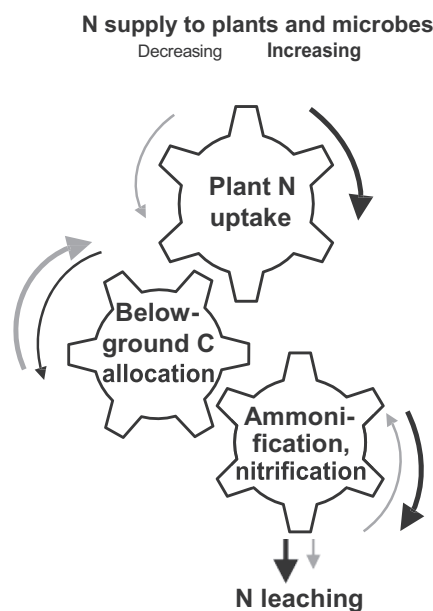


Fig. 8. On the relation between N supply and leaching of N as affected by the interactions between N supply and the tree belowground C allocation to mycorrhizal fungi and associated microbes (TCAM) (Högberg et al., 2013, by permission of Springer). When N supply increases (upper wheel, black thick arrow) ectomycorrhizal fungi and other rhizosphere microbes become progressively more and more C limited due to lower TCAM (middle wheel, black thin arrow), soil fungi/bacteria ratio decreases, and microbial assimilation of N diminishes whereas N mineralization increases (lower wheel, thick black arrow). Subsequently, the increasing ammonium levels may stimulate autotrophic nitrification, and nitrate leaching. In contrast, when tree and microbial N supply decreases (upper wheel, thin grey arrow) the TCAM increases (middle wheel, thick grey arrow), the fungi/bacteria ratio increases, and subsequently N mineralization, nitrification and N leaching diminishes (lower wheel, thin grey arrow).

N mineralization rates (Högberg et al., 2014b). Interestingly, the ratio fungi/bacteria increased as did ε (see Section 3.5.), indicating that the function of ECM was restored; this ratio and ε remained low in the on-going N treatments (Högberg et al., 2011). In fact, molecular data showed that the ECM fungal community, but not the bacterial community, was largely restored when the high N treatment had been terminated for 14 years (Högberg et al., 2011, 2014a).

In summary, these findings suggest that N-poor Fennoscandian boreal forests are rather resilient to modest N additions, and that other factors than the total amount of N added ultimately constrain plant productivity in the long term.

6.4. How will global changes affect the Fennoscandian boreal forests? Will we see N enrichment through N deposition or progressive N limitation (PNL) driven by increasing $[\text{CO}_2]$?

Most Fennoscandian boreal forests receive fairly modest to low levels of N deposition (Sponseller et al., 2016; Binkley and Högberg, 2016). Such low N addition rates are rarely tested in experiments in boreal forests. However, From et al. (2016) reported based on 10 years of additions of N that rates $\leq 6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ had no positive effect on tree stem growth in Scots pine forests. Thus, it is less likely that we will see significant N enrichment of these forests in general. In fact, the decreasing runoff of inorganic N during the last decades (Lucas et al., 2016) supports this suggestion.

But how will these forests respond to global warming and to increasing atmospheric $[\text{CO}_2]$? An important feature of these ecosystems is that the soil is frozen for a long time in the spring although days are long and sunny (Goulden et al., 1998), and air temperatures may be sufficiently high to support substantial rates of photosynthesis (Bergh et al., 2003; Hall et al., 2013; Wallin et al., 2013). It is, therefore, of considerable interest that observations of increasing intra-annual variations in atmospheric $[\text{CO}_2]$ at high latitudes (Graven et al., 2013) suggest that the C sink strength of northerly ecosystems has increased.

It has been predicted that increasing $[\text{CO}_2]$ could cause progressive N limitation (PNL) by increasing NPP, and thus the C supply to soil biota, but without simultaneously promoting the release of N in available forms (e.g., Luo et al., 2004). Elevated $[\text{CO}_2]$ and TCAM will have marginal positive effects on ECM root colonization levels in boreal forests, where colonization is almost total (Taylor et al., 2000), but perhaps larger effects on the abundance of and C supply to the extramatrical ECM mycelium extending out from the roots can be expected (Cairney, 2012). Elevated atmospheric $[\text{CO}_2]$ also has the potential to change the ECM community composition in the root tips of boreal forest trees (Fransson et al., 2001), but the importance at the ecosystem scale of putative community shifts is not fully known (Cairney, 2012).

Perhaps the decreasing runoff of inorganic N (see above) is indicative of an increased N sink strength of ECM fungi (Högberg et al., 2013) because of a CO_2 -induced increase in TCAM? However, data on tree growth support the contrary (Swedish National Forest Inventory), as do the increasing intra-annual variations in atmospheric $[\text{CO}_2]$ over the northern hemisphere. A recently highlighted mechanism can increase plant growth in response to elevated $[\text{CO}_2]$ without the need for more N, and that is suppression of photorespiration (Ehlers et al., 2015). Moreover, it was recently proposed that the transfer of N from the soil through ECM fungi to the trees was greater under higher $[\text{CO}_2]$ (Terrer et al., 2016), but the vast majority of the experiments included in that analysis were much richer in N than the N-poor forests discussed here.

Clearly, the many complex interactions between the C and N cycles in boreal forests deserve much further exploration. At this

stage it seems the N-limitation will prevail in the foreseeable future.

6.5. On the relations among N supply, plant biodiversity and plant growth

How shall one interpret the positive relation between nutrient supply (especially of N) and the number of species of vascular plants? We submit to the classic opinion of species richness and the associated presence of certain plant species primarily as indicators of site fertility (e.g., Cajander, 1909; Dahl et al., 1967; Ellenberg, 1977; Lahti and Väisänen, 1987) rather than the intrinsic cause of it. We do not deny the existence of feed-backs, i.e. that under tree canopies the floras, soil microbial communities and degradation of litter and release of N can differ depending on tree species, but we propose that the greater variation in N supply and tree growth across landscapes relate to edaphic soil factors, which are amplified by fundamental plant and microbial physiological feed-backs, in particular the one through variations in TCAM (see Sections 4.2 and 5.2).

However, in a recent analysis of data from the Swedish National Forest Inventory (SNFI) Gamfeldt et al. (2013) proposed the reverse, i.e. tree species diversity as a cause of higher forest production. In their analysis, they made no reference to previous studies using SNFI data, especially Högglund and Lundmark (1977). Gamfeldt et al. used soil moisture classes, but not the classes related to hill-slope hydrology (i.e. lateral flow), which were important according to Högglund and Lundmark and others (Tamm, 1991; Holmgren, 1994; Giesler et al., 1998; Zinko et al., 2005; see also Fig. 7).

Thus, there is a risk of confusing cause and effect, i.e. if number of tree species and other plant species is an indicator of more fertile soils, or the driver of soil fertility. Clearly, moving the highly diverse assemblages of plant species in a GDA to a GRA up-slope will not increase the forest productivity there (nor would most of the plants survive there) because this will not change simultaneously the flux of groundwater and nutrients to be up-slope rather than down-slope. We note also that the analysis by Gamfeldt et al. (2013) did not reveal a cause of the proposed higher growth in mixed forests as compared to single species stands.

Moreover, it appears that they in their model compared the average growth of single species stands with the average growth of multi-species stands. This means that a single species with a high growth (e.g., *Picea abies* in S. Sweden) was not alone compared with the multi-species stands growing on the same soil, i.e., there was no real test of over-yielding, which is common in this type of research (Beckage and Gross, 2006). The possibility of errors due to the well-known “sampling effect” was not accounted for, i.e., that a mix of several species is likely to include a single species with particularly high growth (Huston et al., 2000).

A higher growth in mixed-species stands under N limitation may occur if there are: (i) variations in the abilities among tree species to take up different N forms or to exploit different soil horizons for N, and thus a greater probability that the soil is more effectively scavenged for N as species diversity increases, (ii) variations in the N-use efficiency among tree species, and the greater probability of including a more efficient species as the number of species increases, (iii) tree-species related variations in the soil below tree-canopies causing variations in the nitrogenase activity of free-living N_2 -fixing bacteria, (iv) species forming symbiosis with N_2 -fixing bacteria, e.g., *Alnus* species, (v) plant-fungal interactions preventing the buildup of high mycelial biomass of ECM fungi that otherwise could aggravate the N limitation.

With regard to the first (i) of these possibilities, some authors have suggested that N source niche separation may occur in boreal forest (e.g., Read, 1991) and in tundra (McKane et al., 2002). How-

ever, we have seen no evidence that boreal tree species and other plants differ fundamentally in their abilities to take up different forms of N *in situ* (e.g., Näsholm et al., 1998; Nordin et al., 2001; Persson and Näsholm, 2001; Persson et al., 2003). Rather, the null hypothesis to be more critically tested must be that trees and other plants take up any form of N available if the N supply is limiting. In our view, preferential uptake of certain N forms only occurs if the N supply is high enough to allow plants to choose among N forms. We propose that this occurs in TH plant communities, e.g., in GDAs, where the high N supply evidently allows many species to close their life cycles, in contrast to in DS communities, where a few stress-tolerant ericaceous species are dominant along with the ECM trees.

A recent comparison between soil supply rates of both organic and inorganic N sources and uptake capacities of these same sources by *Pinus sylvestris* roots showed the uptake capacities to be one-to-two orders of magnitude higher (Oyewole et al., 2016), suggesting soil N supply rate to be the determinant of both amounts and forms of N acquired by plants (cf. Nye and Tinker, 1977). An exception to this general pattern was found for NO_3^- , for which root uptake rates of N fertilized trees was similar, or even lower than soil supply rates.

With regard to the second (ii) possibility, variations in N-use efficiency are possible among species, but also within species depending on N supply (e.g., Mäkelä et al., 2008, 2016; Vicca et al., 2012). A classic hypothesis is that evergreens dominate nutrient poor soils because of their low loss rates of N (Aerts, 1995). The major factor explaining the low N-loss rates of evergreen compared to deciduous trees is the difference in leaf longevity while nutrient (N and P) resorption rates are as high, or even higher for deciduous trees (Aerts, 1995, and references therein). Retention of N in the canopy could be greater in conifers, but they are represented by two species only in Fennoscandian boreal forests; greater species diversity is contributed by the many deciduous species, but their presence could indicate better site conditions, i.e. that they are in or close to GDAs, rather than greater N-use efficiency being a competitive advantage of these species. Notably, broad-leaved deciduous tree species have higher rates of maximum photosynthesis and a steeper slope of the relation between net photosynthesis and leaf N than needle-leaved evergreens (Reich et al., 1995), suggesting broad-leaved deciduous trees should have higher NUE. However, enhanced maximum rates of photosynthesis may have little bearing on *in situ* canopy photosynthesis rates and hence on NUE. This is because light levels inside canopies are often much lower than those required for differences in rates of photosynthesis to occur (Brix, 1981; Tarvainen et al., 2016).

However, a higher NUE of photosynthesis does not automatically translate to a higher NUE of growth, because deciduous high-NUE leaves also incur greater C and N costs in terms of higher leaf turnover rate (Wright et al., 2004) and increased herbivory compared to conifer needles. Because soil N availability determines the costs of N uptake for leaf construction, it has a strong influence on which leaf strategy dominates in a site as well as on productivity. This suggests that leaf-strategy and other traits that determine NUE of growth are effects rather than causes of higher or lower site productivity under N limited conditions.

As regards the third (iii) possibility, it is possible that the soil microorganisms under different tree canopies differ in nitrogenase activity, e.g., as a result of properties of the canopy or its litterfall. However, there is a lack of data from tree species trials, especially trials comprising mixes of species, in the Fennoscandian boreal region.

With regard to the fourth (iv) possibility, *Alnus* forms a distinct zone along the coast of the Gulf of Bothnia, where new land is created by isostatic rebound (Vestøl, 2006), and along rivers and in

ditches along roads, but is very rare in older undisturbed forests. Thus, it is unlikely that N_2 -fixing tree species contribute to greater productivity in older forest systems in general.

The fifth (v) possibility suggests that the outcome of fungal interactions could alter the distribution of nutrients between plants and soil. There are antagonistic effects between saprotrophic and ECM fungi as indicated by inhibited growth in natural soil of both ecological groups of fungi in the zones where the mycelia interact (Leake et al., 2002), and the size of the C supply to the respective fungal guild determines the outcome of the interaction (Lindahl et al., 2001). There are also tree-species related variations in amounts of soil organic material produced on the same site (Berg et al., 2001), which could affect the balance between ECM fungi and saprotrophic fungi and hence the extent to which ECM fungi will immobilize N under different plant species mixtures (Högberg et al., 2003). A hypothesis worthy of testing is if a reduction in the proportion of ECM tree species, by increasing the diversity of tree species with arbuscular mycorrhiza (AM) can increase site productivity via reduced fungal N immobilization.

Forest regeneration based on clear-felling and planting has reduced tree species diversity in forest stands, while at the same time forest productivity has almost doubled the last 90 years in Sweden according to the SNFI. Creating more species-rich stands is attractive from many points of view, as raised by Gamfeldt et al. (2013), but their argument that increasing tree species diversity would increase the average tree growth by as much as 50% is contradicted by the positive trend in tree growth in forests, which are dominated by one or two conifer species.

We conclude that there is an unfortunate lack of direct experiments comparing the growth of single species as compared to mixes of species in Fennoscandian boreal forests. The few species trials available in Sweden do not show that more diverse forests are more productive than all single species (i.e. yields more than the most productive species alone) when grown on the same soil. At the same time there is strong evidence that more diverse and productive plant communities occur at lower positions along slopes (Hägglund and Lundmark, 1977), corroborating the idea that plant diversity and productivity indicate favorable edaphic conditions, e.g., a higher N supply, rather than the other way around.

7. Conclusions and suggestions for future research

Based on the arguments discussed above, we propose that a more complex and integrating organismal physiology perspective should be adopted in the analysis of N supply from plant litter to plant root N uptake. Such a perspective should be allowed to question the prevailing focus on decomposing organisms and the idea that the rate of decomposition always limits the rate of N supply to plants.

The analysis should encompass the role of strong biological sinks for N in the soil (we find that biotic N immobilization dominates over abiotic immobilization, see Fig. 4). We propose, that with decreasing N availability, trees respond with greater TCAM_R , which in turn results in a greater biological sink for available N in the soil, mainly composed of ECM fungi and associated microbes in the mycorrhizosphere. Under conditions of low soil N availability, the efficiency of transfer of N from the soil, through the ECM fungi to the plants, may very well limit the rate of N supply to the plants. In our view, the organisms with the best supply of C will always be the superior competitors for available N. Sites with a high availability of N are sites where N is present in excess of the C supply to the organisms.

Our proposals need much further discussions and critical testing, for example of our hypothesis that the rate of litter decomposition may not always determine the rate of N supply to the

mycorrhizal plants. This, and further elucidation of the role of N immobilization by ECM fungi can be addressed by powerful combinations of modern molecular and stable isotope methods.

Another critical question is to what extent the plant community composition is an indicator of soil fertility rather than the cause of it. We have proposed that the role of edaphic site conditions is overlooked in some contemporary research, and argue that there is a need in this context for careful field experiments, in which designed plant communities are grown under initially identical soil conditions. Only then can the feed-back effects of plants on soils be distinguished.

We see strong reasons to revisit the forest vegetation type classification schemes and use them as a framework for addressing critical questions pertinent to interrelations among soils, microbes and plants, but using the modern toolboxes now available to researchers. Further in-depth exploration of national soil survey data is recommended, but a note of caution is that some parameters, especially those that refer to lateral water flow are rather subjective as based on the judgment of field surveyors. Long slopes sometimes lack rich GDAs, while surprisingly rich GDAs may occur, albeit rarely, in rather flat areas away from major slopes as shown at Betsele (Giesler et al., 1998) and Varjisån (Giesler et al., 2002). We suspect that the microtopography and especially that of the underlying bedrock plays a role in this context.

We have suggested that the variations in flux of N down hill-slopes are pivotal as being the edaphic basis for the interactions between soils, microbes and plants, which ultimately shapes the local N cycles. The flux down slopes needs to be quantified. More samples of N and other solutes in the lateral water flow are needed along with modelling of hill-slope hydrology.

It is evident that not all boreal forests are strongly N-limited; this condition prevails in the majority of forests, but there are high N supply hotspots in the landscape. This seems to be forgotten today when much research focusses on human perturbations like global warming and N deposition. We propose that researchers should recognize and explicitly address the natural variations in N supply in these forests; much can be learned from such comparisons and the effects of global warming and N deposition may vary depending on the forest type. Edaphic variations should also be considered and evaluated in studies of silvicultural systems (Hynynen et al., 2005).

We have favored evidence from field studies and have, with few exceptions, ignored results from laboratory studies. A reason for this is our concern that simplified experimental set-ups in the laboratory may not mimic at appropriate scales the important interactions between organism physiologies in the field; especially the plant and microbial community compositions and their respective rates of C and N supply. Ecosystem manipulations in forests require sizeable plots, not least because of the lateral spread of tree roots (e.g., Göttlicher et al., 2008). We recommend that quadratic plots should be at least 30 m wide, to allow a central 20 m wide plot to be used for measurements.

Factorial perturbations in the field of the supplies of N and base cations are tempting, e.g., as means to partition effects of N from those of pH, but in the real world these are not separate, because N and base cations are components of the same water flux and ecosystem nutrient cycling. There is a need to try to better distinguish the effect of acidity *per se*, which perhaps can be done by comparing till soils with and without an influence of calcareous bedrock.

Finally, we would like to heighten the observation by Leuzinger et al. (2011) that manipulation experiments frequently show strong initial responses to treatments, but weaker and less significant responses in the longer term. Thus, experimental attempts to separate factorially the determinants of ecosystem functions may

not yield reliable results unless we are patient and wait for many years before we draw our conclusions.

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