

# Carbon and Nitrogen Relations among Soils, Microbes and Plants in Boreal Forests

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Doctoral thesis  
Swedish University of Agricultural Sciences

Uppsala 2004

**Acta Universitatis Agriculturae Sueciae**

Silvestria 314

ISSN 1401-6230

ISBN 91-576-6548-6

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Printed by: SLU, Grafiska enheten, Umeå, Sweden, 2004

## Abstract

Nordström Högberg M. 2004. Carbon and Nitrogen Relations among Soils, Microbes and Plants in Boreal Forests. Doctor's dissertation.  
ISBN 91-576-6548-6, ISSN 1401-6230

Across Fennoscandian boreal forests, variations in hill-slope hydrochemistry cause regular patterns in vegetation composition and forest productivity. The turnover of N, the nutrient limiting plant growth, should be a result of interactions between soils, microbes and plants. This thesis represents a first attempt to describe variations in microbial biomass, community structure and activity associated with these patterns. The main study area was a local 90-m-long natural productivity gradient in northern Sweden, site Betsele, representing three coniferous forest types with increasing productivity: dwarf-shrub (DS), short-herb (SH) and tall-herb (TH) types. Destructive tree girdling was conducted in a DS forest type at another site, Åheden, to enable estimation of the biomass of mycelium of ectomycorrhizal (ECM) fungi. The gradient encompassed the range in soil chemistry and plant community composition in Fennoscandian boreal forests and is thus a useful model for soil-microbial-plant interactions. There was also, at this local landscape level, associations between certain soil conditions and plants with specific mycorrhizal types, as proposed typical of different biomes on a continental scale. At Åheden, extramatrical ECM mycelium contributed one third of soil microbial biomass and produced, together with ECM roots, half the dissolved organic carbon. Neither microbial biomass nor soil (root plus microbial) respiration rates varied along the forest productivity gradient. This was unexpected because of the large variation in forest productivity. Gross N mineralization rate increased roughly in proportion to this three-fold increase in productivity. Thus, in contrast to the conventional view, higher rates of N mineralization were not caused by higher microbial activity in general. The susceptibility of the microbial activity of mor soils to physical disturbance decreased when soil fertility increased from the DS to the TH forest type; this was paralleled by a drastic decrease in fungal biomass. Variations in the contribution of mycorrhizal fungi to the microbial biomass confound interpretations of relations among soil and microbial C/N ratios and soil N mineralization. I hypothesize that low N supply and plant productivity, and hence low litter C supply to saprotrophs are associated with a high plant C supply to mycorrhizal fungi, while the reverse occurs under high N supply. Thus, effects of N availability on C supply to these functional groups of microbes acts in opposing directions.

Key words: forest productivity gradient, mor-soils, microbial; biomass, activity, community structure, mycorrhizal fungi, saprotrophs, C allocation, N supply, gross N mineralisation/immobilisation, N-limitation.

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*To my beloved family:*

*Gunvor*

*John*

*My dear husband Peter and our children*

*Axel, Jon-Isak and Fredrika*

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# Appendix

## Papers I-V

The thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Giesler, R., Högberg, M. and Högberg, P. (1998). Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. *Ecology* 79, 119-137.
- II. Högberg, M. N. and Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist* 154, 791-795.
- III. Högberg, M. N., Bååth, E., Nordgren, A., Arnebrant, K. and Högberg, P. (2003). Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - a hypothesis based on field observations in boreal forest. *New Phytologist* 160, 225-238.
- IV. Högberg, M. N., Nordgren, A. and Högberg, P. (0000). Large variability in sensitivity of microbial activity to physical disturbance among mor-layers from boreal forest. (Manuscript).
- V. Högberg, M. N., Myrold, D. D., Giesler, R. and Högberg, P. (0000). Shifts in soil nitrogen dynamics along a local plant productivity gradient in boreal forest. (Manuscript).

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Academic thesis to be publicly defended for the degree of Doctor of Philosophy. Faculty opponent: Dr. Clare Robinson, Department of Life Sciences, Kings College, University of London, UK. Evaluation committee: Professor Åsa Frostegård, Dept. of Chemistry and Biotechnology, Agricultural University of Norway; Ass. Professor Stefan Olsson, The Royal Veterinary and Agricultural University, Dept. of Ecology and Molecular Biology, Copenhagen, Denmark; Professor Tryggve Persson, Inst. för ekologi och naturvård, SLU, Uppsala. Location: HVC FU 26. Date: 040908 Time: 9.15.

## Introduction

In the Fennoscandian boreal forest landscape, there are typically regular variations in vegetation composition and forest productivity (Cajander, 1926). Plant productivity and composition increase down-slope longer slopes along with changes in species composition (Hägglund & Lundmark, 1977) and the edaphic cause of this variation is the hill-slope hydrochemistry (Högberg, 2001a). Nitrogen has been experimentally identified as the nutrient that most strongly influences forest growth and plant species composition in these forest (Tamm, 1991).

Plants characteristics such as certain carbon allocation patterns between above- and below-ground parts (Hansen *et al.*, 1997; Aerts, 1999), life-spans and biochemical composition of tissues (Lambers *et al.*, 1998; Berg & McLaugherty, 2003) in turn influence microbial turnover of C and nutrients (Vitousek, 1982; Chapin III *et al.*, 1986; van Breemen & Finzi, 1998) through effects on activity and composition of the microbial community (Wardle, 1992; Zak *et al.*, 2003). Thus, there are cause-effect relationships leading to soil-microbial-plant interactions, because microbially driven nutrient turnover is a result of both the site hydrochemistry and the plants.

Several processes can lead to a higher N supply to plants in groundwater discharge areas than in the up-slope recharge areas (Chapin III *et al.* 1988; Tamm, 1991; Giesler *et al.*, 1998; Högberg 2001a): (1) transport of N towards toe-slope areas, i.e. a flux of N from recharge to discharge areas, (2) smaller losses of N during fires because of wetter conditions and lower position in the landscape, (3) conditions more conducive for N<sub>2</sub>-fixation, (4) higher rates of N mineralization and higher *in situ* flux of solutes to roots and mycorrhizas because of more favourable moisture conditions, and (5) a higher pH and other conditions more favourable for autotrophic nitrification (which produces NO<sub>3</sub><sup>-</sup>, the most mobile soil solute N form) and possible also N mineralisation. This thesis focuses on the relations between forest productivity and soil microbiology and N turnover in soils.

### C and N links soil, microbes, and plants

Traditionally, large stores of organic C in boreal forest soils are attributed to negative effects of low temperature, soil acidity and plant residue recalcitrance upon microbial activity. While plants use solar energy to fix atmospheric CO<sub>2</sub>, derived from an almost unlimited and simple C source, into carbohydrates to build up their biomass, saprotrophic soil microbes rely on C in plant debris and in diverse and often complex C compounds constituting soil organic matter. In most ecosystems plant photosynthesis and production is often N-limited, especially outside the tropics (Tamm, 1991), while on the contrary, soil microbes are often said to be C-limited.

This assumption, which is not valid for all organisms and conditions, is primarily based on frequent observations of a strongly enhanced respiratory activity after C additions. That a part of the microbial community is C-limited is easily

demonstrated by additions of labile C to the soil, e.g., sugars (Anderson & Domsch, 1978a), but this effect can also be found after additions of more complex C sources (Anderson & Domsch, 1978b; Wu *et al.*, 1993). The C available to soil microorganisms is ultimately derived from plant photosynthesis and it could, therefore, be expected that processes or factors, which determine the availability of soil C, have the proximal control on microbial activity.

A coupling between plant production and the microbial biomass in the forest floor was found by Zak *et al.* (1994) in a study of a continent-wide gradient in North America. Myrold (1987, 1989) reported positive correlations between net primary production and microbial biomass C and N in the mineral soil. Zak *et al.* (1990) also suggested that patterns of C and N cycling within an old-field chronosequence were predictable and positively correlated to the accretion of plant and microbial biomass. It is expected that low soil microbial biomass and activity should be found when plant production or foliar litter production is low and the soil C/N ratio is high (Wardle, 1992), which is often associated with slowly degradable litter with high lignin/N ratio. In contrast, when high plant productivity is found, saprotrophic microbes are expected to gain C and N from a higher supply of litter (Wardle, 1992) (often easily degradable foilar litter with low lignin/N ratio) and the microbial release of C and mineral N should be higher. This in turn should favour plant growth and further litter production. Thus, the conventional theories link high microbial activity to high turnover of nutrients. Differences in plant productivity and plant community structure are therefore often thought to be associated with differences in microbial activity, in particular. However, this assumption has not been tested with regard to the large local landscape variation in forest productivity found in Fennoscandian boreal forest.

Plant roots proliferate preferentially in the humus beneath the litter layer, and this is the soil horizon where most of the plant N is taken up (Vogt *et al.*, 1983). The importance of the forest floor in nutrient cycling has long been recognized; (e.g., Hesselmann, 1926; Romell, 1935; Flanagan & Van Cleve, 1977; Cole, 1981; Covington, 1981; Federer, 1984; Prescott *et al.*, 2000).

Roots sensing low nutrient supply induce production of growth promoting cytokinins, alternatively, more carbohydrates in the leaves implies that more photosynthate can be translocated to the roots (Lambers *et al.*, 1998). Under limiting nutrient supply there is considerably higher C allocation to the below-ground parts, i.e. to root growth, root respiration, N<sub>2</sub> fixation, root exudation and mycorrhizas (van der Werf *et al.*, 1994). Thus, the supply of the plant limiting nutrient in soil significantly affects plant growth and biomass allocation. The ectomycorrhizal symbiosis is known to be sensitive to the N availability in soil and changes in the plant C allocation to the ECM fungus will affect its performance (Wallenda & Kottke, 1998). In an experiment in a *Pinus* forest, fertilization led to a 50% decline in relative allocation of photosynthates to roots, while total net photosynthesis doubled (Cannell, 1989). In addition to soil nutrient status, the season determines patterns of C allocation in trees. In temperate coniferous tree species root growth activity shows a bimodal course with maxima in autumn and spring prior to bud break (Hansen *et al.*, 1997).

In response to low N supply, compared to optimum nutrient supply, plants also increase their production of tannins and lignins (Lambers *et al.* 1998). These substances are well known to slow down litter decomposition in soil, which may further aggravate the N shortage in the environment. It has been found that N concentration is a good predictor of limit values for litter decomposition (Berg & McClaugherty, 2003). Hence, plant species composition and the metabolic reaction of plants to soil nutrient status affects the build-up of the organic mor layer.

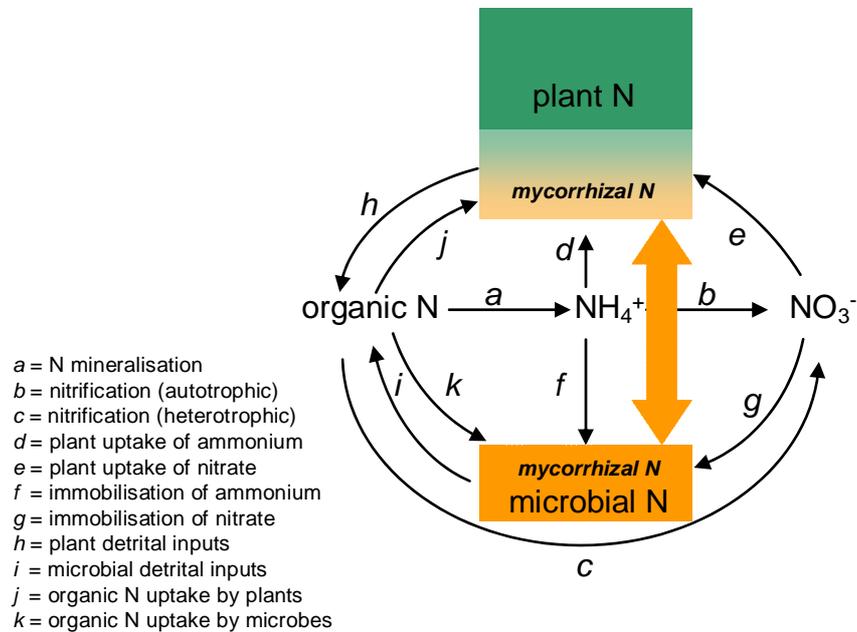
One of the first plant-microbe interactions observed, N immobilisation, was detrimental for plants and suggested strong competition between soil microbes and plants for soil N (Jansson, 1958). This is still an important subject for research (e.g., Kaye & Hart, 1997; Freeman *et al.*, 1998; Lipson *et al.*, 1999; Korsæth *et al.*, 2001). On contrary, the mycorrhizal symbiosis (Frank, 1877; Harley & Smith, 1983; Smith & Read, 1997) was found to be beneficial also for the plants. Romell (1935; 1938) studied natural forest mor-soils and found dense mycorrhizal fungal mycelium in the mor-layer of boreal forest soils and associated the abundance of mycorrhizal sporocarps directly to the presence of hyphal connections to trees. Thick mycelial mats with high respiratory activities were also found in similar mor soils in Finland by Hintikka & Näykki (1967) and provide evidence of substantial local activity by mycorrhizal fungi. Ectomycorrhizal hyphal mat soils may contain 30-50% fungal rhizomorph material per g dry forest floor material equivalent to 2 to 600 km mycelium (Ingham *et al.*, 1991).

## **Mycorrhizal fungi and the N cycle**

Mycorrhizas are involved in nutrient uptake by most land plants (Harley & Smith, 1983). Nearly all tree root tips (>98%) in temperate and boreal forests are ectomycorrhizal (Taylor *et al.*, 2000). The external mycelium of all mycorrhizal types play a key role in uptake of nutrients by plants (Smith & Read, 1997). However, the mycorrhizal symbioses and their potential roles in nutrient turnover are often not considered in studies of N transformations. Jansson and Persson (1982) presented a detailed N cycle, which was divided into elemental, autotrophic and heterotrophic N cycles. Symbiotic N<sub>2</sub>-fixation was considered in the elemental N cycle, but mycorrhizal fungi were not included as an important component in any of the cycles. The most likely reason for this is that mycorrhizal fungi are difficult to characterize, since on one hand, with respect to their major C supply, which is photoassimilates, they are autotrophic organisms, but on the other hand, with respect to their taxonomic position, they are heterotrophic organisms. The symbiotic relationship links two kingdoms and two biologically distinct organisms.

Thus, the mycorrhizal fungi lie within an autotrophic-heterotrophic continuum which is illustrated (arrows) in the simplified N cycle (Fig. 1). Here, mycorrhizal fungi are intimately associated with the autotrophic plant (upper half), while mycorrhizal N at the same time cannot be distinguished from other microbial N (lower half) by available microbiological methods. Mycorrhizal N is not only reserved for its own N metabolism, which is the case for heterotrophic fungi, but partly also represents N on its way to the plant (Fig. 1). Mycorrhizal interactions

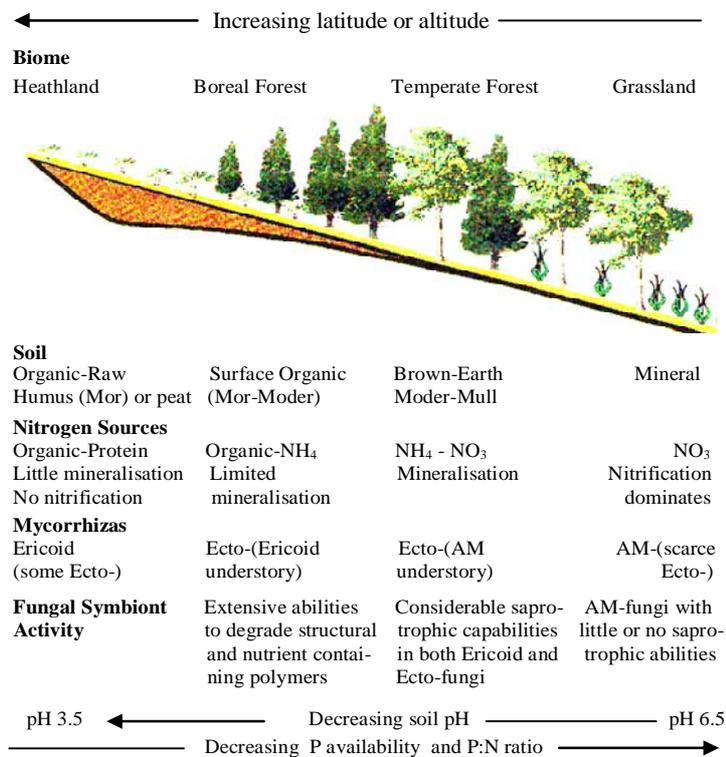
and their role in nutrient cycling are difficult to study since they involve complex interrelationships between the physiology of the plant and the physiology the fungal partner.



**Figure 1.** A simplified soil N cycle for terrestrial ecosystems (modified from Davidson *et al.*, 1992 and Hart *et al.*, 1994a); mycorrhizal N and organic N uptake by microbes and plants (Näsholm *et al.*, 1998) are introduced by the author. Note the presence of mycorrhiza in two positions (thick arrow), and that methods of soil microbiology in general are unable to differentiate between mycorrhizal fungi and conventional heterotrophs.

Another gap in our knowledge is the uncertainty about the ecological role played by the mycorrhizal fungi in N cycling. Read (1983; 1986; 1991; 1993) presented a model, which suggests that the distinctive changes in plant community composition along gradients of increasing altitude or latitude are paralleled by certain changes in the type of mycorrhizal association of the characteristic dominant plants (Fig. 2).

Read proposed that under cold and wet conditions, decomposition of organic matter will be slow and incomplete, resulting in the formation of acid peaty soils or thick mor-layers with slow mineralisation of N. Under these conditions, ericaceous dwarf shrubs with ericoid mycorrhiza, with a high capacity to use organic N sources, dominate. Further towards warmer and drier conditions, decomposition is more complete, resulting in thinner and less acid organic mor-layers, in which  $\text{NH}_4^+$  is a significant plant N source along with organic sources. These conditions are thought to be typical of the boreal forest, where trees are commonly ectomycorrhizal. Under still warmer conditions, in nemoral forests, higher soil pH and mull formation prevails, and  $\text{NO}_3^-$  becomes more important as a plant N source at the same time as arbuscular mycorrhiza becomes important.



**Figure 2.** Relationships, on a northern hemisphere based continental scale, among the distribution of biomes along environmental gradients and the roles of the prevailing mycorrhizal association in facilitation of N and P capture by the characteristic functional groups of plants, as suggested by Read and co-worker (Read 1983, 1986, 1991, 1993, Read & Perez-Moreno, 2003). Redrawn from Read & Perez-Moreno (2003). Note that soil pH is from Read (1991). With permission from *New Phytologist*.

Recent research in boreal forest has been unable to detect differences in the capacity to take up amino acids among plants with ericoid, arbuscular and ectomycorrhiza (Näsholm *et al.*, 1998). However, these different types of mycorrhiza might still differ in their capacity to use more complex N sources.

## Objectives

The microbiology underlying the large differences in forest productivity across Fennoscandian boreal landscapes has not been explored. This thesis represents a first attempt to describe variations in microbial activity, biomass and community structure associated with shifts in soil chemistry and plant productivity and community composition in this context. The main study area was a local natural gradient through a coniferous forest, representing three forest types typical of the Fennoscandian boreal landscape. A separate destructive study, involving forest girdling, was conducted to enable an estimate of the biomass of ectomycorrhizal fungi, in a low productivity forest, similar to the poor nutrient supply end of the the main study area.

The specific objectives were:

- To describe, in detail, soil conditions and plant community composition along a nutrient supply gradient (**Paper I**).
- To estimate the contribution by ectomycorrhizal extramatrical fungal mycelium to microbial biomass (**Paper II**) by a tree girdling experiment.
- To describe the soil microbiology (**Paper III**) and assess the contribution by ectomycorrhizal roots and extramatrical mycelium to microbial activity and microbial biomass along the natural N supply and forest productivity gradient (**Papers III-IV**).
- To link soil N dynamics to soil chemistry, plant community composition, mycorrhizal associations and microbial community composition in general (**Paper V**).

## Methodological considerations

### Study site descriptions

#### *The natural forest productivity gradient: Site Betsele*

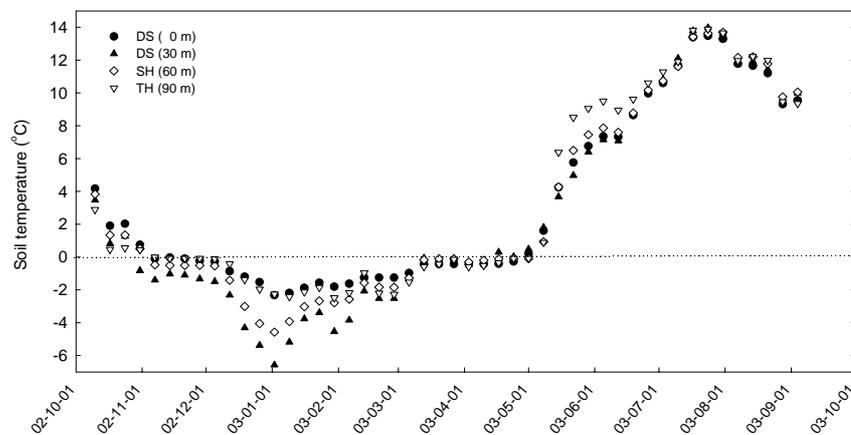
Studies (**Papers I, III-V**) were conducted along a 90-m-long forest productivity gradient, located at the bottom of the Umeå River valley close to the village Betsele, northern Sweden (64°39' N, 18°30' E, 235 m above sea level). The soils along the gradient have the same texture and are sandy till soils classified as Haplic Podzols (FAO, 1988) and contain many boulders.



**Figure 3.** End-points of the landscape sequence in northern Sweden; the low productive dwarf-shrub forest type (DS) from 0 to 40 m (left) dominated by Scots pine and *Vaccinium* dwarf shrubs; to the high productive tall-herb forest type (TH) at 90 m with 36 m tall Norway spruce and a diverse herbaceous layer with, e.g., *Actaéa spicata*, *Aconitum septentrionale* and *Rubus idaeus* (right).

The poor end of the forest productivity gradient is located in an extensive recharge area with c. 130-yr-old open *Pinus sylvestris* (nomenclature of species follows Tutin *et al.* (1964-1980) forest and towards the high productive discharge area there is an increasingly dense *Picea abies* forest of similar age (Fig. 3).

Mean annual temperature and precipitation are 1.0°C and 570 mm, respectively. The period with snow cover averages 170 days between late October and early May. The gradient does not encompass a complete slope spanning from the water divide to the discharge area. Some of the water may have flowed 700-800 m through the soils and bedrock up-slope of the discharge area. The slope is only 2%. The discharge area comprises only the last 10 m of the transect. Surface discharge usually occurs for about one or a few weeks during snow melt, but may also occur under exceptionally wet conditions in summer and autumn. Discharge events can be short and very dynamic. The groundwater level may rise and fall several decimeters in a day. No studies were performed during discharge events. The more open forests in the beginning, 0 m, and in the end of the gradient, 90 m, showed higher soil temperatures during the coldest winter period compared with the rest of the transect (Fig. 4). At 90 m, flush of discharge water may enhance soil thawing in the spring, leading to somewhat higher soil temperatures earlier in the season.



**Figure 4.** Soil temperatures during 48 weeks between 10 October 2002 and 4 September 2003 at c. 7 cm depth between the boundary between the mineral soil and the mor layer at 0, 30, 60 and 90 m along the forest productivity gradient at Betsle, northern Sweden. Data are weekly mean temperatures.

Three forest types have been defined along the gradient (**Paper I**). At the poor upper portion (0-40 m) in the dwarf shrub forest type (DS), plants with either ectomycorrhiza (ECM) or ericoid (EM) mycorrhiza dominate. The field layer is dominated by ericaceous dwarf shrubs; e.g., *Vaccinium myrtillus*, *V. vitis-idaea*, *Empetrum hermaphroditum*. In the short herb forest type (SH) at 50-80 m, plants with arbuscular mycorrhiza (AM) become prominent along with ECM and EM species. Several short herbs are abundant, e.g., *Oxalis acetosella* *Maianthemum bifolium* and *Solidago virgaurea*. At 90 m in the tall herb forest type (TH), which is now a glade caused by tree falls, AM or non-mycorrhizal species totally dominate, e.g., *Aconitum septentrionale*, *Actea spicata* and *Rubus idaeus* (**Paper I**). The *Pinus* forest (here represented by 0-40 m) has an average productivity (as estimated by two independent methods applied to areas >1 ha) of 2.9 m<sup>3</sup> stem wood ha<sup>-1</sup> yr<sup>-1</sup>, while the *Picea* forest (50-90 m) has an average productivity of 8.0 m<sup>3</sup> stem wood ha<sup>-1</sup> yr<sup>-1</sup> (P. Högberg and L. Kinell, unpubl. data). Around the low end of the gradient (90 m) windfalls have opened up the forest and the productivity cannot be accurately quantified. The potential productivity should, however, be

even higher at the very end of the gradient (90 m) as the tallest trees (>36 m) with stem diameters of 0.75 m (at breast height) are found close to that area.

### *The forest girdling experiment: Site Åheden*

#### Site characteristics

This study (**Paper II**) was conducted in a low productive, naturally regenerated 45-55-year-old Scots pine (*Pinus sylvestris* L.) forest (Fig. 5) located on a weakly podzolized sandy silt sediment at the forest site called Åheden, northern Sweden (64°14'N, 19°46'E, 175 m above sea level). The climate is similar to that at site Betsele (see above) with a mean annual temperature of 1.0°C (1980-99), and a mean annual precipitation of 600 mm (1981-97). Usually there is a snow cover for six months between late October and early May. For detailed soil temperature data see Högberg *et al.* (2001b). There is a sparse understorey of *Calluna vulgaris* and *Vaccinium vitis-idaea*. The bottom layer consists of mainly *Cladonia* spp. lichens and *Pleurozium schreberi* moss (Fig. 5). The mor-layer (F+H horizons) is only 2 cm thick.



**Fig. 5.** The Scots pine forest at Åheden, 100 km east of the main study area (forest site Betsele), in northern Sweden. The photograph was taken shortly after the large-scale girdling, during which c. 120 stems per plot were girdled in the summer of 2000.

## Methods applied

### *Site Betsele*

#### Microbial biomass C and N (**Paper III**)

The principle for the fumigation extraction (FE) method is an exposure of soil microorganisms to chloroform vapor, which destroys the cell membranes. The cytoplasmic C and N leaks out into the soil, from which it is extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub>, passed through a filter and analysed. A soil sample not treated by chloroform, but extracted in the same way as the fumigated soil, acts as a control (Brookes *et al.*, 1985a; Brookes, 1985b; Vance *et al.*, 1987). Microbial biomass C/N ratios presented in this thesis are based on FE. In another of the methods applied, substrate induced respiration (SIR), the glucose-induced increase in respiration of incubated soil was used as a measure of microbial biomass (Anderson & Domsch, 1978a; Nordgren *et al.*, 1988a; Nordgren, 1988b). Extracted adenosine triphosphate (ATP) was also used as a measure of microbial

biomass. ATP in soil pre-incubated for a week at 25 °C was determined by using the reaction luciferin-luciferase (Eiland, 1983; Arnebrant & Bååth, 1991). The total amount of membrane bound phospholipid fatty acids (total PLFA) was extracted according to (Frostegård *et al.*, 1991; Frostegård & Bååth, 1996) and used as another estimate of microbial biomass. Bacteria were counted microscopically after AO staining of bacteria extracted from soil (Söderberg & Bååth, 1998). Analyses of ATP, PLFA and counting (and measurements of activity) of bacteria were performed in another laboratory.

#### Microbial community structure (**Paper III**)

The composition of the microbial community was determined by analysis of group specific PLFAs in soil (Tunlid & White, 1992; Frostegård *et al.*, 1993; Frostegård & Bååth, 1996; Frostegård *et al.*, 1996). Specifically, the PLFA 18:2 $\omega$ 6, 9 was used as an indicator of fungal biomass (Federle, 1986), and the sum of the specific bacterial PFLAs as an indicator of the bacterial biomass. Lipids were extracted from fresh soil (1 g) using the Bligh & Dyer (1959) extraction technique as modified by Frostegård *et al.* Polar lipids (phospholipids) were separated on silica acid columns, dried under N<sub>2</sub> and subjected to mild alkaline methanolysis. The resulting fatty acid methyl esters were analysed on a gas chromatograph with a flame ionisation detector. Methyl nonadecanoate fatty acid was used as the internal standard.

#### Microbial activity (**Paper III**)

The total microbial activity in sieved root-free soil samples was estimated by measuring basal respiration at 20 °C (Nordgren *et al.*, 1988a; Nordgren, 1988b). Bacterial activity was estimated as the rate of thymidine incorporation (TdR) into bacteria extracted from soil after homogenization and centrifugation (Bååth, 1992). To examine the extent, to which the bacterial community in the field is adapted to the pH of the bulk soil (Bååth, 1996a), bacteria were separated from soils (Bååth, 1996b), incubated in two different buffers (pH 7 and pH 4), and the ratio between the rates of thymidine incorporation at the two pH levels was used as an index of the pH tolerance of the bacterial community. The rate ratio was then related to the pH in soil (soil:water ratio, 1:5). A high ratio was taken to indicate a community well adapted to high pH conditions, and vice versa.

#### Field basal soil respiration (**Paper III**)

Gas was sampled sequentially from headspaces placed on the ground after removal of mosses and plant shoots. Analyses of CO<sub>2</sub> was performed on an ANCA-NT (Europa Scientific Ltd, Crewe, U.K.) (Högberg & Ekblad, 1996; Högberg *et al.*, 2001b). Note that these measurements also include root respiration.

#### Microbial activity response to trenching (**Paper IV**)

Ten-cm diameter plots were trenched through the mor-layer down to the uppermost mineral soil with an auger at every tenth meter along three 10 m long transects perpendicular to the 90-m-long forest productivity gradient at site Betsele. Sampling sites were chosen to exclude plant shoots. To ensure that all roots were cut, each soil core was lifted up and then placed back into its initial

position. After 24 days of pre-incubation *in situ* in the field, the trenched mor-layer samples and control samples were recovered. During transportation and manual sorting out of roots (>2mm), samples were kept at temperatures of  $10.3 \pm 0.6$  °C (mean $\pm$ SD). The microbial activity ( $\mu\text{g CO}_2\text{-C h}^{-1}\text{g}^{-1}\text{o.m.}$ ) in 10 g of fresh root free soil from each sample was measured at  $20 \pm 0.1$  °C in a respirometer (Respicond V, Nordgren Innovations, Djäkneboda, Sweden) during a period of 258 h. The relative decrease in microbial activity after trenching was calculated using the following equation:

$$\text{Relative decrease (\%)} = 100 \times [(\text{microbial activity}_{\text{control}} - \text{microbial activity}_{\text{trenched}})] / \text{microbial activity}_{\text{control}} \quad (\text{eq. 1})$$

#### Net mineralisation and nitrification rates (**Paper V**)

Rates of net N mineralisation were determined in the absence of plant N-uptake and plant N-inputs (Nadelhoffer *et al.*, 1984). Long-term net mineralization and nitrification rates were determined in root free soil equivalent to 25 g (d.m.) in O<sub>2</sub> permeable polyethylene bags and incubated in the field beneath the mor-layer. After 7 weeks, pool sizes of ammonia and nitrate were used to estimate mineralisation rates (Eno, 1960). The same methodology was used for short-term net mineralisation and nitrification rates, but these soils were incubated 24 h only. Time zero denotes initial levels of inorganic N and t denotes inorganic pool size at the end of the incubation period.

$$\text{Net N mineralisation} = [(\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_t - (\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N})_{t_0}] / t \quad (\text{eq. 2})$$

Similarly, net rates of nitrification were calculated from the net change in the soil NO<sub>3</sub><sup>-</sup> pool size:

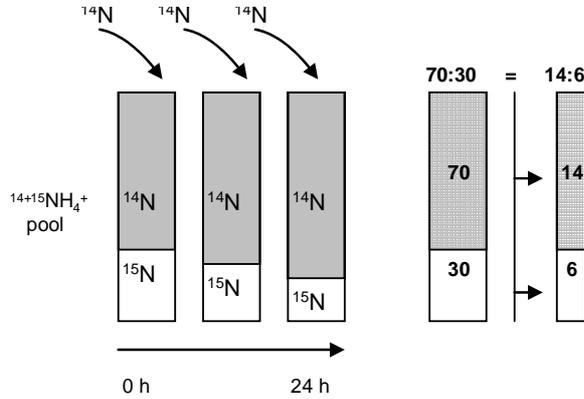
$$\text{Net nitrification} = [(\text{NO}_3^-\text{-N})_t - (\text{NO}_3^-\text{-N})_{t_0}] / t \quad (\text{eq. 3})$$

#### Gross N turnover rates (**Paper V**)

Actual N transformation rates, i.e. gross N mineralisation or gross nitrification rates include the whole production of NH<sub>4</sub><sup>+</sup> (or NO<sub>3</sub><sup>-</sup>) in soil and microbial immobilisation of NH<sub>4</sub><sup>+</sup> (or NO<sub>3</sub><sup>-</sup>). Gross N mineralisation thus encompasses all consumptive processes of NH<sub>4</sub><sup>+</sup>, while gross nitrification includes all consumptive processes of NO<sub>3</sub><sup>-</sup>. This technique relates the changes in <sup>15</sup>N abundance of the labelled inorganic N pool to the rate of N mineralisation (or nitrification) (Fig. 6).

We established a simple field laboratory. Mor layer soil, F+H (approximately corresponding to Oe + Oa), were sampled and roots were sorted out, whereafter the remaining soil was mixed and fresh soil equivalent to ~75 g (f.w.) was put into plastic bags. A total of 3.5 mL 98 atom % <sup>15</sup>NH<sub>4</sub>Cl or Na<sup>15</sup>NO<sub>3</sub> was dispersed slowly into each soil sample by three separate injections using a syringe and needle. The amount injected corresponded to  $2.1 \pm 0.3$  (mean $\pm$ SD)  $\mu\text{g N/g}$  dry organic matter. Within 30 seconds after injection (time zero = t<sub>0</sub>), 125 mL 2 M KCl was added. Samples and the extractant were gently mixed by hand at intervals. Extracts were filtered after 1 h extraction time in a nearby indoor laboratory. A parallel sample, denoted t<sub>24</sub>, was placed in O<sub>2</sub> permeable polyethylene bags and incubated in the field under the mor-layer in the forest type, from which the soil was sampled, for 24 hours before extraction. A diffusion method served the

purpose of concentrating and transferring dilute inorganic  $^{14+15}\text{N}$  from the soil extract as  $\text{NH}_3$  onto a solid substrate for subsequent  $^{15}\text{N}$  analysis. Diffusion was performed using the methods by Sørensen & Jensen (1991) and Stark & Hart (1996).



**Figure 6.** Principle of the pool-dilution technique in which the product pool is labelled (soil ammonium or nitrate pool) and the  $^{15}\text{N}$  abundance of that pool will decline when  $^{14}\text{N}$  is released during N mineralization (Left panel). Note that proportional exploitation of the soil ammonium (or nitrate) pool containing labelled N ( $^{15}\text{N}$ ) and unlabelled N ( $^{14}\text{N}$ ), will not alter the  $^{15}\text{N}$  abundance of the pool under study, i.e. there is no preferential use of added  $^{15}\text{N}$  (Right panel). Figures are redrawn from Powlson & Barraclough (1993).

Gross mineralization ( $m$ ),  $\text{NH}_4^+$  immobilization ( $c$ ), gross nitrification, and  $\text{NO}_3^-$  immobilization rates were calculated using equations (4) and (5) of Kirkham & Bartholomew (1954), which are described below. The term immobilization, as used in the original article, is here replaced by consumption and refers to the sum of all consumptive processes of the labelled pool (Davidson *et al.*, 1990; Davidson *et al.*, 1991).

$$m \text{ (or } n) = [(M_0 - M_1)/t] \log(H_0M_1/H_1M_0)/\log(M_0/M_1) \quad (\text{eq. 4})$$

$$c_a \text{ (or } c_n) = [(M_0 - M_1)/t] \log(H_0H_1)/\log(M_0/M_1) \quad (\text{eq. 5})$$

Where:

- $M_0$  = initial  $^{14+15}\text{NH}_4$  pool size ( $\mu\text{g N g}^{-1}$  o.m.)
- $M_1$  = post-incubation  $^{14+15}\text{NH}_4$  pool size ( $\mu\text{g N g}^{-1}$  o.m.)
- $H_0$  = initial  $^{15}\text{NH}_4$  pool ( $\mu\text{g N g}^{-1}$  o.m.)
- $H_1$  = post-incubation  $^{15}\text{NH}_4$  pool ( $\mu\text{g N g}^{-1}$  o.m.)
- $m$  = mineralization rate ( $\mu\text{g N g}^{-1}$  o.m.  $\text{d}^{-1}$ )
- $n$  = nitrification rate ( $\mu\text{g N g}^{-1}$  o.m.  $\text{d}^{-1}$ )
- $c_a$  =  $\text{NH}_4^+$  consumption rate ( $\mu\text{g N g}^{-1}$  o.m.  $\text{d}^{-1}$ )
- $c_n$  =  $\text{NO}_3^-$  consumption rate ( $\mu\text{g N g}^{-1}$  o.m.  $\text{d}^{-1}$ )
- $t$  = time (1 d for the present study)

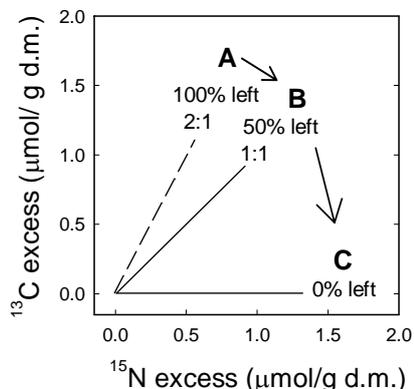
We labelled and analysed the soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools separately, i.e. for samples that received  $^{15}\text{NH}_4^+$ , the  $\text{NH}_4^+$  pool was used for M and H, respectively. For samples that received  $^{15}\text{NO}_3^-$ , the  $\text{NO}_3^-$  pool was used for M and H in Eqns. (4) and

(5) and the symbol  $m$  was replaced by  $n$ , which is the nitrification rate ( $\mu\text{g N g}^{-1}$  o.m.).

#### Decomposition of universally labelled ( $^{15}\text{N}$ , $^{13}\text{C}$ ) amino acids (**Paper V**)

This novel method enables studies of *in situ* decomposition of amino acids (or proteins) in the soil (Näsholm *et al.* 1998; 2001; Persson *et al.* 2003). Carbon of carboxy groups is rapidly lost as  $\text{CO}_2$  (g) during the decomposition of amino acids in soil (Fokin *et al.* 1993, Näsholm *et al.* 2001). Injection of the double-labelled compound into the soil results in a certain  $^{13}\text{C}/^{15}\text{N}$  molar ratio (calculated in excess of the natural backgrounds of  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ , respectively). When it is added to soil, the tracer will be diluted differently in different samples depending on their original C and N contents and isotopic ratios. The excess ratio will be that of the original tracer compound, unless it has started to decompose.

The use of linear regressions  $^{13}\text{C}$  vs.  $^{15}\text{N}$  made it possible to determine the rate of decomposition with no need to make a precise budget for the tracer. The schematic drawing below illustrates the principle of the method (Fig. 7). In the case of universally labelled glycine, the relationship between  $^{13}\text{C}$  and  $^{15}\text{N}$  for intact amino acid (slope) is 2. Thus, when half of the amino acid decomposed, the slope is 1 for glycine (Fig. 7). Lastly, when all of the amino acid is mineralized the slope reaches zero. For example, if the slope changes from an expected value of 2 to 1.077, the decrease in abundance of intact glycine in soil was equal to  $(2-1.077)/2$  or 46%. When studying decomposition of  $\alpha$ -glycine ( $\text{H}_2\ ^{15}\text{N}^{13}\text{CH}_2\ ^{12}\text{COOH}$ ), in which only the  $\alpha$ -C is labelled, the  $^{13}\text{C}$  and  $^{15}\text{N}$  relationship is 1/1 when decomposition starts after injection and minor changes are, therefore, expected.



**Figure 7.** Schematic drawing illustrating changes of the  $^{13}\text{C}/^{15}\text{N}$  excess ratio during decomposition of a labelled amino acid injected into soil, here exemplified by universally labelled glycine with an  $^{13}\text{C}/^{15}\text{N}$  excess ratio of 2/1. A; theoretical slope is 2.0 when the amino acid is still undecomposed in soil. B; when 50% of the amino acid is decomposed the slope=1.0. C; when all amino acid is decomposed the slope=0.

Within three minutes (in the DS forest type only), 4 h, 24 h, and 6 days after injection, F+H soil was harvested from around each premarked individual injection point (core diam. 44 mm). Roots ( $>1$  mm) were quickly sorted out by hand. Root-free soil samples were placed in plastic bags and then immediately frozen between blocks of dry ice ( $-80^\circ\text{C}$ ), less than 3 minutes after sampling. During this work, disposable latex gloves were used to avoid cross contamination between samples. Dried ( $70^\circ\text{C}$ , 48 h) and milled soils were analysed on an ANCA-MS 20-20 (Europa Scientific Ltd, Crewe, U.K) for  $^{13}\text{C}$  and  $^{15}\text{N}$  abundances. Soil samples were analysed, in dual isotope mode, i.e.  $^{15}\text{N}$  and  $^{13}\text{C}$  abundances were measured sequentially on the same sample (Ohlsson & Wallmark, 1999). Atom % excess values were calculated by subtracting the mean natural abundances of  $^{13}\text{C}$  and  $^{15}\text{N}$

of water treated control soils (n=24) from the atom % value of  $^{13}\text{C}$  or  $^{15}\text{N}$  of the amino acid treated soil sample in each forest type separately.

Potential denitrification enzyme activity (**Paper V**)

Mor layer soil was sampled at every tenth meter along the gradient. In the laboratory, ten grams of root-free fresh soil from each position and 80 mL DEA solution (1mM glucose, 1mM  $\text{KNO}_3$ , 200 mg chloramphenicol/L) were added to a 125-mL serum bottle, which was sealed, evacuated and flushed with  $\text{N}_2$  and filled with acetylene equivalent to 10% of gas volume. Samples were shaken continuously. Gas samples were taken at 0, 0.5, 1.0, and 1.5 hours and analysed for  $\text{N}_2\text{O}$  (g) on a gas chromatograph (ECD-GC). Total  $\text{N}_2\text{O}$  concentration was calculated including  $\text{N}_2\text{O}$  dissolved in the solution, using a Bunsen coefficient of 0.632 (Tiedje, 1982).

### *Site Åheden*

Biomass of ECM, production of DOC (**Paper II**)

In early June 2000, the Scots pine trees on three square plots (900m<sup>2</sup>) were girdled 1.3 m above ground, by complete removal of the bark over 0.3-m long sections around circumferences of the stems (early girdled plots). In mid-August 2000, trees on three more plots were girdled (late girdled plots) (Högberg *et al.*, 2001b) (Fig. 5). Girdling completely stops the flow of photosynthates in the phloem from the canopy to the roots and ectomycorrhizal fungi, but water and nutrients can still be taken up from soil via the xylem for some time. Girdling enabled estimates in the field of the contribution of extramatrical mycelium of ECM fungi to soil microbial biomass and by ECM roots and extramatrical ECM mycelia to production of dissolved organic carbon (DOC). Determination of microbial C and microbial N in root-free mor layer soil was performed by using the fumigation-extraction (FE) technique. Extractable DOC was determined on filtered extracts of non-fumigated soil.

## **Results and Discussion**

### **The natural forest productivity gradient: Soil chemistry and plants**

#### *Soils*

Selected soil and microbiological characteristics of the superficial mor-layer (F+H horizon) in the dwarf shrub (DS), short herb (SH) and tall herb (TH) forest types, are presented in Table 1. Soil C/N ratios ranged from 41 in the DS type (at 0 m) to 19 in the TH type at 90 m and pH in the soil solution increased 2.6 pH units (Table 1). The total N pool in the mor-layer, in kg per hectare, increased c. 3.6 times from the DS to the TH forest type,  $\text{NH}_4^+$  in the soil solution increased from 0 to 80 m, but from 80 to 90 m,  $\text{NO}_3^-$  became the most abundant inorganic N form in the soil solution (**Paper I**). However, in soil extracts (soil:water) the concentration of amino acids exceeds that of inorganic N in the DS type, as shown by Nordin *et al.*,

(2001) is equal to inorganic N in the SH type and lower than inorganic N in the TH type.

**Table 1.** Selected characteristics of the mor-layer of the two study sites, namely the forest productivity gradient (site Betsele) and the Scots pine forest (site Åheden). Abbreviations for different forest types at Betsele; dwarf shrub type (DS) between 0 and 40 m, short herb type (SH) between 50 and 80 m and tall herb type (TH) at 90 m. The forest type at Åheden is a dwarf shrub (DS) forest type and data are from control plots. Data are means  $\pm$  1 SE.

Soil Property	Forest types			
	Betsele			Åheden
	DS	SH	TH	DS
<i>Chemical</i>				
pH in soil solution †	3.8 $\pm$ 0.0a	4.9 $\pm$ 0.2b	6.4 $\pm$ n.d.	n.d.
pH (soil:water, 1:3) ††	4.2 $\pm$ 0.0a	5.3 $\pm$ 0.2b	6.0 $\pm$ n.d.	4.0 $\pm$ 0.0a
Organic matter (% of d.s.) ‡	91.0 $\pm$ 1.4a	71.0 $\pm$ 2.1b	49.6 $\pm$ 2.0c	75.0 $\pm$ 2.0b
Organic matter (kg/m <sup>2</sup> ) ‡	2.6 $\pm$ 0.3ab	2.3 $\pm$ 0.2ab	4.6 $\pm$ 1.0a	1.8 $\pm$ 0.0b
N (% of o.m.) †††	1.2 $\pm$ 0.0a	2.3 $\pm$ 0.1bc	3.2 $\pm$ 0.2b	1.6 $\pm$ 0.1ac
C : N ratio †††	40.8 $\pm$ 1.2a	24.5 $\pm$ 0.5b	18.8 $\pm$ 0.9b	39.8 $\pm$ 1.8a
Total N (g/m <sup>2</sup> ) †††	31.7 $\pm$ 1.2a	51.6 $\pm$ 1.7b	144.2 $\pm$ 9.4c	29.1 $\pm$ 1.0a
<i>Microbiological</i>				
Microbial C (mg/g o.m.) §	10.9 $\pm$ 2.2a	10.2 $\pm$ 3.5a	11.0 $\pm$ 3.5a	10.0 $\pm$ 1.4a
Microbial N (mg/g o.m.) §	0.9 $\pm$ 0.1a	1.4 $\pm$ 0.3a	2.3 $\pm$ 0.1b	1.1 $\pm$ 0.0a
Microbial C : N ratio §	11.7 $\pm$ 2.0a	6.9 $\pm$ 1.6b	4.8 $\pm$ 1.3b	8.9 $\pm$ 2.4a
DEA (mg C h <sup>-1</sup> m <sup>-2</sup> ) ¶	0.0 $\pm$ 0.0a	0.7 $\pm$ 0.3b	34.6 $\pm$ n.d.	n.d.
totPLFA microbial C (mg/g o.m.) *	6.7 $\pm$ 0.5a	8.0 $\pm$ 0.3b	8.0 $\pm$ n.d.	n.d.
Fungal biomarker (mol %)*	14.8 $\pm$ 1.5a	8.8 $\pm$ 1.7b	0.9 $\pm$ n.d.	n.d.
Fungi : bacteria ratio*	0.44 $\pm$ 0.09a	0.18 $\pm$ 0.02b	0.02 $\pm$ n.d.	n.d.
bactAODC (x 10 <sup>7</sup> ml <sup>-1</sup> )**	8.62 $\pm$ 0.91a	9.55 $\pm$ 0.07b	9.61 $\pm$ n.d.	n.d.

*Note:* Statistical comparisons between the three different forest types were not possible for soil pH, DEA, PLFAs and Bacterial AODC, since the TH forest type was represented by only one composite sample. However, statistical differences between the DS and SH forest types were tested by t-tests using the means of the F and H horizons, when these were measured separately, or the F+H, when they were not physically separated. Statistical analyses on microbial biomass C and N, microbial C/N ratio (n=5) and soil chemistry (n=9-10) were performed by one-way ANOVA (SIGMASTAT@3.0 SPSS Science, Chicago, IL, USA), followed by multiple comparison tests (Tukey's, Dunn's or Holm-Sidak test).

† Betsele (n=4-5), (**Paper I**).

†† pH in soil:water (1:3) (unpublished); Betsele n=4-5, 4 June 1994. Åheden n=9, 12 September 2000.

‡ Organic matter (o.m.) mass of FH layer, defined as % loss of dry matter after ignition of dried soil (n=1). Betsele (**Paper III**); Åheden (**Paper II**).

††† Betsele, unpublished n=10; Åheden, unpublished (n=9).

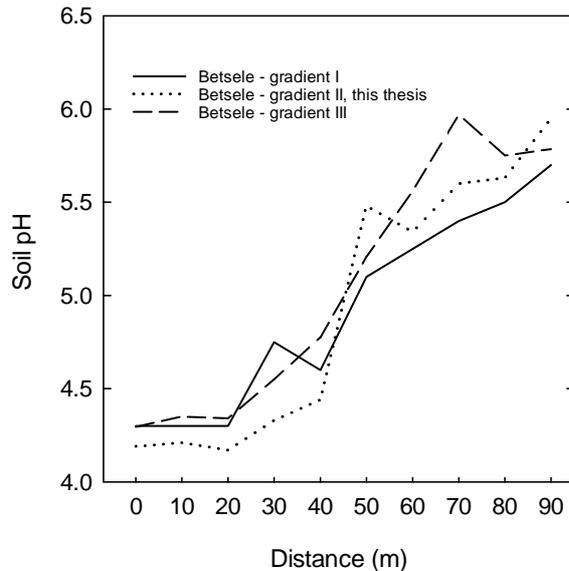
§ Fumigation extraction; Betsele seasonal means 1997, n=3 (**Paper III**); Åheden 12 September 2000, n=3. (**Paper II**).

¶ Denitrification enzyme activity potential (n=4-5) (**Paper V**)

\* Phospholipid fatty acid (PLFA) 18:2 $\omega$ 6,9 in the F horizon (**Paper III**). PLFA 18:2 $\omega$ 6,9 (mol %) / PLFAs bacteria (mol %) n=4-5 (**Paper III**).

\*\* Acridine orange direct counts (AODC); counting of stained bacteria extracted from soil (n=4-5) (**Paper III**).

We found strong positive correlations between N concentration in the mor layer and the soil solution pH ( $r=0.98$ ) and base saturation ( $r=0.88$ ) (**Paper I**). Also in two other 90-m-long gradients established 1989 and 2000 in the same area, there were correlations between N concentration and soil pH ( $r=0.92$  and  $0.87$ , respectively).



**Figure 8.** Soil pH (soil:water) at every 10th m along three parallel gradients (50 m apart) established in 1989 (I), 1990 (II) and 2000 (III) at Betsela. Note that the *soil solution* pH values reported in **Paper I** and in Table 1 are not included; those pH-values showed a similar, but steeper increase, with lower pH at 0 m and higher at 90 m as compared to the data displayed here.

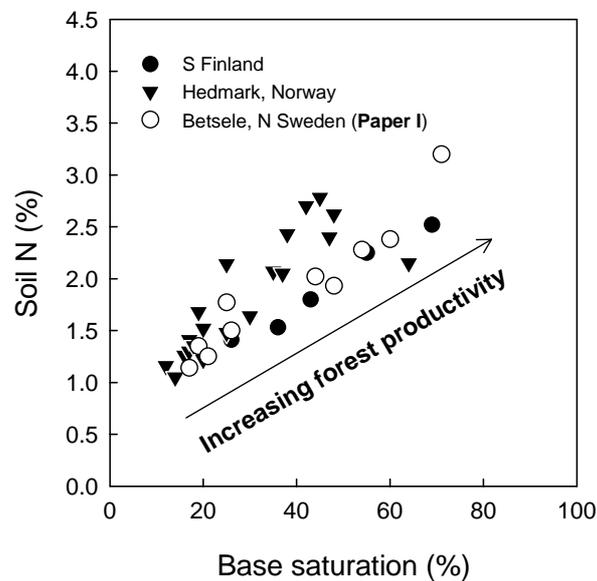
### Plants

Foliar analyses of N concentration showed a continuous increase along with the increase in soil N concentration. At 0 m, in three species out of six sampled, N concentration was below 1%, whereas at 90 m N concentration in all six sampled species exceeded 2.5%. The percentage P increased slightly between the DS and SH forest types. Thereafter, from the SH to the TH forest type, there was a significant decrease in P concentration. The quotient P/N also decreased significantly between SH to TH forest type to average ratio 0.1 for all species. A P/N ratio below 0.1 is considered to be a threshold for P limitation (Linder, 1995). These observations gave the first indications of P limitation among the plants in the TH forest type. The low P/N ratio and a  $^{32}\text{P}$  root uptake study both indicated the presence of P limitation in the TH forest type. The very low P availability in the soil in this forest type was confirmed in a subsequent study (Giesler *et al.*, 2002). The total fine root biomass of trees and understorey plants declined from c. 3700 kg ha<sup>-1</sup> in DS through c. 1650 kg ha<sup>-1</sup> in SH to c. 590 kg ha<sup>-1</sup> in the TH forest; the major portion of this decline was due to a loss of fine roots of ericaceous

understorey plants (**Paper III**). A decline of root density with N availability has been found both in fertilization experiments in red pine plantations (Haynes, 1995), Norway spruce stands (Majdi & Persson, 1995) and in natural forests with different site productivities (Vogt *et al.*, 1983).

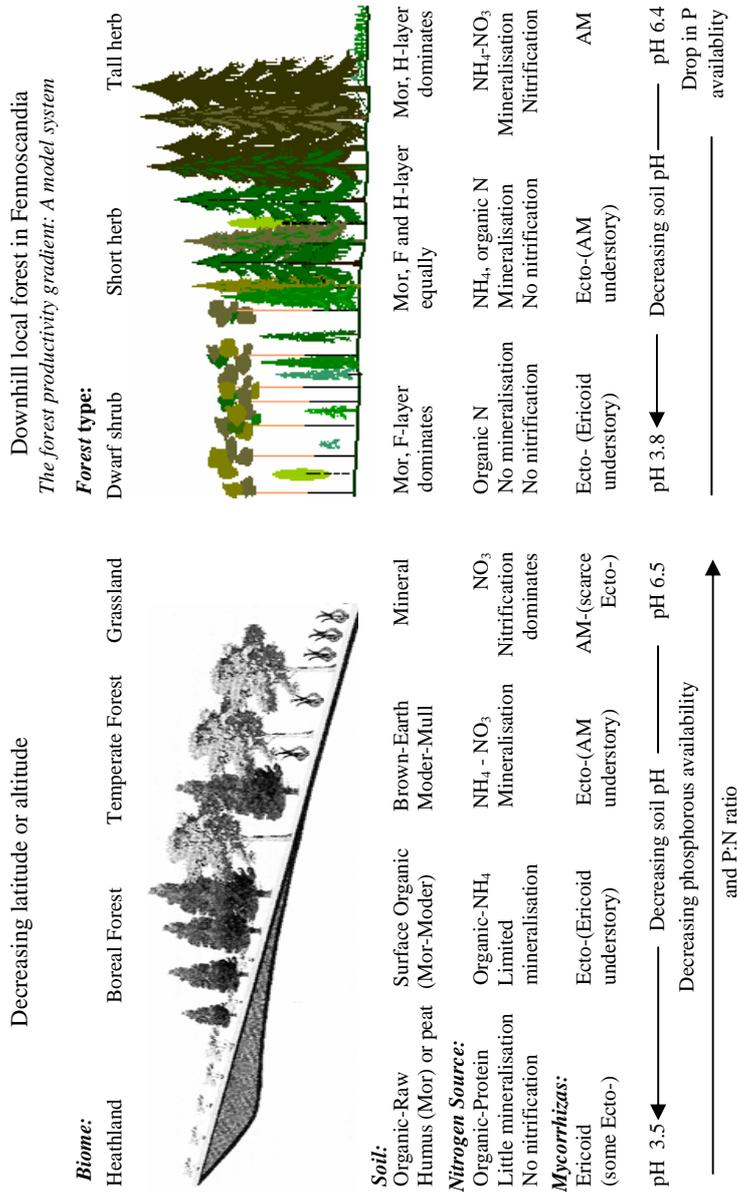
*A landscape perspective on soil conditions and plant community composition*

The gradient at Betsela apparently represents most of the range in soil pH, base saturation, N concentrations, forest production and plant community composition found in Fennoscandian boreal forests (Fig. 9). The previous regional surveys have demonstrated strong correlations between N concentration and base saturation in soils of different forest types in Norway (Dahl *et al.*, 1967) and Finland (Lahti & Väisänen, 1987). The data from the N supply gradient at Betsela clearly showed the same pattern of increasing forest productivity along with increasing N concentration and base saturation (Fig. 9) (**Paper I**).



**Figure 9.** The linear relationship between %N and % base saturation in the mor layer in different forest types representing increasing forest productivity in Fennoscandia (**Paper I**). Norwegian data are from Dahl *et al.* (1967) and represent forest types in the Hedmark province. Finnish data are from Lahti and Väisänen (1987) and represent mean values of major forest types in 921 forest stands in southern Finland.

We also found associations between certain soil conditions and plants with specific mycorrhizal types, as previously proposed as typical of different major biomes by Read (1983; 1986; 1991; 1993) (Fig. 10). It is noteworthy that such a large variability in these associations can be found within very short distances at a local landscape level (Fig. 10), and hence without any major variations in climate (Fig. 4). The large variations in soil conditions and plant community composition, and the generality of these variations in the context of the Fennoscandian boreal forests, make the Betsela transect a very valuable object of study.



**Figure 10.** Left panel; the proposed relationship between the distribution of biomes along gradients and the prevailing mycorrhizal associations with the characteristic functional groups of plants on a northern hemisphere based scale, as suggested by Read and coworker (Read & Perez-Moreno, 2003; Read (1983, 1986, 1991, 1993). Redrawn from Read & Perez-Moreno (2003). Note that potential fungal symbiont activity is not included and that soil pH is from Read (1991). With permission from *New Phytologist*. Right panel; a local scale (this thesis), where forest productivity gradients are often found along slopes in the Fennoscandian landscape. In this thesis the 90-m-long forest productivity gradient in northern Sweden is used as a model system for studies of soil-microbial-plant interactions in Fennoscandian boreal forests.

## The forest girdling experiment

### *The contribution by ectomycorrhizal mycelium to soil microbial biomass and to DOC*

A month after the late girdling treatment, soil microbial C was 41% lower in late girdled plots than in control plots, whereas at the same time, three months after early girdling, the soil microbial C was 23% lower in early girdled than in control plots (**Paper II**). Microbial N did not change significantly after girdling. A 30-50% decline in microbial C was also found in another recent forest girdling experiment in Norway spruce forest in Germany (Subke *et al.*, 2004). Thus, based on the average loss of microbial C in the early and late girdling treatments, at least 32% of the microbial biomass C was contributed by extramatrical ECM mycelium. This contribution was calculated to be equivalent to 145 kg ha<sup>-1</sup>, corresponding to 58 kg C ha<sup>-1</sup> at a fungal biomass C content of 40% by weight. This is lower than the 160 to 800 kg ha<sup>-1</sup> ECM extramatrical mycelium and mantles found by Wallander *et al.* (2001) after using decreases in PLFA18:2ω6,9 after trenching as an estimate of ECM mycelium in soil. However, Wallander *et al.* (2001) studied more productive forests in southern Sweden. Our estimate of a 32% contribution by extramatrical ECM fungi to microbial biomass should be a conservative estimate, primarily because the necromass of ECM mycelium in soil in girdled plots could be used as a substrate by other soil organisms.

Levels of extractable DOC were 45% lower in girdled than in control plots. In the mor layer of boreal forests the abundance of DOC is high (van Hees *et al.*, 2000). For the first time (**Paper II**), it was shown that ECM roots and fungi were directly involved in production of DOC at this scale in the field. This has also been found more recently in a girdling experiment in Wetzstein, Germany, where 37 and 75% lower DOC levels was found in the mineral soil in girdled plots in a young and an old Norway spruce stand, respectively, 3 months after girdling (Michael Andersson, personal communication). Griffiths *et al.* (1994) compared ectomycorrhizal mats soils with non-mat soils in forests dominated by Douglas-fir and found 5 to 360 times higher DOC levels in mat soils and the highest levels were recorded in autumn.

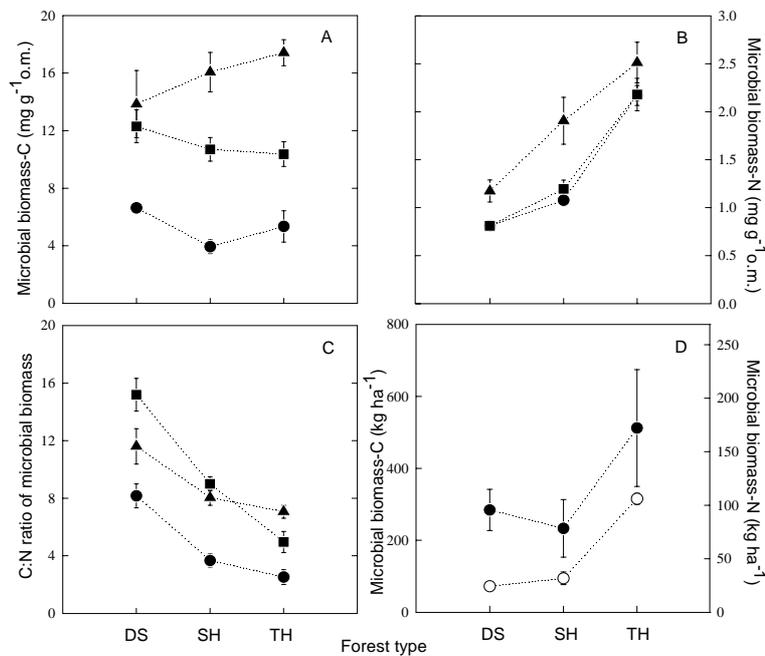
In boreal mor layer soil, low molecular organic acid C contribute 5 to 10% of DOC (van Hees *et al.*, 2000). The production of low molecular weight organic acids by roots is promoted by mycorrhiza and contributes to weathering of minerals (Lundström *et al.*, 2000; van Breemen *et al.*, 2000). One common view is that most of the DOC constitutes more or less recalcitrant substances left over from decomposition or is a refractory product of decomposition. However, the DOC produced is a potential C source for other microbes in the mycorrhizosphere (eg., Nurmiaho-Lassila *et al.*, 1997; Bonkowski, 2004). It is well known that microbial activity is very high in the rhizosphere as compared with the bulk soil.

## The natural forest productivity gradient: Soil microbiology

### Microbial biomass C and N

Levels of SIR, ATP and total PLFA increased over the gradient from the low productive DS to the SH forest type with the SH forest type having 20-30% higher microbial biomass (**Paper III**). The same order of change was found between the H and F horizons of the mor layer. In the high productivity TH forest type, the microbial biomass was similar to or lower than in the SH forest type. Mean microbial biomass as estimated by the total PFLA, SIR, ATP, and FE methods was 6.7, 5.9, 5.7, and 5.3 mg biomass-C g<sup>-1</sup> o.m., respectively.

Microbial biomass C per g o.m. as determined by the FE method did, however, not change along the gradient (Fig. 11A) (discussed later). Microbial biomass N (FE) increased from the DS to the SH forest type by 49% and from SH to the TH forest type by 64% when expressed as g<sup>-1</sup> o.m. Mean microbial biomass N was 0.9, 1.4 and 2.3 mg N g<sup>-1</sup> o.m. in the DS, SH, and the TH forest type, respectively (Fig.11B). The fractions of N immobilised in the microbial biomass in these forest types during the vegetation period were 7.6, 6.9, and 7.3% of total soil N, respectively.



**Figure 11.** Microbial biomass g<sup>-1</sup> organic matter in the mor-layer, estimated by the (fumigation extraction) FE technique along the Betsele forest productivity gradient June 17 (circle); August 4 (triangle); September 27 (square). A; microbial biomass C. B; microbial biomass N. C; microbial C/N ratio. D; seasonal means of microbial biomass C kg per ha (closed circle) and N kg per ha (open circle). Data are means ± SE. A-C; n=5; D, n=3.

The microbial biomass C and N and the microbial C/N ratios were within the range found in other coniferous forests. The mean microbial C along the gradient at Betsele, estimated by the four methods, was 1.4±0.1% (SD) of total organic C.

This was close to the 1.7% found in a coniferous boreal forest of similar age (Bauhus *et al.*, 1998), the 1.2% in Finnish Scots pine forests (Martikainen & Palojarvi, 1990), the 1.6% in Finnish Norway spruce stands (Smolander & Mälkönen, 1994), but slightly lower than the 2.2% based on FE (64 studies), SIR (9 studies) and microcalorimetry (1 study), previously found for boreal forests (Bauhus & Khanna, 1999). The mean contribution of microbial N to total N was 7.3%; almost the same concentration (7.5%) was found by Bauhus *et al.* (1998) in the forest floor of coniferous boreal forests. Thus, microbial C and N constitute rather constant fractions of total C and N along the gradient. The mean microbial C/N ratio over the Betsel gradient was c. 8 (Fig. 11C) compared to 9 in the study of boreal forests by Bauhus *et al.* (1998). In fact, the mean microbial C/N ratio of 4.8 in the TH forest is slightly below values found in many agricultural soils of 5.3 and 5.5 estimated by the FI and FE technique, respectively (Jenkinson, 1987; Joergensen *et al.*, 1994). However, Smith and Paul (1990) reported a microbial C/N ratio of 3.7 for cultivated soils. Microbial C and N expressed per area was similar in the poor DS and the SH forest type, whereas the TH forest type contained twice as much microbial C and almost four times more microbial N per area. This is because of accumulation of soil organic matter in this forest type.

In the following I will concentrate on differences in microbial biomass, community structure and activity between the DS and SH forest types, which show a three-fold difference in forest productivity. The TH forest type has significantly different forest and soil conditions (more open, periodically water-logged) compared to the rest of the gradient. Periodic anaerobic conditions and/or very high concentrations of N (Berg & McLaugherty, 2003) may, for example, explain the greater soil organic matter accumulation in the TH forest.

There were significantly higher microbial biomass C and N (FE) values in August than in June and the mean C/N ratio of the microbial biomass was highest in the autumn. (Fig. 11). Estimates of microbial biomass were found to increase in both a forest and a grass ecosystem when <sup>14</sup>C flow to roots increased in a bimodal pattern of early spring and late autumn, at the same time as soil water content increased during these periods, which was suggested to have a contributing effect on microbial activity (Smith & Paul, 1986). Soil fungal biomass, determined by the fluorescein diacetate (FDA) method (Bååth & Söderström, 1982) was studied in a mature and a clear-cut pine forest in Sweden, with and without slash. Bååth and Söderström observed a seasonality, that was most obvious in the treatment without slash, where FDA-active fungal length had the same seasonal pattern as soil moisture with high values during early spring and autumn. Biomass peaks of FDA active fungal mycelium in a Scots pine forest (Sweden) were registered in autumn and early spring (Söderström, 1979). Seasonality in microbial biomass may thus be linked not only to soil moisture but also to the suggested bimodal course of root growth activity, with maxima in autumn and spring (Smith & Paul, 1986) and prior to bud break (Hansen *et al.*, 1997). However, seasonality in microbial biomass was not found in a hardwood forest in northwestern Lower Michigan, after applying the fumigation incubation technique (Holmes & Zak, 1994).

All estimates of microbial biomass are based on root-free soils and all four

methods applied (SIR, ATP, total PLFA, FE) require corrections of raw data to be converted to soil microbial biomass data. The SIR, ATP and PLFA were calibrated against the SIR method, which in turn is calibrated to the fumigation incubation (FI) technique. The correction factors applied in the FE method was calibrated by microscopic counting (Martikainen & Palojärvi, 1990) (**Paper III**). As is discussed above, the microbial activity of mor soils seems to be very sensitive to disturbance and responds differently to incubation (**Paper IV**). Some important distinctions can be made between the four methodologies applied in **Paper III**, i.e. SIR, ATP, total PLFA, FE: i) whether they require a preincubation period (20 °C, c. 7-10 days for SIR, at 25 °C c. 5-7 days for ATP) or not (FE and total PLFA), ii) if they measure microbial activities in a direct manner (after glucose addition, SIR) or in an indirect manner (ATP, total PLFA), and iii) if the method is applied shortly after sampling (FE) or after different periods of storage of soil at 4 °C (SIR, ATP and PLFA). Hence, the FE method is valuable to use as the method can be applied directly after soil sampling without pre-incubation.

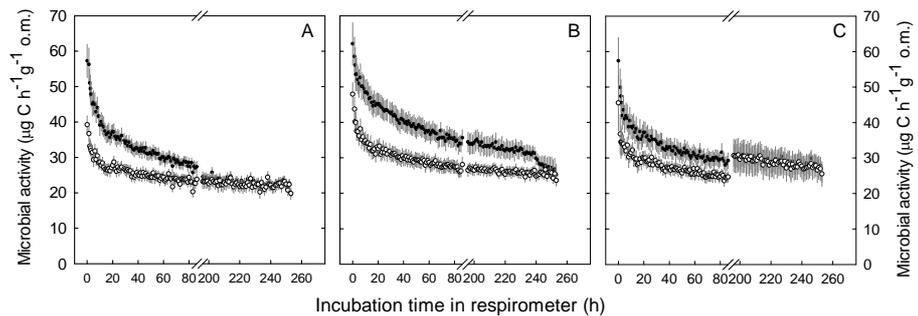
### *Microbial activity*

Microbial activity determined as CO<sub>2</sub> released g<sup>-1</sup> o.m., i.e. basal respiration, was significantly higher in sieved root-free soil from the intermediate SH compared to the low productivity DS forest type with increases between 28 and 37% depending on the sampling occasion. In soil samples from the TH forest type, the microbial activity was generally lower than in the SH forest type. However, there were no significant differences in field respiration rates, which comprise both root activity and microbial activity, between forest types. Field rates showed a seasonal trend with significantly higher rates throughout the gradient late in the summer. Interestingly, this high soil respiration rate coincided with the season when C allocation to the roots is thought to be highest in these forests (Hansen *et al.*, 1997). The weekly mean soil temperature increased c. 2.5 °C between late June and early August. At Åheden the heterotrophic respiration declined after a 20-day-long 6 °C decline in soil temperature, whereas root respiration did not decline (Bhupinderpal-Singh *et al.*, 2003). However, sometimes also a respiration peak in spring has been observed elsewhere (Smith & Paul, 1986; Griffiths, 1990). The larger above-ground plant biomass and three-fold higher productivity towards the productive end of the gradient at Betsele does not support a higher soil microbial activity (via root allocation and above-ground litter fall).

However, the stand girdling experiment (Högberg *et al.*, 2001b) suggests that the respiration of mycorrhizal roots is equal to or even exceeds saprotrophic microbial respiration in strongly N-limited boreal forests. Hence, a reduction in the activity of mycorrhizal fungi towards the high productivity end of the gradient could be significant. In another girdling experiment, fertilized and non-fertilized plots were compared, and the effect of girdling on soil respiration was clearly smaller in fertilized plots (Olsson, P; Högberg, P; Linder, S, unpubl.). This demonstrated that trees, which experience a higher nutrient supply, allocate less C below-ground (Cannell & Dewar, 1994). I propose that the same occurs as the nutrient availability increases along the Betsele gradient (**Paper III**). The result is that the microbial biomass C and overall microbial respiratory activity varies remarkably little along the gradient (**Paper III**), despite the large suspected

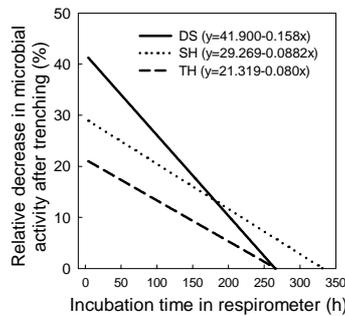
variation in rates of N cycling and the two-and-half fold increase in microbial N and three-fold increase in forest productivity.

In the trenching experiment (**Paper IV**), the microbial activity measured in root-free soil declined both in control soil excavated 6.5 h before measurements and in soil trenched 24 days earlier and preincubated in the field, initially by up to 38% during the first day of measurement (Fig. 12A-C). This may be caused by the soil handling, often called the sampling effect, since both treatments showed the same pattern. However, trenching decreased microbial activity by  $41.8 \pm 2.3\%$ ,  $28.2 \pm 2.0\%$  and  $21.8 \pm 3.0\%$  (means  $\pm$  SD) in DS, SH and TH, respectively, during the first 20 h of laboratory incubation (Fig. 12).



**Figure 12.** Microbial activity ( $\mu\text{g CO}_2\text{-C h}^{-1}\text{g}^{-1}\text{o.m.}$ ) in root-free mor-layer soil under controlled laboratory conditions at 20 °C. Soils were from the different forest types along the forest productivity gradient with increasing N supply from 10, 60 to 90 m. A; dwarf-shrub forest (10 m). B; short-herb forest (60 m). C; tall-herb forest (90 m). Control soil (closed circles) and trenched soils (open circles). Data are means  $\pm$  S.E., n=9-10.

Simple modelling of data on relative decreases in microbial activity predicted that after c. 11, 14 and 11 days in DS, SH and TH soils, respectively, the microbial activity in control soils would reach the same level as in trenched soils (Fig. 13).



**Figure 13.** Models predicting the time when control soils equal trenched soil in microbial activity *ex situ*, based on linear regressions of data on relative decrease in microbial activity after trenching. These simple models explained 85, 68, and 46 % of the total variation found in the relative decrease in microbial activity in the DS, SH, and TH forest soil, respectively (see also Fig. 17B).

In reality, the difference between control and trenched soils was gone after c. 8, 10 and 8 days in DS, SH and TH soils, respectively (Fig. 12A-C). The rates of decline in SH and TH soils were approximately the same, while the microbial activity in the DS soil declined twice as fast. i.e. 1.8-2.0 times faster (steeper slope) (Fig. 13). The difference between the modelled and the experimental data in number of days until the difference is gone, may be related to uncertainties in the estimates of microbial activity at the starting point of the experiment. Alternatively, an equation

taking into account shifts in respiration rate with time (see Fig. 18B) could improve the model.

Thus, the negative response increased with decreasing soil N supply but increasing ratio fungi/bacteria. The large variability in sensitivity to physical disturbance and incubation may complicate the interpretation of studies based on the activity of microbes, for example net and gross N mineralisation studies and microbial biomass estimates. As predicted, the soils with the highest predicted contribution of mycorrhizal extramatrical mycelium seemed to be the most sensitive to physical disturbance (**Paper IV**).

### *Microbial community structure*

The most dramatic change observed was the decrease in the fungal PLFA 18:2 $\omega$ 6,9, from around 15 % in the DS forest to around 1% in the TH forest (**Paper III**). Soil fungi have C/N ratios of 10 to 12 and bacterial ratios generally range from 4 to 6. The mean C/N ratio of the microbial biomass varied along the gradient with values of 11.7, 6.9, and 4.8 for DS, SH and TH forest types, respectively, indicating a much lower abundance of fungi in the SH and especially in the TH forest type. One reason for this decrease could be the pH increase across the gradient, since fungi are thought to be favoured compared to bacteria at low pH (Alexander, 1977; Hartel, 1999). In beech-oak forest, Bladogatskaya and Anderson (1998) found an increase in the ratio fungi/bacteria (measured with the selective inhibition technique) with decreasing pH. However, elsewhere using the PLFA or ergosterol methods to estimate fungal abundance, only small effects were found after considerable pH changes by liming, ash-treatment or alkaline pollution of coniferous forest soil (Fritze & Bååth, 1993; Frostegård *et al.*, 1993; Bååth *et al.*, 1995). In a recent study Bååth and Anderson (2003) found that the effect of pH on the ratio fungi/bacteria depended on the method used (PLFA or selective inhibition). Thus, in natural soils representing a range of over 4 pH units, there was no correlation between pH of the bulk soil and the fungal indicator PLFA 18:2 $\omega$ 6,9 (Bååth & Anderson, 2003).

Hence, the decreasing fungal biomass observed in direction of the groundwater discharge area at Betsele may not be a direct effect of soil pH *per se*. Alternatively, the decrease in fungal biomass reflects a decrease in extramatrical mycelium of mycorrhizal fungi, as a result of decreasing plant below-ground allocation in response to increasing nutrient supply. Changes in the bacterial community structure, as revealed by PLFA, however, appeared pH-related, e.g., variations in the PLFA 10Me18:0, indicative of actinobacteria, which are known to prefer higher pH. Several PLFAs indicative of bacteria increased in relative amounts along the gradient, e.g., 18:1 $\omega$ 7, common in gram negative bacteria, and 10Me18:0, indicative of actinobacteria. This increase was most marked with the PLFA br18:0 (indicative of gram positive bacteria), which was not found in several samples from the DS forest type, but increased to almost 1% of the total PLFAs in the TH forest type. The PLFA 16:1 $\omega$ 5, which has been suggested as an indicator of arbuscular mycorrhiza in soil (Olsson *et al.*, 1995; Olsson, 1999), accounted for c. 2% of total PLFAs in the DS forest type, and doubled in relative abundance in the TH forest type. The fungi to bacteria ratio decreased along with

decrease in soil pH and organic matter content in soil. The ratio of 0.02 found in the TH forest soil was similar to the lowest values reported among forest, grassland, arable, and garden soils in Frostegård & Bååth (1996).

Pennanen *et al.* (1999) studied the structure of the microbial community in Finnish coniferous forests and its relation to site fertility. Their gradient spanned variations in soil pH and C/N ratio of 3.95-4.22 and 37.4-27.9, respectively, i.e., a much smaller range than observed at Betsele. They observed no differences in SIR, total PLFA or basal respiration. However, they observed a narrowing in the ratio fungi to bacteria from 0.6 to 0.4. Thus, like me they found no variations in microbial biomass, but variations in community structure.

#### *Comparison between the F and H horizons*

We found almost twice as high microbial activity and about 30% higher microbial biomass (ATP, SIR, total PLFA) in the F than in the H horizon (**Paper III**). Community structure differed between the F and H layers. There was a lower fungi/bacteria ratio in the H horizon compared to the F horizon throughout the gradient. The F horizon had relatively higher amounts of the PLFAs 16:1 $\omega$ 7c, 18:1 $\omega$ 7 and 20:4 (eucaryotic PLFA), while the H horizon, especially, had relatively higher amounts of the PLFAs 19:1b, 20:0, cy19:0, 10Me16:0, 10Me17:0 and 10Me18:0 (**Paper III**). In **Paper I**, the mor layer was divided in two equally thick parts, O<sub>1</sub> and O<sub>2</sub>. This was not optimal since the contribution of F and H layer changes remarkably through the gradient. At the poor end, the H layer constitutes about 1/7, but at the rich end almost the entire mor is H. Hence, the contribution of the F horizon, the most active horizon, decreases along the gradient.

#### *Nitrogen turnover in soils*

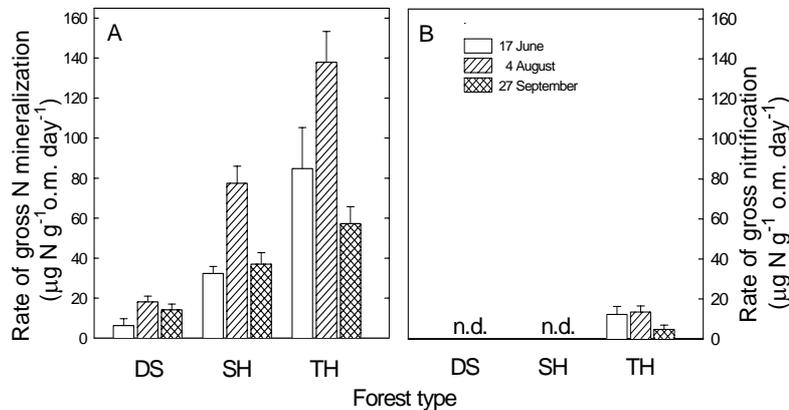
In the DS forest type, where the soil C/N ratio is 41, there was no net mineralization and NH<sub>4</sub><sup>+</sup> consumption exceeded the gross N mineralization rate. This system was, therefore, classified as strongly N-limited.

In the SH forest type, with a soil C/N ratio of 25, the pool size of NH<sub>4</sub><sup>+</sup> was larger than in the DS type, while gross NH<sub>4</sub><sup>+</sup> consumption still exceeded gross N mineralization. Net N mineralization occurred after >1 month long incubations. The SH type was thus classified as less N-limited than the DS forest type.

The TH type, with a soil C/N ratio of 19, was the only forest type, in which gross N mineralization exceeded gross NH<sub>4</sub><sup>+</sup> consumption; here net N mineralization was found already after 1 day of incubation. I thus classify this forest type as not limited by N. Gross nitrification was high although gross NO<sub>3</sub><sup>-</sup> consumption was higher in the TH forest type, which could be explained by strong abiotic and biotic sinks for nitrate; we found high potential denitrification enzyme activity in this forest type only. In the TH forest soil, P is strongly bound to sesquioxides (**Paper I**). Hence, N is not limiting because of a limitation by P and/or C. The very low C/N ratio of microbial biomass in this forest type, c. 5, supports the latter.

Seasonal means of gross NH<sub>4</sub><sup>+</sup>-N mineralisation rate were 12.8±3.5, 48.8±14.4, and 93.3±23.7  $\mu\text{g g}^{-1}$  o.m. day<sup>-1</sup> (mean±SE) in the DS, the SH, and the TH forest

type, respectively (Fig. 14). Seasonal means of  $\text{NH}_4^+$  consumption rates were  $15.6 \pm 3.1$ ,  $55.7 \pm 15.1$ , and  $76.1 \pm 30.3 \mu\text{g NH}_4^+\text{-N g}^{-1}\text{ o.m. day}^{-1}$  (mean  $\pm$  SE) in the DS, the SH, and the TH forest type, respectively (Fig. 14A).

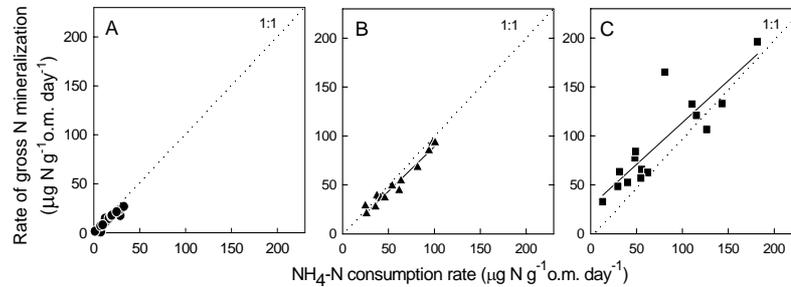


**Figure 14.** Gross rates of N cycling along the forest productivity gradient at Betsele. Abbreviations: (DS); dwarf-shrub (SH); short herb, (TH); tall herb forest type. A; gross N mineralisation  $\mu\text{g N g}^{-1}\text{ o.m. day}^{-1}$ . B; gross nitrification rate ( $\mu\text{g N g}^{-1}\text{ o.m. day}^{-1}$ ) in the tall herb (TH) forest type.

Gross N mineralisation data from a primary successional transect (grey alder/rowan, downy birch, downy birch/Norway spruce and Norway spruce) typical for the up-lift land coast in Western Finland (Merilä *et al.*, 2002) were close to the rates found in the DS forest type, c.  $20 \mu\text{g NH}_4^+\text{-N g}^{-1}\text{ o.m. day}^{-1}$  (mean  $\pm$  SE). Arctic soils and temperate hardwood forest soils (Fisk *et al.*, 1998; Fisk & Fahey, 2001) also show similar rates as found in the DS forest type. The rates in the SH and in particular in the TH forest type are very high, but similar rates were found in O<sub>2</sub>-layer soil at the Blodgett Research forest in California (Davidson *et al.*, 1992). After recalculating our highest rates in August to per g dry substance, our gross N mineralisation rates in the SH and TH forest type were  $55 \pm 6$  and  $68 \pm 8 \mu\text{g NH}_4^+\text{-N g}^{-1}\text{ d.s. day}^{-1}$  (mean  $\pm$  SE), respectively. This was slightly below the rate of  $81 \pm 10 \mu\text{g NH}_4^+\text{-N g}^{-1}\text{ d.s. day}^{-1}$  (mean  $\pm$  SE) found in the forest floor of a aspen/coniferous mixed forest in the Boreal Plain in eastern Canada (Carmosini *et al.*, 2002). In a study of N cycling in an evergreen forest in S. Chile, Perakis and Hedin (2001) found gross N mineralisation rates of c.  $42 \mu\text{g g}^{-1}\text{ o.m. day}^{-1}$ .

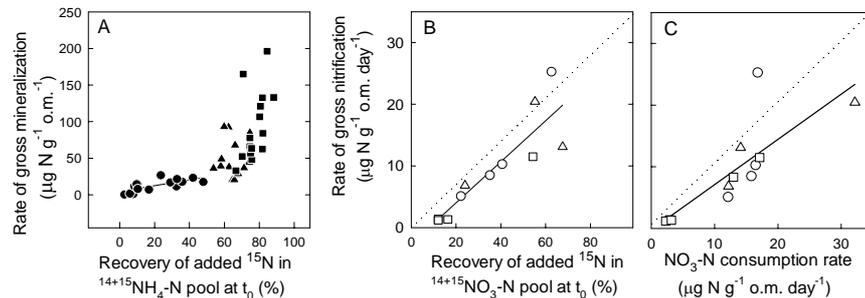
Many studies on gross N turnover in various forests and tundra ecosystems have shown that  $\text{NH}_4^+$  production (N mineralisation) seldom exceeds gross N consumption (all processes consuming  $\text{NH}_4^+$ ) (e.g., Davidson *et al.*, 1992; Fisk *et al.*, 1998; Fisk & Fahey, 2001; Perakis & Hedin, 2001; Carmosini *et al.*, 2002). This was also found at Betsele in the DS and SH, but not in the TH forest type (Fig. 15A-C). Data below the 1/1 line show that gross N consumption rate, c, exceeds gross N mineralization rate, m, (Fig. A-B), while data above the 1/1 line show that  $m > c$  (Fig. 5C). Hence, in the TH forest, the gross N mineralisation rate was higher than the gross  $\text{NH}_4^+$  consumption rate (Fig. 15C), whereas the  $\text{NO}_3^-$  consumption exceeded the gross nitrification rate (Fig. 16C) (Paper V). Seasonal

mean of gross nitrification in the TH forest type was  $13.0 \pm 2.4 \mu\text{g g}^{-1} \text{o.m. day}^{-1}$ . Interestingly, the gross nitrification rate in the TH forest type was similar to the gross N mineralisation rate in the DS forest type.



**Figure 15.** Relationships among rates of gross N mineralization and  $\text{NH}_4^+$  consumption along the N supply gradient at Betsele. Dotted lines show the 1/1 ratio. A; the nutrient poor dwarf-shrub forest type (DS). B; in the intermediate short-herb forest type (SH). C; in the rich tall-herb forest type (TH).

The gross nitrification rates were in the lower range of data reported elsewhere (e.g., Davidson *et al.*, 1992; Stark & Hart, 1997), but may be explained by extremely high potential denitrification activity (DEA) and a high potential abiotic immobilisation of  $\text{NO}_3^-$  in this forest type (Fig. 16B) (**Paper V**) leading to a rapid consumption of labelled  $\text{NO}_3^-$ , and hence very low atom % excess of  $\text{NO}_3^-$  already at the first (time zero) measurement.

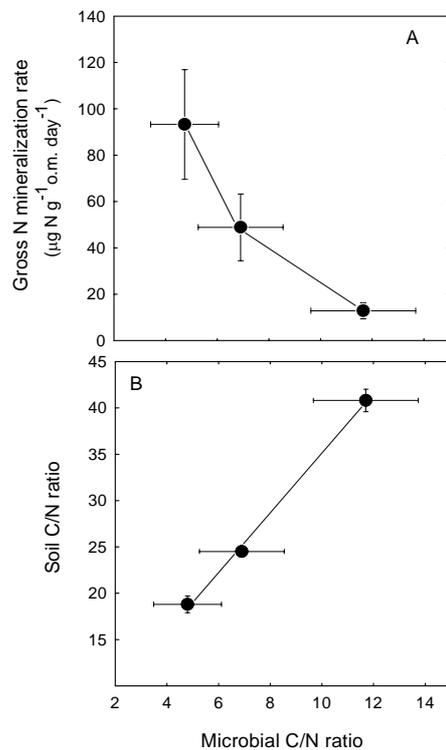


**Figure 16.** Extractability of  $^{15}\text{N}$  in the soil  $^{14+15}\text{NH}_4^+$  or  $^{14+15}\text{NO}_3^-$  pool immediately after addition to the soil, along the N supply gradient at Betsele. Recovery represents the portion of added tracer in the soil extract which will be “diluted” by newly mineralised  $^{14}\text{NH}_4^+$  or  $^{14}\text{NO}_3^-$  during the experiment. Dwarf shrub forest type (DS) (circle); short herb forest type (SH) (triangle); tall herb forest (TH) (square). Dotted lines show the 1/1 ratio. A; gross N mineralization rate vs. recovery of  $^{15}\text{N}$  in the soil  $^{14+15}\text{NH}_4^+$  pool along the gradient at Betsele. B; gross nitrification rate vs. recovery of  $^{15}\text{N}$  in the soil  $^{14+15}\text{NO}_3^-$  pool in the (TH) tall-herb forest type. C; gross nitrification rate vs.  $\text{NO}_3^-$  consumption rate in the TH forest type

Low recoveries of added  $^{15}\text{NH}_4^+$  tracer immediately after addition to the soil (Fig. 16A) further strengthen the idea that the DS forest is a very N-limited forest type. Here, gross N mineralisation rates are positively correlated to the recovery of added  $^{15}\text{NH}_4^+$  (time zero). Recovery increased along with increases in pool sizes and N turnover in the gradient (Fig. 16A). Low recovery of added  $^{15}\text{NO}_3^-$  were found in the TH forest type (Fig. 16B).

The mean residence time (average number of days an N atom resides in a pool) for N was generally 10 h to 1 day for  $\text{NH}_4^+$ -N along the gradient but  $>2$  days for  $\text{NO}_3^-$ -N in the TH forest type. Davidson *et al.* (1992) found similar residence times, which varied (depending on sampling time) between 7 h to 2 days for  $\text{NH}_4^+$ -N and 7 h to 4 days in the  $\text{O}_2$ -layer in an  $>100$  yr old forest at the Blodgett Research forest (composed by Douglas fir, white fir, ponderosa/sugar pine, incense cedar, and California black oak). The turnover rate for chloroform labile N (here uncorrected, i.e. the N-flush after fumigation) spanned between 7 to 17 days in the SH and TH forest types. Consequently, microbial N turned over within 18 to 43 days. This was close to the 23-55 days found by Davidson *et al.* (1992). There was a tendency towards longer pool turnover times for both inorganic N and microbial N in the DS forest type than in the SH and TH forest types, but not significantly so, due to exceptionally high turnover rates in DS in June.

Apparently, our studies of gross and net turnover of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools confirmed the presence of profound differences along this forest productivity gradient in *in situ* N cycling. Obviously, the changes in N cycling occur along with variations in base saturation and other soil chemical properties. However, soil N is turned over by microorganisms, and should proximally be controlled by the supplies of C and N to the organisms. This was supported by a strong correlation between soil and microbial C/N ratios (Fig. 17A); these two ratios were negatively correlated with gross N mineralization rates (Fig. 17B). This seemingly supports the classical view of strong links between saprotrophs, their substrates and soil N turnover. A problem is, however, that the relationship between soil and microbial



**Figure 17.** Stoichiometry among soil and microbial C/N ratios and gross N mineralization along the forest productivity gradient at Betsale (**Paper V**). A; relationship between gross N mineralization and microbial C/N ratio. B; linear relationship between soil C/N ratio and microbial C/N ratio. Note that the microbial C/N ratio is based on the same correction factors for microbial C and N, consequently, microbial C/N is equal to C/N of the microbial cytoplasm (the FE method extracts mainly the cytoplasmic content of microbial cells (Tate *et al.*, 1988; Jenkinson *et al.*, 2004)).

C/N ratios is confounded by large variations in the direct C supply from plants to mycorrhizal fungi in response to variations in plant N limitation. The supply of labile C to this particular fraction of the microbial community is in the opposite direction in the sense that it is high when a high soil C/N ratio implies a poor quality substrate for saprotrophs and *vice versa*. There were thus no major differences in microbial C, despite large variations in plant productivity and microbial N. The ratio C/N in soil-to-C/N in microorganisms was very narrow,  $3.7 \pm 0.2$  (mean  $\pm$  SD, n=3) over a wide range of soil conditions and large variability in the ratio fungi/bacteria (Fig. 17). This also implies a constant microbial efficiency (or growth yield) of about 0.27 along the gradient (**Paper V**). This fact, and the shift in microbial community from a large fungal contribution (assumed to have a yield coefficient c. 0.44), to more of bacteria (yield coefficient c. 0.32) (Myrold, 1999), indicates that there may be no major differences in the substrate quality or use, alternatively, no differences in metabolic traits among functional groups along the gradient. However, this apparent constancy may hide shifts between low N supply and poor substrate quality for heterotrophs, but a large supply of high quality (photosynthate C mainly) for mycorrhizal fungi and high N supply and high substrate quality for heterotrophs, but low substrate supply to mycorrhizal fungi.

Classically, soil microbiologists have used the soil C/N ratio to predict whether N is likely to be mineralized or immobilized. Commonly, the ratio of 20 is regarded as the threshold, below which net N mineralization occurs (Swift *et al.*, 1979; Killham, 1994; Myrold, 1999). The critical C/N ratio is thought to depend on the relative contributions from fungi and bacteria to soil microbial biomass, since their N contents differ (Myrold, 1999). It is of particular interest that bacteria are relatively homeostatic and maintain ratios narrowly around 5, while fungi display higher ratios and greater variations in their C/N ratios depending on the quality of their substrates (Serner & Elser, 2002).

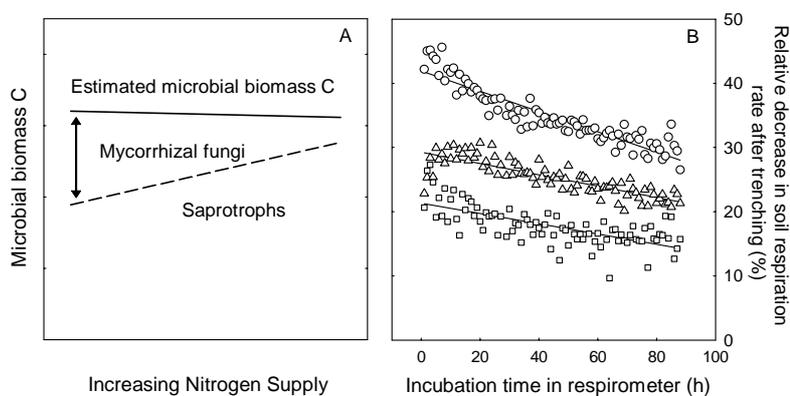
We found no major differences in rates of decomposition of  $^{13}\text{C}$ ,  $^{15}\text{N}$  labelled amino acids *in situ* across the gradient (**Paper V**), which appears to contradict the results of studies of net and gross turnover of inorganic N. Thus, the turnover of amino acids seems to be the same along the gradient, whereas the turnover of inorganic N increases considerable. One should note, however, that the amino acids added could have been decomposed by both saprotrophs and mycorrhizal fungi.

## Hypothesis

The higher N availability in toe-slope positions in the Fennoscandian forests has been suggested to be due to fluxes of N down slopes, less losses of N by fire, higher inputs of N from  $\text{N}_2$  fixation and higher rates of N turnover *in situ*, e.g. higher rates of N mineralization and nitrification (Högberg, 2001a). This thesis addresses the latter aspect, in particular.

High rates of gross and net N mineralization and gross and net nitrification were found in the groundwater discharge area (**Paper V**). However, the higher apparent rates of N cycling are not caused by higher microbial activity in general;

laboratory incubations as well as field measurements did not show that microbial respiration is higher in the discharge area than elsewhere along the gradient (**Paper III**). Since microbial biomass C was rather constant, and variations in respiration were comparatively small, we suggest that the higher rates of N cycling in the direction of the discharge area are caused by decreasing C/N ratios of substrates used by the microbial community as whole (Fig. 17). I also suggest that the inclusion of mycorrhizal fungi among other microbes, generally thought of as classical saprotrophs in the contexts of biomass and activity, has to be considered in this context. I argue that mycorrhizal fungi should be viewed as integral parts of plant root systems, which experience the same decrease in allocation of C from plant shoots as roots do when the soil nutrient supply increases (Nylund, 1988; Wallander & Nylund, 1992).



**Fig. 18.** A; A model of changes in the size of the microbial biomass and shifts between functional groups when soil N supply increases (**Paper III**). The arrow illustrates temporal variation in the size of the soil microbial biomass and the suggested sensitivity of the mycorrhizal fungal component to disruption of its major C supply caused by physical disturbance, e.g., as a consequence of soil sampling. B; the calculated relative decreases (%) in microbial activity after trenching (**Paper II**), assessed by hourly measurements, in mor-layer soil from the dwarf-shrub at 10 m (circles), short-herb at 60 m (triangles) and tall-herb forest at 90 m (squares) along the Betsela gradient.

We thus hypothesized that effects of soil N availability on the availability of C to mycorrhizal fungi and heterotrophs acts in opposing directions (**Paper III**). We also proposed that such variations in C supply from plants to mycorrhizal fungi can have a decisive effect on soil N dynamics. Saprotrophs increase their biomass when their supply of C from plants is enhanced in response to greater N-supply; on the contrary, increased N-supply seems to cause a reduction of the C-supply to plant roots and their mycorrhizal symbionts (Fig. 18). The latter is because plants allocate less C to their roots and their mycorrhizal fungi under conditions of high nutrient supply (Nylund, 1988; Wallander & Nylund, 1992). Thus, the high microbial C/N ratio in the DS forest type may not solely reflect the low C/N ratio of litter substrates, but also a large C flux to mycorrhizal fungi. This is supported by the finding of a very constant microbial yield coefficient along the gradient.

In opposition of the paradigm of a common C-limitation in soil microbes, Kaye and Hart (1997) suggested that microbes might be N- rather than C-limited, when

the soil C/N ratio is high. This may very well be the case in the DS forest type, and especially for mycorrhizal fungi, which have access to a large C supply. Read (1991) emphasized, in his model of relations between soils and plant communities, exclusive capacities of certain mycorrhizal types to access specific soil N sources. I would rather like to emphasize the quantitative importance of the direct C flow from plants to their mycorrhizal fungi. This large C supply gives the latter a unique position in nutrient-poor systems. However, when plants decrease their below-ground C allocation in response to a higher nutrient supply, mycorrhizal fungi become less important. In the nutrient-poor system, they are a strong sink for N, because they can maintain a high C/N ratio, but their importance as N sink should decrease drastically as the soil N supply increases. This in turn leads to even higher N availability to other soil organisms, and may be one explanation of the sharp increase in inorganic N towards the discharge area at Betsele.

## Concluding remarks and future perspectives

- Local plant productivity gradients, like the one at Betsele, can be used as model systems for soil-microbial-plant interactions in forest ecosystems commonly found in Fennoscandia.
- In a poor Scots pine forest extramatrical ectomycorrhizal (ECM) mycelium contributed one third of microbial biomass and produced together with ECM roots half the dissolved organic carbon in the soil.
- The abundance of mycorrhizal extramatrical mycelium, based on the decline in activity after trenching, decreased with increasing soil fertility from 42, 28, to 22% in the DS, the SH, and the TH forest types respectively. This confirmed the finding of a drastic decline in biomarkers indicative of fungi along the gradient.
- Neither mean microbial biomass nor soil respiration rates varied significantly along the forest productivity gradient, which was totally unexpected because of the three-fold increment in plant production.
- Gross N mineralisation rate increased 2.6 to 5.6 times between the DS and SH forest type, i.e. roughly in proportion to the three-fold increase in forest productivity along the gradient, while there was net mineralization in the TH forest only. Gross nitrification was only measurable in the TH forest type. Net nitrification was absent in DS, detectable in the SH and significant in the TH forest type.
- The higher apparent rates of N cycling are not caused by higher microbial activity in general.
- The surprisingly high microbial biomass C and respiratory activity in the poor DS forest is suggested to be caused by a high C allocation by trees to their ectomycorrhizal fungal symbionts in response to a strong soil N limitation.

- The large fractional contribution of mycorrhizal fungi to the microbial community confounds the interpretation of relations among soil and microbial C/N ratios and soil N mineralization.
- Susceptibility of the microbial activity of mor soils to physical disturbance decreases when soil fertility increases, which may further confound the interpretation of measures of soil microbial activity in these kind of soils.

Declining microbial activity after sampling thus complicate the interpretation of estimates of microbial biomass and N cycling. My primary concern is that mycorrhizal fungi, in particular, quickly run out of C during incubations after sampling as pointed out by (Lindahl *et al.*, 2002). It is, therefore, of considerable interest to develop methods taking the effects of the mycorrhizal mycelium into account. There is also a need to clarify the mechanisms of the rapid immobilisation of N to soils, especially in N-limited soils. Further advances in isotopic and molecular methods will likely enable distinctions to be made between different metabolic traits among soil microbes in general and, in particular they will likely help to distinguish the role played by mycorrhizal fungi. This is most important, but may be difficult because of the many complex potential interactions between saprotrophs and mycorrhizal fungi (Lindahl *et al.*, 2001).

## References

- Aerts R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedback. *Journal of Experimental Botany* 50.
- Alexander M. 1977. *Introduction to Soil Microbiology*. 2<sup>nd</sup> edn. New York, USA: John Wiley & Sons.
- Anderson JPE & Domsch KH. 1978a. A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biology and Biochemistry* 10: 215-221.
- Anderson JPE & Domsch KH. 1978b. Mineralisation of bacteria and fungi in chloroform-fumigated soils. *Soil Biology and Biochemistry* 10: 207-213.
- Arnebrant K & Bååth E. 1991. Measurements of ATP in forest humus. *Soil Biology and Biochemistry* 23: 501-506.
- Bauhus J & Khanna PK. 1999. The significance of microbial biomass in forest soils. In: Rastin N and Bauhus J, eds. *Going underground-ecological studies in forest soils*. Trivandrum, India: Research Signpost, 77-110.
- Bauhus J, Pare´ D & Côte´ L. 1998. Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. *Soil Biology and Biochemistry* 30: 1077-1089.
- Berg B & McLaugherty C. 2003. *Plant litter. Decomposition. Humus Formation. Carbon Sequestration*. Heidelberg, Berlin, Germany: Springer Verlag.
- Bhupinderpal-Singh, Nordgren A, Ottosson-Löfvenius M, Högberg MN, Mellander P-E & Högberg P. 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant Cell and Environment* 26: 1287-1296.
- Blagodatskaya EV & Anderson T-H. 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO<sub>2</sub> of microbial communities in forest soils. *Soil Biology and Biochemistry* 30: 1269-1274.
- Bligh EG & Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37: 911-917.

- Bonkowski M. 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* 162: 617-631.
- Brookes PC. 1985b. Chloroform fumigation and the release of soil nitrogen: the effect of fumigation time and temperature. *Soil Biology and Biochemistry* 17: 831-835.
- Brookes PC, Landman A, Pruden G & Jenkinson DS. 1985a. Chloroform fumigation and release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* 17: 837-842.
- Bååth E. 1992. Thymidine incorporation into macromolecules of bacteria extracted from soil by homogenization-centrifugation. *Soil Biology and Biochemistry* 24: 1157-1165.
- Bååth E. 1996a. Adaptation of soil bacterial communities to prevailing pH in different soils. *FEMS Microbiology Ecology* 19: 227-237.
- Bååth E. 1996b. Thymidine incorporation of bacteria sequentially extracted from soil using repeated homogenization-centrifugation. *Microbial Ecology* 31: 153-166.
- Bååth E & Anderson T-H. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry* 35: 955-963.
- Bååth E, Frostegård Å, Pennanen T & Fritze H. 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. *Soil Biology and Biochemistry* 27: 229-240.
- Bååth E & Söderström B. 1982. Seasonal and spatial variation in fungal biomass in a forest soil. *Soil Biology and Biochemistry* 14: 353-358.
- Cajander AK. 1926. The theory of forest types. *Acta Forestalia Fennica* 29: 1-108.
- Cannell MGR. 1989. Physiological basis of wood production: a review. *Scandinavian Journal of Forest Research* 4: 459-490.
- Cannell MGR & Dewar RC. 1994. Carbon allocation in trees - A review of concepts for modelling. *Advances in Ecosystem Research* 25: 59-104.
- Carmosini N, Devito KJ & Prepas EE. 2002. Gross nitrogen transformations in harvested and mature aspen-conifer mixed forest soils from the Boreal Plain. *Soil Biology and Biochemistry* 34: 1949-1951.
- Chapin III FS, Fetcher N, Kielland K & Everett KR. 1988. Productivity and nutrient cycling of Alaskan tundra: Enhancement by flowing soil water. *Ecology* 69: 693-702.
- Chapin III FS, Vitousek PM & Van Cleve K. 1986. The nature of nutrient limitation in plant communities. *The American Naturalist* 127: 48-58.
- Cole DW. 1981. Nitrogen uptake and translocation by forest ecosystems. *Ecological Bulletins* 33: 219-232.
- Covington WW. 1981. Changes in forest floor organic matter and nutrient content following clear cutting in northern hardwood. *Ecology* 62: 41-48.
- Dahl E, Gjems O & Kjelland-Lund JJ. 1967. On the vegetation of Norwegian conifer forest in relation to the chemical properties of the humus layer. *Meddelelser fra det Norske Skogsforsøksvesen* 85: 501-531.
- Davidson E, Hart SC, Shanks CA & Firestone MK. 1991. Measuring gross nitrogen mineralization, immobilization, and nitrification by <sup>15</sup>N isotopic pool dilution in intact soil cores. *Journal of Soil Science* 42: 335-349.
- Davidson EA, Hart SC & Firestone MK. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. *Ecology* 73: 1148-1156.
- Davidson EA, Stark JM & Firestone MK. 1990. Microbial production and consumption of nitrate in an annual grassland. *Ecology* 71: 1968-1975.
- Eiland F. 1983. A simple method for quantitative measurements of ATP in soil. *Soil Biology and Biochemistry* 15: 665-670.
- Eno CF. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. *Soil Science Society America Proceedings* 24: 277-279.
- FAO. 1988. Food and Agriculture Organization of the United Nations (FAO). FAO/UNESCO Soil Map of the World. Revised legend. World Resources Report. In. Rome, Italy: FAO.

- Federer CA. 1984. Organic matter and nitrogen content of the forest floor in even-aged northern hardwoods. *Canadian Journal of Forest Research* 14: 763-767.
- Federle TW. 1986. Microbial distributions in soil - new techniques. In: Megusar F and Gantar M, eds. *Perspectives in Microbial Ecology*. Ljubljana, Slovenia: Slovene Society for Microbiology, 493-498.
- Fisk MC & Fahey TJ. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. *Biogeochemistry* 53: 201-223.
- Fisk MC, Schmidt SK & Seastedt TR. 1998. Topographic patterns of above- and belowground production and nitrogen cycling in alpine tundra. *Ecology* 79: 2253-2266.
- Flanagan PW & Van Cleve K. 1977. Microbial biomass, respiration and nutrient cycling in a black spruce taiga ecosystem. *Ecological Bulletins* 25: 261-273.
- Fokin AD, Knyazev DA & Kuzyakov YV. 1993. Destruction of <sup>14</sup>C- and <sup>15</sup>N-labelled amino acids and nucleic bases in soil and the supply of their transformation products to plants. *Eurasian Soil Science* 25: 109-122.
- Frank A. 1877. Über die biologischen Verhältnisse des Thallus einiger Krustenflechten. *Beiträge Zur Biologie der Pflanzen* 2: 123-200.
- Freeman C, Baxter R, Farrar JF, Jones SE, Plum S, Axhenton TW & Stirling C. 1998. Could competition between plant and microbes regulate plant nutrition and atmospheric CO<sub>2</sub> concentrations. *The Science of the Total Environment*: 181-184.
- Fritze H & Bååth E. 1993. Microfungal species composition and fungal biomass in a coniferous forest soil polluted by alkaline deposition. *Microbial Ecology* 25: 83-92.
- Frostegård Å & Bååth E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* 22: 59-65.
- Frostegård Å, Bååth E & Tunlid A. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry* 25: 723-730.
- Frostegård Å, Tunlid A & Bååth E. 1991. Microbial biomass measured as total lipid phosphate in soil of different organic content. *Journal of Microbiological Methods* 14: 151-163.
- Frostegård Å, Tunlid A & Bååth E. 1996. Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. *Soil Biology and Biochemistry* 28: 55-63.
- Giesler R, Högberg M & Högberg P. 1998. Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. *Ecology* 79: 119-137.
- Giesler R, Petersson T & Högberg P. 2002. Phosphorus limitation in boreal forests: effects of aluminum and iron accumulation in the humus layer. *Ecosystems* 5: 300-314.
- Griffiths RP. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation rates. *Canadian Journal of Forest Research* 20: 211-218.
- Griffiths RP, Baham JE & Caldwell BA. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biology and Biochemistry* 26: 331-337.
- Hansen J, Türk R, Vogg G, Heim R & Beck E. 1997. Conifer carbohydrate physiology: updating classical views. In: Rennenberg H, Eschrich W and Ziegler H, eds. *Trees-Contributions to Modern Tree Physiology*. Leiden, The Netherlands: Backhuys Publishers, 97-108.
- Harley JL & Smith SE. 1983. *Mycorrhizal symbiosis*. London, UK: Academic Press.
- Hart SC, Stark JM, Davidson EA & Firestone MK eds. 1994a. *Nitrogen mineralization, immobilization and nitrification*. Madison, USA: Soil Science Society of America.
- Hartel PG. 1999. The soil habitat. In: Sylvia DM, Fuhrmann JJ, Hartel PG and Zuberer DA, eds. *Principles and Applications of Soil Microbiology*. New Jersey, NJ, USA: Prentice Hall, 21-43.
- Haynes BE. 1995. Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiology* 15: 317-325.

- Hesselmann H. 1926. Studier över barrskogens humustäcke, dess egenskaper och beroende av skogsvården. Stockholm: Statens skogsförsöksanstalt. 169-552.
- Hintikka V & Näykki O. 1967. Notes on the effects of the fungus *Hydnellum ferrugineum* (Fr.) Karst. on forest soil and vegetation. *Communicationes Instituti forestalis Fenniae* 62: 1-22.
- Holmes W & Zak DR. 1994. Soil microbial biomass dynamics and net nitrogen mineralization in northern hardwood ecosystems. *Soil Science Society of America Journal* 58: 238-243.
- Hägglund B & Lundmark J-E. 1977. Site index estimations by means of site properties, Scots pine and Norway spruce in Sweden. *Studia Forestalia Suecica* 103.
- Högberg P. 2001a. Interactions between hillslope hydrochemistry, nitrogen dynamics, and plants in Fennoscandian boreal forest. In: Schulze E-D, Heimann M, Harrison S, Holland E, Lloyd J, Prentice IC and Schimel D, eds. *Global Biogeochemical Cycles in the Climate System*. San Diego, USA: Academic Press, 227-233.
- Högberg P & Ekblad A. 1996. Substrate-induced respiration measured *in situ* in a C<sub>3</sub>-plant ecosystem using additions of C<sub>4</sub>-sucrose. *Soil Biology and Biochemistry* 28: 1131-1138.
- Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M & Read DJ. 2001b. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411: 789-792.
- Ingham ER, Griffiths RP, Cromack K & Entry JA. 1991. Comparison of direct vs fumigation incubation microbial biomass estimates from ectomycorrhizal mat and non-mat soils. *Soil Biology and Biochemistry* 23: 465-471.
- Jansson SL. 1958. Tracer studies on nitrogen transformations in soil with special attention to mineralization-immobilization relationships. *Annals of the Royal Agricultural College of Sweden* 24: 101-361.
- Jansson SL & Persson J. 1982. Mineralization and immobilization of soil nitrogen. In: Stevenson FJ, ed. *Nitrogen in Agricultural Soils*. Madison, Wisconsin, USA: Soil Science Society of America, Inc., 229-252.
- Jenkinson DS. 1987. Determination of microbial biomass carbon and nitrogen in soil. In Wilson JR, ed. *Advances in Nitrogen cycling in Agricultural Ecosystems*. Brisbane, Qld, Australia: CAB International, Wallingford 1988, 368-386.
- Jenkinson DS, Brookes PC & Powlson DS. 2004. Measuring soil microbial biomass. *Soil Biology and Biochemistry* 36: 5-7.
- Joergensen RG, Meyer B & Mueller T. 1994. Time-course of the soil microbial biomass under wheat: A one year field study. *Soil Biology and Biochemistry* 26: 987-994.
- Kaye JP & Hart SC. 1997. Competition for nitrogen between plants and soil microorganisms. *TREE* 12, 139-143.
- Killham K. 1994. *Soil Ecology*. Cambridge, UK: Cambridge University Press.
- Kirkham D & Bartholomew WV. 1954. Equations for following nutrient transformations in soil, utilizing tracer data. *Proceedings of the Soil Science Society of America* 18: 33-34.
- Korsaeth A, Molstad L & Bakken LR. 2001. Modelling the competition for nitrogen between plants and microflora as a function of soil heterogeneity. *Soil Biology and Biochemistry* 33: 215-226.
- Lahti T & Väisänen RA. 1987. Ecological gradients of boreal forests in South Finland: an ordination test of Cajander's forest type theory. *Vegetatio* 68: 145-156.
- Lambers H, Chapin III FS & Pons TL. 1998. *Plant Physiological Ecology*. New York: Springer-Verlag.
- Lindahl B, Stenlid J & Finlay R. 2001. Effects of resource availability on mycelial interactions and <sup>32</sup>P transfer between a saprotrophic and an ectomycorrhizal fungus in soil microcosms. *FEMS Microbiology Ecology* 38: 43-52.
- Lindahl BO, Taylor AFS & Finlay RD. 2002. Defining nutritional constraints on carbon cycling in boreal forests - towards a less "phytcentric" perspective. *Plant Soil* 242: 123-135.
- Linder S. 1995. Foliar analysis for detecting and correcting nutrient imbalances in Norway spruce. *Ecological Bulletins* 44: 178-190.

- Lipson DA, Raab TK, Schmidt SK & Monson RK. 1999. Variation in competitive abilities of plants and microbes for specific amino acids. *Biology and Fertility of Soils* 28: 257-261.
- Lundström U, van Breemen N & Bain D. 2000. The podzolization process. A review. *Geoderma* 94: 97-107.
- Majdi H & Persson H. 1995. Effects of ammonium sulphate application on the chemistry of bulk soil, rhizosphere, fine roots and fine-root distribution in a *Picea abies* (L.) Karst. stand. *Plant and Soil* 168-169: 151-160.
- Martikainen PJ & Palojarvi A. 1990. Evaluation of the fumigation-extraction method for the determination of microbial C and N in a range of forest soils. *Soil Biology and Biochemistry* 22: 797-802.
- Merilä P, Smolander A & Strömmer R. 2002. Soil nitrogen transformations along a primary succession transect on the land-uplift coast in western Finland. *Soil Biology and Biochemistry* 34: 373-385.
- Myrold DD. 1987. Relationship between microbial biomass nitrogen and a nitrogen availability index. *Soil Science Society of America Journal* 51: 1047-1049.
- Myrold DD. 1999. Transformations of nitrogen. In: Sylvia DM, Fuhrmann JJ, Hartel PG and Zuberer DA, eds. *Principles and Applications of Soil Microbiology*. New Jersey, USA: Prentice Hall, 259-294.
- Myrold DD, Matson PA & Peterson DL. 1989. Relationships between soil microbial properties and aboveground stand characteristics of conifer forest in Oregon. *Biogeochemistry* 8: 265-281.
- Nadelhoffer KJ, Aber JD & Melillo JM. 1984. Seasonal patterns of ammonium and nitrate uptake in nine temperate forest ecosystems. *Plant and Soil* 80: 321-335.
- Nordgren A. 1988b. Apparatus for the continuous, long-term monitoring of soil respiration rate in large number of samples. *Soil Biology and Biochemistry* 20: 955-957.
- Nordgren A, Bååth E & Söderström B. 1988a. Evaluation of soil respiration characteristics to assess heavy metal effect on soil microorganisms using glutamic acid as a substrate. *Soil Biology and Biochemistry* 20: 949-954.
- Nordin A, Högberg P & Näsholm T. 2001. Soil nitrogen form and plant nitrogen uptake in a boreal forest along a forest productivity gradient. *Oecologia* 129: 125-132.
- Nurmiaho-Lassila E-L, Timonen S, Haahtela K & Sen R. 1997. Bacterial colonization patterns of intact *Pinus sylvestris* mycorrhizospheres in dry pine forest soil: an electron microscopy study. *Canadian Journal of Microbiology* 43: 1017-1035.
- Nylund J-E. 1988. The regulation of mycorrhiza formation - carbohydrate and hormone theories reviewed. *Scandinavian Journal of Forest Research* 3: 465-479.
- Näsholm T, Ekblad A, Nordin A, Giesler G, Högberg M & Högberg P. 1998. Boreal forest plants take up organic nitrogen. *Nature* 392: 914-916.
- Näsholm T, Huss-Danell K & Högberg P. 2001. Uptake of glycine by field grown wheat. *New Phytologist* 150: 59-63.
- Ohlsson KEA & Wallmark H. 1999. Novel calibration with correction for drift and non-linear response for continuous flow isotope ratio mass spectrometry applied to the determination of delta N-15, total nitrogen, delta C-13 and total carbon in biological material. *Analyst* 124: 571-577.
- Olsson PA. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. MiniReview. *FEMS Microbiology Ecology* 29: 303-310.
- Olsson PA, Bååth E, Jakobsen I & Söderström B. 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research* 99: 623-629.
- Pennanen T, Liski J, Bååth E, Kitunen V, Uotila J, Westman CJ & Fritze H. 1999. Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. *Microbial Ecology* 38: 168-179.
- Perakis SS & Hedin LO. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. *Ecology* 82: 2245-2260.

- Persson J, Högberg P, Ekblad A, Högberg MN, Nordgren A & Näsholm T. 2003. Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field. *Oecologia* 137: 252-257.
- Powlson DS & Barraclough D. 1993. Mineralization and assimilation in soil-plant systems. In: Knowles R and Blackburn TH, eds. *Nitrogen Isotope Techniques*. San Diego, USA: Academic Press, 209-242.
- Prescott CE, Maynard DG & Laiho R. 2000. Humus in northern forests: friend or foe? *Forest Ecology and Management* 133: 23-36.
- Read DJ. 1983. The biology of mycorrhiza in the Ericales. *Canadian Journal Botany* 61: 985-1004.
- Read DJ. 1986. Non-nutritional effects of mycorrhizal infection. In: V. Gianinazzi-Pearson and Gianinazzi S, eds. *Physiological and Genetical aspects of Mycorrhizae*. Paris, France: Institut National de la Recherche Agronomique (INRA), 169-176.
- Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* 47: 376-391.
- Read DJ. 1993. Mycorrhiza in Plant Communities. In: Ingram DS, Williams PH and Tommerup IC, eds. *Advances in Plant Pathology: Mycorrhiza Synthesis*. London, UK: Academic Press, 1-31.
- Read DJ & Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? *New Phytol* 157: 475-492.
- Romell LG. 1935. Ecological problems of the humus layer in the forest. *Cornell University Agricultural Experimental Station Memoir No. 170*: 3-28.
- Romell LG. 1938. A trenching experiment in spruce forest and its bearing on problems of mycotrophy. *Svensk Botanisk Tidskrift* 32: 89-99.
- Smith JL & Paul EA. 1986. The role of soil type and vegetation on microbial biomass and activity. In Megusar F and Gantar M, eds. *Perspectives in Microbial Ecology*. Ljubljana, Slovenia: Slovene Society for Microbiology, 460-466.
- Smith JL & Paul EA. 1990. The significance of soil microbial biomass estimations. In: Bollag J-M and Stotsky G, eds. *Soil Biochemistry*. New York, USA: Dekker, 357-396.
- Smith SE & Read DJ. 1997. *Mycorrhizal symbiosis*. 2<sup>nd</sup>. London, UK: Academic Press.
- Smolander A & Mälkönen E. 1994. Microbial biomass C and N in limed soil of Norway spruce stands. *Soil Biology and Biochemistry* 26: 503-509.
- Stark JM & Hart SC. 1996. Diffusion techniques for preparing salt solutions, Kjeldahl digests and persulphate digests for nitrogen-15 analysis. *Soil Science Society of America Journal* 60: 1846-1855.
- Stark JM & Hart SC. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385: 61-64.
- Sterner RW & Elser JJ. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. New Jersey, USA: Princeton University Press.
- Subke J-A, Hahn V, Battipaglia G, Linder S, Buchmann N & Cotrufo MF. 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia* 139: 551-559.
- Swift MJ, Heal OW & Anderson JM. 1979. Decomposition in Terrestrial Ecosystems. In: *Studies in Ecology*. Oxford, UK: Blackwell.
- Söderberg KH & Bååth E. 1998. Bacterial activity along a young barley root measured by the thymidine and leucine incorporation techniques. *Soil Biology and Biochemistry* 30: 1259-1268.
- Söderström B. 1979. Seasonal fluctuations of active fungal biomass in horizons of a podzolized pine-forest soil in central Sweden. *Soil Biology and Biochemistry* 11: 149-154.
- Sörensen P & Jensen ES. 1991. Sequential diffusion of ammonium and nitrate from soil extract to a polytetrafluoroethylene trap for <sup>15</sup>N determination. *Analytica Chimica Acta* 252: 201-203.
- Tamm C-O. 1991. *Nitrogen in Terrestrial Systems*. Berlin, Germany: Springer-Verlag.

- Tate KR, Ross DJ & Feltham CW. 1988. A direct extraction method to estimate soil microbial C: effects of experimental variables and some different calibration procedures. *Soil Biology and Biochemistry* 20: 329-335.
- Taylor AFS, Martin F & Read DJ. 2000. Fungal diversity in ectomycorrhizal communities of Norway spruce (*Picea abies* (L.) Karst.) and beech (*Fagus sylvatica* L.) along north-south transects in Europe. In: Schulze E-D, ed. *Carbon and Nitrogen Cycling in European Forest Ecosystems Ecological Studies*. Berlin, Germany: Springer-Verlag, 343-365.
- Tiedje JM. 1982. Denitrification. In: Page AL, ed. *Methods in Soil Analysis, Part 2. Chemical and Microbiological Properties - Agronomy Monograph no. 9*. 2<sup>nd</sup> ed. Madison, USA: ASA - SSSA, 1011-1026.
- Tietema A. 1998. Microbial carbon and nitrogen dynamics in coniferous forest floor material collected along a European nitrogen depositing gradient. *Forest Ecology and Management* 101, 29-36.
- Tunlid A & White DC. 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Stotzky G and Bollag J-M, eds. *Soil Biochemistry*. New York, USA: Dekker, 229-261.
- Tutin TG, Heywood VH, Burges NA, Valentine SM, Walters SM & Webb DA eds. 1964-1980. *Flora Europea*. Cambridge, UK: Cambridge University Press.
- Wallander H, Nilsson L-O, Hagerberg D & Bååth E. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist* 151: 753-760.
- Wallander H & Nylund J-E. 1992. Effects of excess nitrogen and phosphorus starvation on the extramatrical mycelium of ectomycorrhizas of *Pinus sylvestris* L. *New Phytologist* 120: 495-503.
- Wallenda T & Kottke I. 1998. Nitrogen deposition and ectomycorrhizas. *New Phytologist* 139: 169-187.
- van Breemen N, Finlay RD, Lundström U, Jongmans AG, Giesler R & Olsson M. 2000. Mycorrhizal weathering: a true case of mineral nutrition? *Biogeochemistry* 49: 53-67.
- van Breemen N & Finzi AC. 1998. Plant-soil interactions: ecological aspects and evolutionary implications. *Biogeochemistry* 42: 1-19.
- van der Werf A, Poorter H & Lambers H. 1994. Respiration as dependent on a species inherent growth rate and on the nitrogen supply to the plant. In: Roy J and Garnier E, eds. *A Whole Plant Perspective of Carbon-Nitrogen Interactions*. The Haag, The Netherlands: SPB Academic Publishing, 91-110.
- van Hees PAW, Lundström U & Giesler R. 2000. Low molecular weight organic acids and their Al-complexes in soil solution - composition, distribution and seasonal variation in three podzolized soils. *Geoderma* 94: 173-200.
- Vance ED, Brookes PC & Jenkinson DS. 1987. An extraction method for measuring microbial biomass C. *Soil Biology and Biochemistry* 19: 703-707.
- Wardle DA. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological Reviews* 67: 321-358.
- Vitousek P. 1982. Nutrient cycling and nutrient use efficiency. *American Naturalist* 119: 553-572.
- Vogt KA, Moore EE, Vogt DJ, Redlin MJ & Edmonds RL. 1983. Conifer fine root and mycorrhizal root biomass within the forest floors of Douglas-fir stands of different ages and site productivities. *Canadian Journal of Forest Research* 13: 429-437.
- Wu J, Brookes PC & Jenkinson DS. 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. *Soil Biology and Biochemistry* 25: 1435-1441.
- Zak DR, Grigal DF, Gleeson S & Tilman D. 1990. Carbon and nitrogen cycling during old-field succession: Constraints in plant and microbial biomass. *Biogeochemistry* 11: 111-129.

Zak DR, Holmes WE, White DC, Peacock AD & Tilman D. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* 84: 2042-2050.

Zak DR, Tilman D, Parmenté RR, Rice CW, Fischer FM, Vose J, Milchunas D & Martin CW. 1994. Plant production and soil microorganisms in late successional ecosystems: a continental-scale study. *Ecology* 75: 2333-2347.

## Acknowledgements

Most of all I am grateful to Professors Roger Finlay, Marianne Clarholm, Torgny Näsholm and Erland Bååth for the support they gave me to finish this thesis.

My gratitude to Professor David Myrold for his contribution to the last paper.

I thank Dr. Björn Berg for introducing me to the field of soil microbiology.

My warmest and deepest thanks goes to my dear Peter and to our lovely children Axel, Jon-Isak och Fredrika, who encouraged me all the way through - together we can accomplish everything we want to!

I give my appreciation to my mother Gun for always being by my side.

Special thanks to my friends Christina Röckner, Eva Lundin, my sister Ingrid and my brothers Bertil, Olof and Per for encouragement.

I am also grateful to past and present staff at the Department of Forest Ecology: Doc. Anders Nordgren, Dr. Bhupinder-Pal Singh (University of Western Australia), Doc. Reiner Giesler (Umeå University), Doc. Ulf Skyllberg, and Dr. Mikael Ottosson-Löfvenius for good collaboration. I thank Doc. Anders Ohlsson, Håkan Wallmark, Birgitta Olsson, Bengt Andersson and Leif Olsson for skilful help with chemical analyses.

The work was financially supported by the Swedish Science Council (VR, formerly the Swedish Natural Science Research Council), the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS, formerly the Swedish Council for Forestry and Agricultural Sciences), and the Commission of the European Community (grants to the projects NiPhys, CANIF, and FORCAST).

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