Microbial mobilization and immobilization of soil nitrogen

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Dissertation
Lund, 2004
A doctoral thesis at a university in Sweden is produced either as a monograph or a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarises the accompanying papers. These have either already been published or are in manuscripts at various stages (in press, submitted or in ms).
This thesis is based on the following papers, which are referred to by their Roman numerals.


II. Bengtson, P., Bengtsson, G., and Falkengren-Grerup, U. Relieving substrate limitation – soil moisture and temperature determine gross N transformation rates. (Submitted)

III. Bengtson, P., Falkengren-Grerup, U., and Bengtsson, G. Spatial distribution of gross N transformation rates and plants – A reciprocal relationship. (Submitted)

IV. Månsson, K.F., Bengtson, P., Bengtsson, G., and Falkengren-Grerup, U. Competition for nitrogen – litter leachate favours microorganisms at the expense of plants. (Manuscript)

V. Bengtson, P. and Bengtsson, G. Rapid turnover of DOC in temperate forest soils accounts for increased CO₂ production at elevated temperature. (Manuscript)

VI. Bengtson, P. and Bengtsson, G. Bacterial immobilization and remineralization of N at different growth rates and N concentrations. (Manuscript)

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Introduction

Microorganisms in forest ecosystems normally recycle nitrogen (N), such that gaseous losses and leaching is quite limited. However, it has been hypothesized that the high anthropogenic N input to forest ecosystems may cause N saturation (Aber, 1992). N saturation has been defined in a number of different ways but all include increases in N availability over time that results in the removal of N limitation on all biological processes in the ecosystem considered (Aber et al., 1998). This would open the N cycle and lead to an increased mobility and leaching of N, soil acidification due to elevated nitrification rates and subsequent aluminum toxicity and loss of base cations.

The theory about N saturation and its consequences is widely accepted. However, a number of N fertilization and removal experiments have been carried out over the last decade or so (Wright and van Breemen, 1995; Wright and Rasmussen, 1998), and the results from these experiments are not as clear-cut and uniform as expected. Many forest ecosystems seem to have a much higher capacity to retain N than originally thought (Emmett et al., 1995; Gundersen et al., 1998; Kjønaas et al., 1998), and the response of an ecosystem to N fertilization is not always as expected. Therefore, the interpretation of the effects of high chronic N deposition rates requires an improved understanding of the biology of the microorganisms involved in N retention and transformation in soils.

N transformation processes

N is the only essential nutrient that is not weathered from soil minerals. This means that all habitats depend on bacterial fixation of molecular N from the atmosphere, or alternatively, on input of NH₃ (NH₃/H₂N) or NOₓ (NO, NO₂, HNO₂, HNO₃), and NO₃⁻ mainly from anthropogenic sources. The ability to fix atmospheric N is limited to a few species of bacteria, and the process requires a lot of energy. N availability in most terrestrial ecosystems is therefore highly dependent on the tight recycling of N carried out by microorganisms. NH₄⁺ can be lost as NH₃ (volatilization) or be taken up by plants or microorganisms (NH₄⁺ immobilization) and converted to organic N. Alternatively, NH₄⁺ is converted to NO₃⁻ via NO₂⁻ by ammonia-oxidizing and nitrite-oxidizing bacteria, respectively (nitrification). NH₄⁺ can also be produced by degradation of organic matter (mineralization), dissimilatory reduction of NO₃⁻ (DRNA), and maybe also leaked or excreted from bacterial cells (mobilization) and fine roots. NO₃⁻ can either be immobilized and converted to organic N in microorganisms (NO₃⁻ immobilization) or taken up by plants, or reduced to gaseous N compounds such as N₂O or N₂ by denitrifying bacteria (denitrification).

Aim of thesis

In forest soils, mineralization of organic N, including microbial N, and immobilization of inorganic N, especially NH₄⁺, are the quantitatively most important N transformation processes (Wessel and Tietema, 1992; Hart et al., 1997; Fisk and Fahey, 2001; Paper I). The aim of this thesis was to elucidate the factors that determine the rate of these processes.
Microbial uptake and assimilation of N

As all other living organisms, bacteria need N for the synthesis of compounds necessary for growth, reproduction and survival, e.g. proteins, DNA and RNA. The assimilation of N can be divided into two separate events: 1. the uptake of N from the extracellular medium, and 2. the intracellular production of N containing compounds. NH$_4^+$ solutions always contain NH$_3$, and there is substantial evidence for rapid diffusion of NH$_3$ across cytoplasmic membranes (Montesinos et al., 1998). Diffusion of NH$_3$ followed by trapping of intracellular NH$_4^+$ could therefore potentially represent a significant process for N acquisition. However, the high pK$_a$ of NH$_3$ (9.25) means that NH$_4^+$ is the predominant form in forest soils, which typically have a pH in the range from 4-7, and diffusion of NH$_3$ followed by intracellular trapping would be a highly inefficient way to acquire N. Furthermore, growing bacteria can maintain an intracellular ammonium-pool against a concentration gradient of at least 100-fold (Jayakumar and Barnes, 1983; Jayakumar et al., 1985), suggesting that bacteria have an active and efficient NH$_4^+$ transport system (Amt) that compensates for outward diffusion of membrane permeable NH$_3$. Such ammonium transport systems are found in bacteria and fungi (Siewe et al., 1996; Marini et al., 1997; Meletzus et al., 1998; Michel-Reydellet et al., 1998; Montesinos et al., 1998), as well as in plants (Ninnemann et al., 1994), and they have a high degree of homology. There are probably three different NH$_4^+$ transport proteins, each one with different affinity for NH$_4^+$ (Marini et al., 1997; Montesinos et al., 1998; Gazzarrini et al., 1999). The three NH$_4^+$ transporters possess distinct substrate affinities, ranging from less than micromolar to millimolar concentrations, the range of NH$_4^+$ concentrations typically found in soil (Marshner, 1995).

The Amt system is energy-dependent and activated when bacteria grow under N limited conditions but repressed when NH$_4^+$ is abundant (Servín-Gonzáles and Bastarrachea, 1984; Siewe et al., 1996). The protein is synthesized only under N starvation and inhibited by internal glutamine (Siewe et al., 1996). Glutamine also represses Amt, and the Amt system therefore seems to be regulated by N availability in a manner similar to that of several amino acid transport systems (Servín-Gonzáles and Bastarrachea, 1984; Merrick and Edwards, 1995). The level of glutamine synthetase (GS) also affects the methylammonium uptake, with a higher uptake at high GS levels (Michel-Reydellet et al., 1998). There are also indications that one of the three Amt transporters is synthesized/activated in response to high intracellular concentrations of C skeletons, possibly providing a link between C and N metabolism (Gazzarrini et al., 1999).

The intracellular production of N containing compounds starts with the incorporation of cellular N into glutamate (Glu) or glutamine (Gln). These compounds then serve as N donors in different biosynthetic reactions. There are two primary pathways that facilitate the synthesis of glutamate (Helling, 1998). It may be formed directly through reductive amination of 2-oxoglutarate (2-OG, α-ketoglutarate) by glutamate dehydrogenase (GDH, gdhA) (Equation 1), or indirectly via glutamine synthetase (GS) which catalyses the addition of ammonia to glutamate to yield glutamine (Equation 2). The amide group of glutamine is then reductively transferred to oxoglutarate by glutamate synthetase (GOGAT) to yield two glutamate molecules (Equation 3). The GS/GOGAT pathway is the most important, and it is ubiquitous in
all bacteria, while the GDH pathway only is present in some bacteria, such as the
enterics (Merrick and Edwards, 1995).

\[ \text{NH}_3 + 2\text{-oxoglutarate} + \text{NADPH} \xrightarrow{\text{GDH}} \text{glutamate} + \text{NADP}^+ \] (1)

\[ \text{NH}_3 + \text{glutamate} + \text{ATP} \xrightarrow{\text{GS}} \text{glutamine} + \text{ADP} + \text{P}_i \] (2)

\[ \text{Glutamine} + 2\text{-oxoglutarate} + \text{NADPH} \xrightarrow{\text{GOGAT}} 2\text{glutamate} + \text{NADP}^+ \] (3)

GDH has a relatively high \( K_m \) of 1 to 4 mM, while both GS and GOGAT have low
\( K_m \) values for their substrates (Merrick and Edwards, 1995; Vanoni and Curti, 1999).
Thus, at low ammonia concentrations the GS/GOGAT pathway is the only pathway
for utilization of ammonia. However, since glutamate dehydrogenase is functioning
when ammonium concentrations are high, ammonia assimilation is shifted from the
glutamine pathway to the glutamate pathway. This might be a way to prevent wasteful
consumption of ATP by glutamine synthetase (Helling, 1998).

The synthesis and/or activation of the proteins for uptake and utilization of different
nitrogenous compounds is tightly regulated in concert with the availability of their
substrates (Merrick and Edwards, 1995). A high intracellular N concentration is
reflected in the intracellular ratio of glutamine to 2-oxoglutarate, such that a high
amount of ammonium results in a high glutamine/2-oxoglutarate ratio, while
ammonium deficiency lowers the ratio. Under N sufficient conditions, the activity of
GS is repressed by adenylylation, while at low intracellular N concentrations, GS is
deadenylylated, resulting in an increase in the GS activity (Vanoni and Curti, 1999).
The expression of the gene encoding GS is also regulated by the intracellular N status.
The result of this is that the transcription is low under N sufficient conditions, while it
is markedly elevated (approximately 10-14 fold) under N deficient conditions
(Schreier et al., 1985; van Heeswijk et al., 2000). Downstream of the gene encoding
GS, a gene homologous to several ammonium transporters is found (van Heeswijk et
al., 1996; Meletzus et al., 1998; Michel-Reydellet et al., 1998). The lack of a clear
termination or promoter sequence between those genes indicates that they are co-
transcribed, which is consistent with the finding that methylammonium uptake is
higher at high GS levels (Michel-Reydellet et al., 1998).

The role of C and N in determining N mineralization and immobilization rates

Soil microorganisms are generally considered to be C limited (Mikan et al., 2000;
Fisk and Fahey, 2001, Vance and Chapin, 2001) and heterotrophic decomposition and
nutrient cycling in soils have traditionally been described using first order kinetics
(Molina et al., 1983; Parton et al., 1987; Kersebaum and Richter, 1990; Sallih and
Pansu, 1993):

\[ \frac{dC}{dt} = -kC \] (4)

where \( C \) is the concentration of available C, and \( k \) the first order rate constant.
Integration of equation X yields:

\[ C_t = C_0 \exp(-kt) \] (5)
where \( C_0 \) is the initial concentration of C and \( C_t \) the concentration of available C at time \( t \). The C mineralised with time, \( C_m \), can then be calculated by substituting \( C_t = (C_0 - C_m) \):

\[
C_m = C_0(1 - \exp(-kt))
\]  

(6)

The rate of C assimilation, \( C_a \), is linearly related to the rate of C mineralization and depends on the C use efficiency \( (C_e) \), i.e. the fraction of the utilized C that is incorporated into the biomass:

\[
C_a = \frac{C_m}{1 - C_e} - C_m
\]  

(7)

The N immobilization, \( N_{imm} \), can then be calculated with:

\[
N_{imm} = \frac{C_a}{C:N_{mic}}
\]  

(8)

where \( C:N_{mic} \) is the C/N ratio of the microorganisms. If C limitation and first order kinetics are assumed, N immobilization rates can be expected to be higher in soils with high C content than in soils with low. Accordingly, Barret and Burke (2000) found that the C content alone could explain more than 60% of the variation in N immobilization at regional scales. The rate of N immobilization in soil profiles decrease in the order litter layer > humic layer > mineral soil (Pullem and Tietema, 1999; Fisk and Fahey, 2001; Puri and Ashman, 1998), and N immobilization rates are higher in forest soils than in agricultural soils, possibly as a result of decreasing C content (e.g. Recous et al., 1999; Fisk and Fahey, 2001) and incorporation of plant material into soils poor in organic matter enhance mineralization and N immobilization rates (Paper IV; Watkins and Barraclough, 1996; Recous et al., 1999; Andersen and Jensen, 2001).

Soil inorganic N pools are small and may have a turnover time of less than a day (Hart et al., 1994; Scott et al., 1998; Verchot et al., 2001; Paper I and II). The validity of Equations 4-8 therefore depends on the assumption that there is enough mineralizable N to support the calculated N immobilization. However, soil microorganisms use the GS/GOGAT rather than the GDH pathway to assimilate NH\(_4\)\(^+\) (Schimel and Firestone, 1989), some soils have net N immobilization (Paper I; Nadelhoffer et al., 1984; Giblin et al., 1991; Wagener and Schimel, 1998), and gross N immobilization rates sometimes increase when soils are fertilized with N (Tietema and van Dam, 1996; Fisk and Fahey, 2001), all indicative of N limitation. The coupling of C and N cycling in soils might therefore be controlled by the concentration of available N rather than by the soil C content, with high N concentrations resulting in high respiration and immobilization rates.

The soil C/N ratio may be important in determining if microorganisms are C or N limited, and several models predicting N turnover and retention in soils include the C/N ratio as an important factor in determining the rate of mineralization, immobilization and nitrification (van Veen et al, 1984; Aber, 1992; Bradbury et al., 1993; Janssen, 1996). The idea that the C/N ratio is important for the immobilization rate rests on the assumption that microorganisms are C limited below a certain C/N ratio and N limited above this ratio (Tate, 1995). The ratio can be calculated from the
C/N ratio of the microorganisms growing on the organic matter and their C use efficiency:

\[ C/N_{\text{lim}} = C/N_{\text{mic}} / C_e \]  
(9)

where \( C/N_{\text{lim}} \) is the C/N ratio where the microorganisms become C or N limited. This ratio is varying between 13 and 30 in the literature depending on the carbon use efficiency and the biomass C/N ratio of the microorganisms, but fungi as well as bacteria are considered to be N limited at substrate C/N ratios above 30 (Killham, 1994; Kaye and Hart, 1997).

**Microbial activity and N mineralization and immobilization**

Independent of C or N limitation, a number of problems exist with using first order kinetics. C and N mineralization of soil organic matter is not spontaneous but catalyzed by extracellular enzymes produced by microorganisms inhabiting the soil, and as discussed above, the uptake and utilization of N is an active process. Catalyzed reactions, as well as substrate utilization by microorganisms, can be described with the Michaelis-Menten equation:

\[ \frac{dS}{dt} = -K E S / (K_m + S) \]  
(10)

where \( S \) is the concentration of the substrate, \( E \) the concentration of the catalyst or the microbial biomass, \( K \) the fundamental rate constant that depends on the quality of the substrate, and \( K_m \) the half-saturation constant. If the concentration of the catalyst is assumed to remain constant, \( K \) and \( E \) can be combined into a new constant, \( V_{\text{max}} \), representing the maximum substrate utilization rate:

\[ \frac{dS}{dt} = -V_{\text{max}} S / (K_m + S) \]  
(11)

If substrate concentrations are low enough (\( S << K_m \)) Equation 11 can be simplified to a linear first order approximation (Bekins et al., 1998):

\[ \frac{dS}{dt} = -V_{\text{max}} S / K_m \]  
(12)

First order kinetics may therefore be useful in describing mineralization and immobilization rates, but it depends on the assumption that the maximum concentration of the substrate is much less than the half-saturation constant. On the other hand, if substrate concentrations are high (\( S >> K_m \)), Equation 11 may be approximated by a zero order equation (Bekins et al., 1998):

\[ \frac{dS}{dt} = -V_{\text{max}} \]  
(13)

The choice of zero order, first order, or Michaelis-Menten kinetics in describing C and N mineralization therefore depends on the concentration of the substrate in relation to the half-saturation constant. In soils, the amount of SOM may be 50-100 times higher than the microbial biomass (Diaz-Ravina, et al., 1993). If a single pool of C and N is assumed, substrate concentrations are likely to be much higher than the half-saturation constant and zero-order kinetics the preferred choice when describing
C and N mineralization rates. On the other hand, soil organic matter is very complex and consists of numerous different compounds with different availability to soil microorganisms (Schulten et al., 1997, Schulten and Schnitzer, 1998) The concentration of compounds such as organic acids are relatively low (Hongve et al., 2000; Strobel, 2001), and if these are considered to be the substrate for microorganisms, the use of first order kinetics might be justified. To my knowledge, no studies have verified the assumption that the concentrations are much lower than the half-saturation constant, probably since the combination of a high microbial diversity, the ability of microorganisms to utilize several different substrates, and the numerous possible substrates makes it virtually impossible.

In Paper I and II I tried to circumvent this problem by examining if N mineralization and immobilization rates were more dependent on and variable with the microbial biomass and activity than on soil C and N concentrations. If first order kinetics is assumed and microorganisms C limited, the respiration rate ($C_m$) and N immobilization rate ($N_{imm}$) should be correlated (Equation 16) and dependent on the concentration of C (Equation 6). On the other hand, if microorganisms are N limited, the N immobilization rate should be dependent on the concentration of inorganic N (Equations 10-12). Since all known NH$_4^+$ transporters isolated from bacteria have a $K_m$ in the micromolar range (Siewe et al., 1996; Montesinos et al., 1998; Soupene et al., 1998), the NH$_4^+$ concentration is sufficiently high to fulfill the assumption that $S\gg K_m$ (Paper I), allowing NH$_4^+$ immobilization at a maximum rate and the use of zero order approximations. However, N immobilization rates are often sufficiently high to deplete the inorganic N pools in less than a day (Hart et al., 1994; Verchot et al., 2001; Paper I and II). Thus, N mineralization may be the rate limiting step if microorganisms are N limited.

In Paper I, I found that N mineralization and immobilization rates among soils were more variable with and dependent on the microbial biomass and activity than on the soil C/N ratio. The N immobilization was of the same magnitude in two of the soils, but considerably lower in the third (Paper I, Fig. 1). The same pattern was found for the ATP content, which reflects both biomass and metabolic state of the microorganisms (Contin et al., 2000), and the respiration rate (Paper I, Fig. 2 and 3). Several other studies have found gross immobilization and mineralization rates to be correlated with respiration rates (Hart et al., 1994; Puri and Ashman, 1998; Tietema, 1998; Barret and Burke, 2000; Paper II). The slope of the relationship between respiration and N immobilization rates varies between 0.032 (Barret and Burke, 2000) and 0.20 (Hart et al., 1994) in the literature. From these slopes the C use efficiency ($C_e$) and the critical soil C/N ratio for N limitation ($C:N_{mic}$, Equation 9) can be roughly estimated.

$$N_{imm} = C_m \times \text{slope}$$  \hspace{1cm} (14)

$$C_a = N_{imm} \times C:N_{mic}$$  \hspace{1cm} (15)

$$C_e = C_a/(C_a + C_m)$$  \hspace{1cm} (16)

If the soil microbial community is assumed to have a C/N ratio of 10, the C use efficiencies will vary between 0.24 and 0.67, and the critical C/N ratio for N limitation becomes 42 and 15, respectively. All the soils in Paper I had C/N ratios
above 15 but below 42, and it is therefore possible that that the same species (C or N) was limiting in all three soils.

Equation 9 suggests the C use efficiency must remain constant C in limited microorganisms, since variations would lead to the conclusion that the critical C/N ratio for C or N limitation can vary from day to day. However, the C use efficiency does not remain constant over time, but may vary several folds (Hart et al., 1994). The C/N ratio of the substrate that is actually utilized is also variable and differs from that of the whole soil (Hendrickson, 1985; van Veen et al., 1985; Hadas et al., 1987; Hart et al., 1994). This may be the reason why there was no influence of the whole soil C/N ratio on the N immobilization rate, and it also demonstrates that the amount of C that is available to and utilized by the microorganisms is not necessarily correlated to the total C content in a soil. Even though N mineralization and immobilization rates were highest in soils with the highest microbial activity in Paper I, suggesting that zero order or Michaelis-Menten kinetics should be used, those soils were also the ones with the highest C and N content, which indicate that first order rates were applicable.

Hence, the results in Paper I were ambiguous with respect to the kinetic order of N mineralization and immobilization, and to the influence of the microbial activity and biomass on the processes. In Paper II and V, I addressed this ambiguity by estimating the utilization of three different soil C fractions. Analysis of δ^13C of the respired CO_2 and the three C pools indicated that microorganisms mainly utilized dissolved organic C (DOC; Paper V, Fig. 1). However, the respiration rate was not dependent on the concentration of DOC (Paper II, Table 3), which would be expected if the microorganisms were C limited and DOC was their C source (Equation 4), and the N immobilization rate was independent of the DOC concentration (Equations 4-8; Paper II, Table 3). The low DOC concentrations compared to the respiration rate meant that the DOC pool would be depleted unless DOC was constantly produced. In addition to microbial C recycled to the DOC pool, soil organic matter (SOM) seemed to be the source of DOC (Paper V, Fig. 2), but the respiration rate was independent of the amount of SOM as well (Paper II, Table 3). However, there was a strong connection between temporal variations of temperature, which affect the rate of metabolic processes, and the respiration rates, and between soil water content and respiration rates (Paper II).

Those results suggested that the microorganisms were not C limited, and that first order approximations, if applicable, should be based on N concentrations. However, Paper I, II and IV gave no evidence for a relationship between in situ concentrations of NH_4^+ and NH_4^+ immobilization rates but supported the suggestion above that the NH_4^+ concentrations was sufficiently high fulfill the assumption that $S \gg K_m$, allowing NH_4^+ to be immobilized at zero order rates. It can be argued that microorganism mainly utilized other N sources than NH_4^+, hence the lack of association between NH_4^+ concentrations and immobilization rates. NO_3^- would be a likely candidate as an alternative N source because of its high mobility in soils compared to NH_4^+, but even though NO_3^- immobilization commonly occurs in forest soils, the ratio between NO_3^- and NH_4^+ immobilization is generally low (Recous and Mary, 1990; Zak et al., 1990; Fisk and Fahey, 2001; Verchot et al., 2001; Paper I and IV). Similarly, microorganisms have the capacity to take up a number of organic N compounds (Merrick and Edwards, 1995), but direct uptake of organic N is small compared to the NH_4^+ uptake (Molina et al., 1990; Hadas et al., 1992). Uptake of NO_3^- and organic N
therefore seem to make a minor contribution to the microbial N demand. Hence, N mineralization seems to be the rate limiting step.

N mineralization rates were dependent on the soil water content, while neither dissolved N concentrations nor total soil N content had any influence (Paper II). I did however find a positive relationship between gross N mineralization rates, respiration rates, and the concentration of phosphate buffer extractable N (PON), in agreement with Matsumoto et al. (2000). PON is a homogeneous, bacterially derived group of protein-like compounds with a size of 8-9 kDa (Matsumoto et al. 2000), which may represent residues of exoenzymes that become abundant and released to degrade organic N compounds during high microbial activity. Accordingly, positive correlations between gross N mineralization rates and soil enzyme concentrations have been found by e.g. Zaman et al. (1999).

Taken together, the lack of influence of the soil C/N ratio (Paper I) and soil C and N pool sizes on the mineralization and immobilization of C and N (Paper II and V), and the positive influence of temperature, soil water content, thymidine incorporation rates (Paper IV), and ATP and exoenzyme concentrations (Paper I and II), suggests that first order approximations (Equation 12) would be inappropriate to describe the mineralization and immobilization of C and N. Turnover of C and N in forest soils rather seems to be tightly controlled by the microbial biomass and activity alone. The correlations between the production and consumption of the primary C and N sources, DOC and NH$_4^+$, (Paper I, II and V; Davidson et al., 1992; Hart et al., 1997; Verchot et al., 2001), and the correlation between respiration and N mineralization and immobilization rates (Paper II; Hart et al., 1994; Puri and Ashman, 1998; Tietema, 1998; Barret and Burke, 2000) seem to confirm this.

The combination of $K$ and $E$ into a single term, $V_{max}$, in Equation 13 rests on the assumption that the concentration of the catalyzer ($E$) is constant. The production of the enzymes necessary for the mineralization and uptake of C and N is however dependent on the microbial activity, and because of its variability this assumption may not be valid. Equation 13 can therefore be modified to:

$$\frac{dS}{dt} = -KE$$  \hspace{1cm} \text{(17)}

which essentially is a first order equation, but dependent on the enzyme concentration rather than the substrate concentration. Since the concentration of $E$ is dependent on the microbial activity Equation 17 can be rewritten to:

$$\frac{dS}{dt} = -KB_{m}A_{m}$$  \hspace{1cm} \text{(18)}

where $B_{m}$ is the microbial biomass and $A_{m}$ the microbial activity per biomass unit. The microbial biomass in soils ($B_{m}$) is well correlated to the soil organic matter content (Zak et al., 1990; Zhang and Zhang, 2003; Milne and Haynes, 2004), and under non-limiting substrate and oxygen conditions, $A_{m}$ is dependent on the temperature and soil water content.

Equation 18 can explain the discrepancy between Paper II, where C and N pool sizes did not seem to have an influence on N mineralization and immobilization rates, and e.g. the study by Barret and Burke (2000), in which the soil C content alone could
explain 60–70% of the variation of N mineralization and immobilization rates. Barret and Burke (2000) used laboratory incubations with high and constant temperature and soil water content to determine N transformation rates. In this way, the variation in \( A_m \) among soils was restrained and the N mineralization and immobilization rates dependent on \( B_m \) alone. Since \( B_m \) is dependent on the soil organic matter content, their results could have been predicted from Equation 18. In paper II, on the other hand, C and N mineralization and N immobilization rates were determined in situ and \( A_m \) was probably more variable than \( B_m \) due to variations in soil water content and temperature, which influence mineralization and immobilization rates (Puri and Ashman, 1998; Zaman et al., 1999). Variations in \( A_m \) may also explain the dependence of N mineralization and immobilization rates on the microbial biomass in some studies (Davidson et al., 1992; Hart et al., 1997), but not in others (Puri and Ashman, 1998; Zaman et al., 1999). The commonly used chloroform fumigation method extracts the total microbial biomass C rather than the active part of the soil microbial community (Ross, 1991; van de Werf and Verstraete, 1987) and would fail to discriminate between two bacterial populations with a similar biomass but varying growth rates (Bloem et al., 1989).

Restrictions on the microbial biomass

Equation 18 includes the microbial biomass and activity as factors determining the substrate utilization rate. Schimel and Weintraub (2003) demonstrated that a model assuming exoenzyme catalysed SOM decomposition only had a knife-edge equilibrium depending on the fundamental rate constant of the substrate \( (K) \). With high \( K \) values, the microbial population grew out of control, and with low it crashed. In reality though, the microbial biomass remains rather constant from day to day and in some cases even from week to week (Witter, et al., 1993; Lovell and Hatch, 1998; Puri and Ashman, 1998), while the microbial activity may vary considerably on the same timescale (Bloem et al., 1989; Lovell and Hatch, 1998). There therefore have to be mechanisms that restrain biomass variations. When conditions are unfavourable, some soil bacteria form spores, and soil microorganisms can remain dormant for a long period of time, resulting in a relatively small decrease in microbial biomass in response to stress. Predation may be an important factor setting an upper limit for the microbial biomass (Ekelund and Rønn, 1994; Zhang et al., 2000; Rønn et al., 2002). The higher microbial biomass in soils with high SOM content might be a result of protection of bacteria against predation by protozoa, since it is generally accepted that fine textured soils offer greater protection than coarse textured soils (Alexander, 1981; Rutherford and Juma, 1992).

The response of soil microorganisms to C and N additions

One objection to use zero order approximations to describe microbial C and N mineralization and utilization may be that addition of C and N to a soil often enhances either respiration or N immobilization rates or both (Mikan et al., 2000; Fisk and Fahey, 2001; Vance and Chapin, 2001). Justus von Liebig's Law of the Minimum states that yield is proportional to the amount of the most limiting nutrient, whichever nutrient it may be. If a deficient nutrient is supplied, yields may be improved to the point that some other nutrient is needed in greater quantity than the soil can provide,
and the Law of the Minimum would apply to that nutrient. Therefore, if mineralization and immobilization rates are assumed to be independent of substrate concentrations, microorganisms cannot be C or N limited and should not respond to C or N additions. However, differences in substrate quality may induce an apparent C or N limitation, although microorganisms are not strictly C or N limited according to the definition. The response of soil microorganisms to C or N additions when zero order kinetics are assumed can be elucidated by dividing Equation 18 into one C part and one N part:

\[
d\frac{C}{dt} = -K_C B_{C,A} A_m
\]

\[
d\frac{N}{dt} = -K_N B_{N,A} A_m
\]

where \(K_C\) is the fundamental rate constant for soil C, and \(K_N\) the fundamental rate constant for soil N. If \(K_C > K_N\), microorganisms would appear to be N limited, and C limited if \(K_C < K_N\). The theoretical response of C and N mineralization and immobilization rates to C and N additions in any of those cases is summarized in Table 1.

Table 1. The theoretical response of C and N mineralization and immobilization rates to additions of C and N with different quality if \(K_C > K_N\) (N limitation) and if \(K_C < K_N\) (C limitation). \(K_{AC}\) is the fundamental rate constant for the added C, and \(K_{AN}\) the fundamental rate constant for the added N.

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<tr>
<td>1. (K_C &gt; K_N) (N limitation)</td>
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<td>5. (K_C &lt; K_N) (C limitation)</td>
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<td>(K_{AC} &lt; K_{CC})</td>
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<td>6.</td>
<td>(K_{AC} &lt; K_{CC}) (2)</td>
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<td>7.</td>
<td>N</td>
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<td>8.</td>
<td>(K_{AN} &lt; K_{NN}) (2)</td>
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(1) Microorganisms growing under nutrient limited conditions take up C compounds, resulting in elevated respiration rates that are not associated with growth (overflow metabolism; Schimel and Weintraub, 2003 and references therein).

(2) No response is detected since the fundamental rate constant is lower for the added C than for soil C or N.

(3) Addition of N results in reduced overflow metabolism, resulting in a lower respiration rate and higher C assimilation rate.

(4) A higher C utilization rate results in higher N utilization rates according to Equation 8.

(5) The synthesis and/or activation of proteins involved in the uptake and utilization of nitrogenous compounds is closely regulated in concert with the availability of their substrates (Merrick and Edwards, 1995 and references therein). That is, if the intracellular N content of the microorganisms is sufficient, no additional N is assimilated, and no enzymes are produced to mineralize organic N. Hence, no response to addition of N, even if \(K_{AN} > K_{NN}\), under C limited conditions.

Table 1 show that addition of an easily available C source can always be expected to increase respiration rates (Assumption 1 and 5). Such responses have been documented numerous times. In fact, the substrate induced respiration method (Anderson and Domsch, 1978), in which the magnitude of the increased CO\(_2\) production in response to glucose additions is used to determine microbial biomass in soils, depends on this assumption. Addition of N to a N limited soil increases the C use efficiency (Fisk and Fahey, 2001; Lovell and Hatch, 1998), resulting in decreased
respiration rates (Assumption 3). Increased respiration rates in response to C additions (Mikan et al., 2000; Vance and Chapin, 2001) and decreased in response to N additions (Fisk and Fahey, 2001) have commonly been interpreted as proof for C limitation of the microbial biomass in soils. If zero order kinetics are assumed and the theoretical responses in Table 1 to N additions valid this might not necessarily be the case, and changes in respiration rates in response to C or N additions may not be a reliable method to assess if microorganisms are C or N limited. Then again, increased N immobilization rates as a response to N additions always seem to be indicative of N limitation (Table 1, Assumption 3 and 7).

The influence of plants on N mineralization and immobilization rates

Theoretically plants may influence N mineralization and immobilization in several ways. Plant litter is the major source of organic C and N in soils. Easily available C sources in litter leachate, such as simple fatty acids, organic acids and carbohydrates may stimulate the microbial activity (Henriksen & Breland, 1999, Hongve et al., 2000). Root exudates also consist of readily available C and N sources, e.g. amino acids, vitamins, enzymes and nucleotides (Graystone et al., 1996; Uren, 2001 in The Rhizosphere eds. Pinton et al), which may have a stimulatory effect on the microbial activity and C and N transformation rates.

Specific compounds in plant litter may also reduce the rate of N mineralization and immobilization. Some polyphenolic compounds have an inhibitory effect on the growth and activity of microorganisms, and the binding of proteins to polyphenolic compounds might render the proteinacious N inaccessible to heterotrophic microorganisms, thereby decreasing the N mineralization rate (Northup, 1995; Krauss et al., 2004). Although plant N uptake generally is much lower than microbial N uptake (reviewed by Kaye and Hart, 1997), it may sometimes even exceed microbial uptake (Paper IV; Norton and Firestone, 1996). Plants may therefore compete with microorganisms for N (Kaye and Hart, 1997), resulting in lower microbial activity C and N transformation rates.

In Paper III and IV, I tried to elucidate the effect of plants on the microbial activity and N mineralization and immobilization rates and vice versa. In Paper IV, the bacterial activity seemed to be the main factor determining the microbial N uptake, while plants had no effect. Plants stimulated the bacterial activity only when fragmented litter had been added to a soil, resulting in low inorganic N concentrations. However, microbial N uptake decreased in one of the three soils in presence of plants but since the decreased microbial N uptake did not coincide with a corresponding increase in plant N uptake or decreased bacterial activity it might be that the plants specifically inhibited fungal activity and N uptake. Hence, the bacterial activity was the main factor determining microbial N immobilization rates, while plants only seemed to have minor effects on the microbial activity and N immobilization rates. This is consistent with the hypothesis that C and N mineralization and immobilization rates are independent of substrate concentrations.

In Paper III, I found that spatial patterns of N transformation rates were more related to the spatial pattern of the cover of plants than to the species composition. Other studies seem to confirm that plants promote N mineralization and immobilization
rates (Jackson and Caldwell, 1993; Schlesinger, 1996), supposedly by root exudates that provide microorganisms with high quality C and N sources (high $K_C$ and $K_N$ values). Similarly, different $K$ values for oak and beech litter, reflecting the lignin content which is higher in beech than in oak (Ferrari, 1999; Sariyildiz and Anderson, 2003a and 2003b) and inversely related to the decomposition rate (Sariyildiz and Anderson, 2003a and 2003b), may explain the different influence of those tree species in N mineralization and immobilization rates (Paper III).

**Abiotic immobilization of N**

N can be immobilized by fixation to soil minerals or direct incorporation into the soil organic matter by chemical reactions. Fixation of NH$_4^+$ to clay minerals has been shown to occur in a wide variety of soils. The fixation process is fast (<30 minutes), but usually <10% of the added NH$_4^+$ is fixed, even though cases of more than 25% NH$_4^+$ fixation have been reported (Drury and Beauchamp, 1991; Trehan, 1996). The NH$_4^+$ fixation is positively correlated to the vermiculate clay content and to the amount of added NH$_4^+$ (Keerthisinghe et al., 1984). Consumption of NH$_4^+$ by biotic immobilization or nitrification seems to cause the release of fixed N (Drury and Beauchamp, 1991; Trehan, 1996), and inhibition of the nitrification process has been reported to enhance the NH$_4^+$ fixation (Juma and Paul, 1983). Therefore, at least a part of the clay-fixed NH$_4^+$ seems to be reversibly bound, but the release of the fixed NH$_4^+$ is much slower than the fixation (Drury and Beauchamp, 1991).

NH$_3$ and other amines can react with soil organic matter to form stable organic N complexes (Thorn and Mikita, 1992). Little is known about the mechanism involved in NH$_3$ fixation, but the reaction is favoured by high pH values and associated with uptake of O$_2$ (Stevenson, 1982). Several possible mechanisms have been proposed, including formation of quinones capable of reacting with NH$_3$ to form complex polymers. NH$_3$ also reacts with reducing sugars, and with a wide variety of ketones, aldehydes and other carbonyl-containing compounds under alkaline conditions (Stevenson, 1982). However, because of the high pK$_a$ of NH$_3$ (9.25) and since NH$_3$ is the reactive form of N (Johnson et al., 2000), these reactions are unlikely to occur in most natural soils.

**Abiotic vs. biotic immobilization of N**

Biotic N immobilization processes rather than chemical reactions seem to be responsible for the majority of N incorporated into the soil organic matter fraction. More $^{15}$N is recovered in the organic matter in $^{15}$NH$_4^+$ treated non-sterile soils compared to autoclaved soils after incubation times as short as 30 minutes (Trehan, 1996). This difference is enhanced with longer incubation times. Nuclear magnetic resonance (NMR) spectra of the organic matter in $^{15}$NH$_4^+$ or $^{15}$NO$_3^-$ treated soils have also revealed that the resulting organic $^{15}$N fraction consists almost exclusively of peptides and proteins (>80%), nucleic acids, and aliphatic amine groups (Clinton et al., 1995). Since the $^{15}$N is recovered in partly humified organic matter rather than in poorly transformed plant fragments, it is possible to attribute the incorporation of $^{15}$N into the organic matter to microbial activity, at least during incubations shorter than a few months. The similarity of $^{15}$N spectra for soils treated with $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$,

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and the enhanced incorporation of $^{15}$N into the organic matter in non-sterile soils compared to autoclaved soils also speaks against direct incorporation of $^{15}$N into organic matter by chemical reactions.

The amount of NH$_4^+$ fixed in clay minerals compared to the amount incorporated in organic matter varies widely depending on soil type. Trehan (1996) found the ratio between clay fixed $^{15}$N and organic $^{15}$N 30 minutes after $^{15}$NH$_4^+$ addition to vary between 0.15 and 2.5 in three agricultural soils. The distribution of $^{15}$N between the clay and organic matter fractions in the three soils also varied widely over the following 20 days, with no repeatable pattern among soils. Drury and Beauchamp (1991) found the fixed and organic matter $^{15}$N fractions to be of equal size 6 hours after $^{15}$NH$_4^+$ addition in two clay loams with different fixation capacity. However, while the amount of $^{15}$N in the organic matter fraction remained fairly constant or only increased slowly after this, the amount of $^{15}$N in the fixed fraction doubled during the following 3 days. Johnson and Burke (2000) found an increase in the relative contribution of abiotic N immobilization to the total N immobilization with increasing N concentrations in a number of forest soils differing in N status. Abiotic N immobilization accounted for 6-90% of the total N immobilization in these soils after 12 hours incubation with $^{15}$N. Because of the high variability in experimental data depending on the soil type used and the lack of theories able to explain this variation, no model exists that can accurately describe the distribution and exchange of N between the two pools.

Mobilization of microbially bound N

The soil microbial biomass often has short turnover times (Joergensen et al., 1990; Hart et al., 1994; Bååth, 1998; Vance and Chapin, 2001). In Paper V we demonstrated the flow of C between the microbial biomass, DOC and SOM was well balanced. Several mechanisms can be responsible for the release of microbial C and N. During bacterial growth there is a high production and breakdown of RNA (Mason and Egli, 1993) and proteins. The resulting compounds can either be reused by the bacteria or excreted as organic nitrous compounds (e.g. urea), or after further degradation as NH$_4^+$. Degradation of mRNA in particular is high during exponential growth (Norris and Koch, 1972), and it is likely that the same is true for proteins. However, as long as the bacteria are not C or N limited, a lot of the degradation products may be used to synthesize new RNA or protein, resulting in a low excretion rate of nitrous compounds. As carbon gets exhausted, the degradation rate of RNA gets even higher (Mason and Egli, 1993), leading to a decrease in bacterial RNA content and a higher excretion rate of nitrous compounds (Therkildsen et al., 1997). In Paper VI, I demonstrated that bacteria have a high ability to recycle N intracellularly, especially at low N concentrations and growth rates. Therefore, remineralization of microbial N by other processes than predation or soil drying/rewetting (discussed below) is likely to occur only when conditions promote high growth rates, i.e. when microorganisms are not substrate limited and temperature and moisture conditions are favourable.

Soil drying and rewetting may be important in mobilizing microbially bound N. There is no active transport mechanism for water in microorganisms, so water flows freely in and out of the cell. In order to avoid lysis bacteria have to maintain turgor in balance with the surrounding environment. During osmotic upshift this is
accomplished by accumulating osmotically active solutes in the cytoplasm, preferably by active uptake or synthesis of so-called compatible solutes; compounds that are highly congruous with cellular functions (Kempf and Bremer, 1998). These include free amino acids and derivates thereof, as well as quaternary amines. If osmoprotected cells are subjected to osmotic downshock, such as a heavy rainfall, water flows into the cells because of the high concentration of solutes in the cytoplasm. This is a very rapid process (<1 min), and to avoid swelling and bursting the microbes will have to respond accordingly. Rapid and extensive solute efflux as a result of osmotic downshift has been shown to occur in several bacterial species (Wood, 1999). This gives a mechanistic explanation to the well-known phenomena that rewetting of an air-dried soil results in a flush of C and N mineralization (Paper I; Birch, 1958; Agarwal et al., 1971; van Gestel et al., 1991; Pulleman and Tietema, 1999; Fierer and Schimel, 2003). Drying and rewetting release physically protected SOM, increasing the amount of extractable C by up to 200% (Fierer and Schimel, 2003). Bacterial cells with a high growth rate and increased efficiency of translation are favoured in rich environments (Nyström, 2002). However, the payoff for this increased rate of reproduction is a reduced ability to withstand starvation (Kurland and Mikkola, 1993), and oxidation of proteins in aging growth-arrested cells may lead to proteolysis (Nyström, 2002). Remineralization of a growth-arrested fast growing microbial community may also explain the secondary peak in respiration and N mineralization rates that is sometimes observed a few days after rewetting of an air-dried soil (Paper I; Pulleman and Tietema, 1999).

Predation may also be an important factor controlling the recycling of microbially bound N. High protozoan activity coincides with high mineralization and immobilization rates and short turnover times of the microbial biomass (Christensen et al., 1996; Rønn et al., 2002). Similarly, there is a positive correlation between the abundance of nematodes and the microbial activity (Mamilov and Dilly, 2002), and addition of earthworms to a soil decreases the total microbial biomass but increases its activity (Zhang et al., 2000).

Environmental concerns

More N is fixed annually by human activities (=140 Tg N yr⁻¹) than by natural processes (110-125 Tg N yr⁻¹; Vitousek, 1994; Jefferies and Maron, 1997). In addition, N have been mobilized from long term storage pools through biomass burning, land clearing and conversion, and drainage of wetlands. Of particular concern are the potential ecological effects of sustained atmospheric nitrogen deposition in natural environments (Jefferies and Maron, 1997). Chronic N deposition rates of up to 50% of the assimilatory N need by trees (van Oene et al., 2000) combined with low in situ net mineralization rates have led to concerns that forest ecosystems may get N saturated (Aber, 1992), resulting in increased mobility and leaching of N.

Large-scale experiments such as NITREX and EXMAN, in which N has been added or removed from the ambient deposition at eight sites spanning a gradient of N deposition across Europe, were initiated during the 1990s to evaluate the effect of N deposition on ecosystem processes (Wright and van Breemen, 1995; Wright and Rasmussen, 1998). Unfortunately, none of 36 or so published NITREX studies
Box 1. A conceptual model describing the flow of C and N (solid arrows) in soil. The main factors determining the rate of these processes are the size of the microbial biomass, its activity, the quality of the substrate they are growing on, predation, and drying-rewetting cycles, as indicated for each process below. The size of the microbial biomass ($B_m$) and its activity ($A_m$) in turn seem to be dependent on soil SOM content ($B_m$) and temperature and soil water content ($A_m$).

Factors affecting rate 1 to 8:

1. Microbial biomass ($B_m$), activity per biomass unit ($A_m$), and the fundamental rate constant for SOM-C ($K_{SOM-C}$).
2. Microbial biomass ($B_m$), activity per biomass unit ($A_m$), and the fundamental rate constant for DOC ($K_{DOC}$).
3. C uptake rate and microbial C use efficiency.
4. Drying-rewetting cycles, predation, and the microbial biomass ($B_m$) and activity per biomass unit ($A_m$).
5. Microbial biomass ($B_m$), activity per biomass unit ($A_m$), the fundamental rate constant for SOM-N ($K_{SOM-N}$), and the intracellular N status of the microorganisms.
6. Microbial biomass ($B_m$), activity per biomass unit ($A_m$), the fundamental rate constant for DN ($K_{DN}$), and the intracellular N status of the microorganisms.
7. Drying-rewetting cycles, predation, and the microbial biomass ($B_m$) and activity per biomass unit ($A_m$).
8. Predation, drought etc.
examined the difference in gross mineralization or immobilization rates between treatment and control plots, but only among sites. Those rates vary between 9-18 mg N kg soil$^{-1}$ d$^{-1}$ (Tietema, 1998). In Paper II and III, I found similar in situ N mineralization rate with an average of 15.3 mg N kg$^{-1}$ d$^{-1}$. If those rates are recalculated to g m$^{-2}$ day (using a soil density of 1 g cm$^{-3}$), the gross N mineralization becomes 0.75 g m$^{-2}$ day in the top 5 cm of the soil. The natural N deposition in southwestern Scania, 0.0054 g m$^{-2}$ day (20 kg ha$^{-1}$, Lagner et al., 1996), and the N addition in the NITREX experiments, 0.0096 g m$^{-2}$ day (Gundersen et al., 1998), only represent 0.7-1.3% of the ammonium that was naturally produced. That is, a reduction in the gross N mineralization rate of about 1% would be enough to compensate for the addition of inorganic N. This decrease would hardly be detectable given the great spatial and temporal variability of N transformation rates (Paper I and III). Chronic N deposition may still be a problem. About 50% of the deposited N is in the form of NO$_3^-$, and although it is now well documented that soil microorganisms immobilize NO$_3^-$ even in the presence of NH$_4^+$, NO$_3^-$ immobilization rates are low compared to NH$_4^+$ immobilization rates. N leakage due to NO$_x$ deposition, especially during periods of low microbial activity, may therefore still be a problem.

Conclusions

From the results in Paper I-VI and the cited literature I conclude that the main factors determining microbial mobilization and immobilization of soil N are the size of the microbial biomass, its activity, and the quality of the substrate they are growing on. Those three factors in turn seem to be dependent on soil SOM content, predation, drying-rewetting cycles, temperature, and soil water content. This conceptual view is summarized in Box 1.

Acknowledgements

I am grateful to Göran Bengtsson and Ursula Falkengren-Gerup for helpful comments on a previous draft. The work included in the thesis was supported by grants from the Foundation for Strategic Environmental Research in Sweden.

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Tack till familj, vänner, handledare och kollegor!
Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity

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Received 18 March 2002; received in revised form 6 September 2002; accepted 18 September 2002

Abstract
A laboratory experiment was designed to challenge the idea that the C/N ratio of forest soils may control gross N immobilization, mineralization, and nitrification rates. Soils were collected from three deciduous forests sites varying in C/N ratio between 15 and 27. They were air-dried and rewetted to induce a burst of microbial activity. The N transformation rates were calculated from an isotope dilution and enrichment procedure, in which 15NH4Cl or Na15NO3 was repeatedly added to the soils during 7 days of incubation. The experiments suggested that differences in gross nitrogen immobilization and mineralization rates between the soils were more related to the respiration rate and ATP content than to the C/N ratio. Peaks of respiration and ATP content were followed by high rates of mineralization and immobilization, with 1–2 days of delay. The gross immobilization of NH4\(^+\) was dependent on the gross mineralization and one to two orders of magnitude larger than the gross NO3\(^-\) immobilization. The gross nitrification rates were negatively related to the ATP content and the C/N ratio and greatly exceeding the net nitrification rates. Taken together, the observations suggest that leaching of nitrate from forest soils may be largely dependent on the density and activity of the microbial community.

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Keywords: Nitrogen; Ammonium; Nitrate; ATP; Isotope pool dilution; Forest; Rewetting

1. Introduction
The low concentration of NO3\(^-\) found in forest soils has often been attributed to low rates of nitrification (Vitousek et al., 1982; Gosz and White, 1986). This interpretation is supported by observations of low net nitrification rates during incubation assays of soil samples. However, occasional measurements of gross nitrification suggest a rapid turnover of a small NO3\(^-\) pool in forest soils, and that the dominant fate of NO3\(^-\), as well as NH4\(^+\), is immobilization in the soil organic matter pool (Davidson et al., 1992; Groffman et al., 1993; Stark and Hart, 1997). In this way, immobilization may prevent nitrogen leakage to ground and surface waters.

The extent of immobilization varies between 35 and 95% from one soil to another (Mead and Pritchett, 1975; Heilman et al., 1982; Melin et al., 1983; Schimel and Firestone, 1989; Hart and Firestone, 1991; Hart et al., 1993). Nitrogen is immobilized either by abiotic or biotic processes. Fixation of NH4\(^+\) to clay minerals is fast (< 30 min) but usually less than 10% of the added NH4\(^+\) is fixed (Drury and Beauchamp, 1991; Trehan, 1996). Most of the immobilization to the soil organic matter is biotic. Early work estimated soil microorganisms to be responsible for 10–50% of NH4\(^+\) immobilization (Brookes et al., 1985) and more recent analyses by NMR of the organic matter of 15NH4\(^+\) or 15NO3\(^-\) treated soils found the organic 15N in microbially derived peptides and proteins (> 80%), nucleic acids, and aliphatic amine groups after several months of in situ incubation (Clinton et al., 1995). The average turnover time of N in microbial biomass is estimated to 1–2 months (Davidson et al., 1992), but some of the immobilized N becomes very stable (Kelley and Stevenson, 1985), as a result of fixation by 2:1 clay minerals (van Veen et al., 1985; Breitenbeck and Paramasivam, 1995), repeated cycles of immobilization and mobilization (Jansson and Persson, 1982; He et al., 1988), and accumulation of persistent residues of microbial cells (Paul and Juma, 1981). Despite the obvious importance of soil microorganisms in the turnover and retention of nitrogen in forest soils, the causes of variation of immobilization of N from one soil to another remain poorly understood.
another and between abiotic and biotic processes are not well known. Some observations suggest that the immobilization is controlled by the concentration of available C (Woodmansee and Duncan, 1980), and others that it is inversely dependent on the amount of available inorganic N (Priha and Smolander, 1995; Bengtsson and Bergwall, 2000).

Mineralization of soil N depends on a wide range of factors, including the C/N-ratio (Frankenberger and Abdelmagid, 1985), the N content (Iritani and Arnold, 1960), lignin content (Frankenberger and Abdelmagid, 1985; De Neve et al., 1994), water soluble N and cellulose content (Iritani and Arnold, 1960; Bending et al., 1998) of the litter, the light fraction organic matter of the soil (Jansen et al., 1992; Sierra 1996), microbial respiration and ATP content (Alef et al., 1988), microbial biomass (Dalal and Meyer, 1987), and microbial N content (Fiuk and Schmidt, 1995). The diversity of factors correlated with N mineralization reflects the variation in substrates and microbial communities being used and temporal changes in substrate quality.

Models predicting nitrogen turnover and retention in soils often include the C/N ratio as an important factor in determining the rate of mineralization, immobilization and nitrification (van Veen et al., 1984; Aber, 1992; Bradbury et al., 1993; Janssen, 1996). The idea to use the soil C/N ratio to predict variations in N mineralization and immobilization rates among soils is based on the fact that heterotrophic soil bacteria usually have a lower C/N ratio than the soil they inhabit. If it is assumed that the cells have a C/N ratio of 10 and respire about 50% of their C uptake, they may be N limited above a soil C/N ratio of 20 and C limited below (Tate, 1995). Soils with a high C/N ratio may then be characterized by rapid immobilization of N and soils with a low C/N ratio by slower N immobilization and a surplus of available NH₄⁺, derived from denitrification of organic carbon sources.

The evidence for a correlation between the soil C/N ratio and nitrogen immobilization and mineralization is equivocal, at least partly because early work was confined to measurements of net rates of N transformation. Within-site variations of the C/N ratios of different pools of organic matter and between-site variations of the C/N ratios and N mineralization efficiency of the microbial biomass may also confound the usefulness of the soil C/N ratio to predict gross nitrogen transformation rates. Therefore, we designed a ¹⁵N pool dilution experiment to simultaneously measure gross immobilization, mineralization, and nitrification, in an effort to challenge the hypotheses of a positive relationship between the C/N ratio and N immobilization and a negative relationship between the C/N ratio and N mineralization and nitrification in three forest soils. Even if the soil C/N ratio may prove useful to predict site-to-site variations of the N transformations, temporal variation of soil temperature and moisture will trigger dynamics of microbial biomass and activity that influence N transformations more than the spatial variation of the C/N ratio. Therefore, we developed the sub-hypothesis that the variation in N transformation rates is controlled by the variations in microbial biomass and activity—a high biomass and activity would correspond (1) to high rates of N immobilization and mineralization, and, as nitrifiers tend to be competitively inferior to heterotrophs (Verhagen and Laanbroek, 1991; Verhagen et al., 1995), (2) to low nitrification rates.

It is well established from laboratory experiments that soil drying and rewetting may bring about a flush of C and N mineralization (Birch, 1958; Agarwal et al., 1971; van Gestel et al., 1991) and an increase in microbial numbers and activity (West et al., 1986). The origin of the mineralized N may be both biomass lyzed by the drying, and non-biomass organic matter that becomes more accessible to microbial attack after drying and rewetting (Jager and Bruins, 1975; Marumoto et al., 1982). We used this evidence to test the sub-hypothesis and induced a microbial growth phase by drying and rewetting the three soils, and then evaluated the subsequent gross N transformation rates. Although short-time laboratory ¹⁵N pool dilution experiments do not necessarily reflect N transformation rates in the field, they facilitate experimental manipulations and help explaining mechanisms causing variation in N transformation rates from one soil to another.

2. Material and Methods

2.1. Soil sampling

Soil material was collected from deciduous forests in southwestern Sweden in July 1998. Soil I was found in the vicinity of Maglo (56°38’N, 13°12’E), and Soil II and III were found in Torup (55°33’N, 13°37’E). After removal of the litter, five samples of the top 5 cm of the soil were collected randomly within a 20 by 20 m square. The samples were sieved through a 4 mm mesh and then pooled. Remaining roots and leaf pieces were removed by hand and the soils were left to air-dry at room temperature for 1 week. All three soils can be described as Dystric Cambisols (FAO system). Properties for the three different soil materials used in the experiment are given in Table 1.

2.2. Experimental design and ¹⁵N additions

A total of 90 acid washed (10% HCl for 1 day) and burnt (400°C for 12 h) 100 ml serum bottles per soil received 10 g of air-dried soil each. The soils were then rewetted to 60% of the water holding capacity by adding autoclaved, deionized water with an automatic pipette, and then gently mixing the soil. The bottles were left over night on an orbital shaker at a temperature of 15°C, and the following day the serum bottles were divided into two groups per soil, one receiving 1.0 ml of ¹⁵NH₄Cl (98% ¹⁵N, Cambridge Isotopic Laboratories) at concentrations of 510, 86, and 17 µM + NaNO₃, at concentrations of 110, 170, and 60 µM for Soil I.
Table 1: Properties for the three different soil materials used in the experiment. All values are on dry weight basis.

<table>
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<tr>
<th>Soil</th>
<th>NO$_3$-N (mg kg$^{-1}$)</th>
<th>NH$_4$-N (mg kg$^{-1}$)</th>
<th>Kjeldahl N (g kg$^{-1}$)</th>
<th>Total C (g kg$^{-1}$)</th>
<th>C/N-ratio</th>
<th>pH</th>
<th>WHC (kg H$_2$O kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.10</td>
<td>7.18</td>
<td>5.24</td>
<td>86.56</td>
<td>Low (17)</td>
<td>4.1</td>
<td>1.29</td>
</tr>
<tr>
<td>II</td>
<td>4.93</td>
<td>4.87</td>
<td>2.44</td>
<td>37.37</td>
<td>Low (15)</td>
<td>3.7</td>
<td>0.64</td>
</tr>
<tr>
<td>III</td>
<td>0.04</td>
<td>0.50</td>
<td>4.32</td>
<td>116.05</td>
<td>High (27)</td>
<td>3.1</td>
<td>1.54</td>
</tr>
</tbody>
</table>

II, and III, respectively, and the other 1.0 ml of Na$_2^{15}$NO$_3$ (99% $^{15}$N, Cambridge Isotopic Laboratories) + NH$_4$Cl at pH 7.5. Of this buffered soil solution, 50 ml were transferred to 3 ml of 250 mM Tris with 4 mM EDTA and a pH of 7.5. Of this buffered soil solution, 50 ml were transferred to a scintillation vial and 50 μl of luciferase–luciferin enzyme (Adenosine 5’-triphosphate (ATP) assay mix, Sigma-Aldrich) added. The light output was immediately measured for 15 s in a Beckman LS5500 scintillator equipped with a single photon meter. The amount of ATP in the samples was calculated from internal standards prepared by adding 19.5 ml of ice-cold extractant and 0.5 ml of ATP standard (5.0 μg per 0.5 ml, disodium salt, Sigma-Aldrich) to each sample prior to extraction.

2.6. Calculation of nitrogen transformation rates

Nitrogen transformation rates were calculated with the FLUAZ-model (Mary et al., 1998). The calculations in this model are based on the isotopic dilution and isotopic enrichment principles (Monaghan and Barrachough, 1995). It uses a numerical fourth order Runge-Kutta algorithm.
with a variable time-step to solve the differential system, and a non-linear fitting program (based on Marquardt’s algorithm) to calculate the unknown N transformation rates between the NH\(_4\)-N, NO\(_3\)-N, organic N and biomass N pools. The gross mineralization, nitrification, and NH\(_4\)\^+-N immobilization rates were calculated from the measurements of the amounts and \(^{15}\)N isotopic excesses of NH\(_4\)-N, NO\(_3\)-N and organic N.

The FLUAZ model minimizes the quadratic weighted error rather than the sum of squares and therefore requires the average value plus the coefficient of variation for the measured variables as input. This accounts for the variance of the measurements, so that the variables with the highest variability have the lowest weight, and yields the nitrogen transformation rates for each treatment rather than for each replicate. Prior to the modeling, outliers of the 95% confidence intervals for the averages were removed. The input data in the model are total organic \(^{15}\)N measurements, but the model simulates the evolution of \(^{14}\)N and \(^{15}\)N in the zymogenous (newly formed) microbial biomass. Biomass N and \(^{15}\)N measurements remain difficult to make and represent the total biomass rather than the zymogenous biomass, which is incorporating the labeled N. Measurements of total organic \(^{15}\)N are also considered to be much more reliable in preparing \(^{15}\)N balances (Mary et al., 1998).

2.7. Statistics

Stepwise multiple regression analysis (forward procedure, F to enter, 4.000, F to remove, 3.996) was used to test the dependence of the gross mineralization-, immobilization-, and nitrification rates on different measured factors. The gross mineralization rate and the ratio between gross total immobilization and gross mineralization were tested against the respiration rate, the net ATP production rate, and the average of the amount of ATP present during 1 day (i.e. the average between the amount measured day 1 and 2, day 3 and 4, and so on). The soil C/N ratio (high or low) was included in the analysis as a dummy variable. The gross NH\(_4\)\^+-N immobilization rates were tested against the same factors, plus the gross mineralization rate, while the ratio between gross NO\(_3\)-N immobilization and gross NH\(_4\)\^+-N immobilization was tested against the gross nitrification rate as well. Gross nitrification and NO\(_3\)-N immobilization rates were tested against the respiration rate, the net ATP production rate, the average of the amount of ATP present during 1 day, the soil C/N ratio, the gross NH\(_4\)\^+-N immobilization rate, and the gross mineralization- or nitrification rates. The respiration rate and the gross total immobilization rate were log\(_{10}\) transformed, while the gross NO\(_3\)-N and NH\(_4\)\^+-N immobilization rates, the gross mineralization rate, and the gross nitrification rates were log\(_{10}\)(X + 1) transformed to meet the assumption of equality of variances.

All three soils were analyzed by multiple regression to test whether the soil C/N ratio was responsible for differences in N transformation rates between the three soils that were not explained by the microbial biomass and activity. The soils were then analyzed separately for the same factors except the soil C/N ratio.

Differences between the three soils in respiration rate and the average of the amount of ATP present at the beginning and end of each experimental day were tested with repeated measurement ANOVA followed by a Scheffé F-test, with day as the within factor. Within each soil, the differences between different days were tested with a one-way ANOVA. All analyses were performed with the StatView 4.53 software (Abacus Concepts, Inc.).

3. Results

3.1. Gross nitrogen transformation rates

The high rates of mineralization and immobilization during day 1–2 and 3–4 were preceded by a burst of microbial activity and then followed by lower rates as the incubation of the soils proceeded (Figs. 1 and 2, Table 2). This pattern coincided in general with the one hypothesized when the experiment was designed, but no significant relationships were found between the gross mineralization and immobilization rates and the microbial biomass/activity in the soils (Tables 3 and 4) due to 1–2 days displacement of peaks for microbial activity and N transformations. The immobilization of NH\(_4\) was dependent only on the gross mineralization rate in the multiple regression analysis (Table 3). When the soils were analyzed separately, the amount of ATP could account for some of the variation of the NH\(_4\)\^+-N immobilization in Soil II (Table 4). Since rapid immobilization of nitrogen in Soil I occurred before the mineralization rate reached its maximum, the dependence of immobilization on mineralization was broken up in this soil.

In Soil III, a time lag of 1 or 2 days was observed between the peaks of the mineralization and immobilization rates and the ATP content (Fig. 3, Table 2).

The cumulative mineralization and NH\(_4\)\^+-N immobilization were of the same magnitude in Soil I and III, but considerably lower in Soil II (Fig. 1). The same pattern was found for the ATP content and the respiration rate (Figs. 2 and 3), suggesting that differences in the mineralization and immobilization potential between the soils were more reflected by those variables than by the soil C/N ratio. The gross NH\(_4\)\^+-N immobilization rate was lower than the gross mineralization rate in Soil I and III, but the resulting net mineralization was small (Fig. 1, Table 2). None of the tested factors could explain the variation in the immobilization/mineralization ratio over time, neither when the soils were analyzed together or separately (Tables 3 and 4).

Even though the immobilization of NO\(_3\)-N was in the order of 1/10–1/100 of that of NH\(_4\)\^+-N, more NO\(_3\)-N was consumed than produced in Soil I and III (Fig. 1, Table 2). When all soils were analyzed together, the immobilization of NO\(_3\)-N...
was independent of all the tested factors (Table 3), but when the soils were analyzed separately, the immobilization of NO$_3^-$ in Soil III was highly dependent on the gross nitrification rate, the net ATP production, and the respiration rate (Table 4). Two cases with gross NO$_3^-$ immobilization exceeding the gross NH$_4^+$ immobilization were also found in this soil (Table 2). The variation of the NO$_3^-$/NH$_4^+$ immobilization ratio was explained by the variation in soil C/N-ratio and ATP production (Table 3).

The gross nitrification rate was low in all three soils, but especially in Soil III (Table 2). Nitrification took off fastest in Soil I, but at the end of the experiment, it was
surpassed by nitrification in Soil II (Fig. 1). When analyzed together, the nitrification rate in the three soils was inversely dependent on the amount of ATP (Table 3). The same dependency of the nitrification rate on the amount of ATP was also found when Soil I was analyzed separately, and, in addition, it was dependent on the gross mineralization rate and the gross NH$_4^+$ immobilization (Table 4). In Soil III, the ATP production, the mineralization rate, and the respiration rate could explain a great deal of the variation of the nitrification rate, whereas no significant relationships between any of the tested variables and the nitrification rate were found in Soil II (Table 4). Net nitrification, that is the difference between gross nitrification and immobilization, was negative in Soil I and III, and about one third of gross nitrification in Soil II (Table 2).

3.2. Soil respiration rates and ATP content

The microbial respiration rates were significantly different in the soils and during the course of the experiment (Fig. 2). It was highest during the first day of incubation, decreased during the second, and maintained a low level during the rest of the incubation. Soil I had the highest respiration rate and Soil II the lowest. Most ATP was found in Soil III and least in Soil II (Fig. 3), that is, the soil with the lowest biomass also had the lowest respiration rate. Although there were irregular variations from 1 day to another, the ATP content seemed to remain constant throughout the experiment in Soil I and decreased in Soil II and III.

4. Discussion

Rather than supporting the assumption that the soil C/N ratio controls potential gross immobilization and mineralization and explains differences in their rates from one soil to another, this study suggests that differences in respiration rate and ATP content are more indicative of the magnitude of the two processes. The soil with the high C/N ratio (Soil III) complied with a number of criteria for high immobilization rates, such as low in situ concentrations of extractable NO$_3^-$ and NH$_4^+$ and essentially no net mineralization, but the latter criteria was true also for the soils with the lower C/N ratio. Those soils clearly had similar C/N ratios, although the total C and N concentrations were different, and yet their ATP content varied by more than a factor of three. Similar inconsistencies in the relationships between the soil C/N ratio and immobilization were found in the NITREX study, where the NH$_4^+$ immobilization was highest at sites with a low C/N ratio (Tietema, 1998). Gundersen et al. (1998a) suggested that the results were influenced by fragmentation of mycorrhiza during sieving of the soils. Mycorrhiza was assumed to be more abundant in the high C/N ratio soils, so sieving would reduce the NH$_4^+$ immobilization rates more in those soils than in those with low C/N ratios.

![Fig. 2. The respiration rate (mg CO$_2$ kg$^{-1}$ soil dry weight) in the three soils during the experiment. The rate differed significantly among soils ($p < 0.001$). Soil I had a higher rate than the two other soils at all times during the experiment, and Soil II had the lowest rate (Scheffe F test, $p < 0.001$). There was also a significant difference in respiration rate among days within each soil ($p < 0.001$). Error bars represent the standard error of the mean.](image)

Table 2

<table>
<thead>
<tr>
<th>Day</th>
<th>Gross mineralization (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>Gross NH$_4^+$ immobilization (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>Gross nitrification (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>Gross NO$_3^-$ immobilization (mg kg$^{-1}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil I</td>
<td>Soil II</td>
<td>Soil III</td>
<td>Soil I</td>
</tr>
<tr>
<td>0–1</td>
<td>22.5 (14.3)</td>
<td>3.9 (1.3)</td>
<td>2.7 (1.6)</td>
<td>13.5 (15.9)</td>
</tr>
<tr>
<td>1–2</td>
<td>53.1 (19.8)</td>
<td>3.2 (2.0)</td>
<td>103 (27.6)</td>
<td>32.1 (10.1)</td>
</tr>
<tr>
<td>3–4</td>
<td>17.4 (5.9)</td>
<td>28.6 (3.5)</td>
<td>10.0 (4.1)</td>
<td>13.9 (9.6)</td>
</tr>
<tr>
<td>4–5</td>
<td>0.0 (13.9)</td>
<td>2.7 (1.0)</td>
<td>0.4 (0.6)</td>
<td>4.2 (15.1)</td>
</tr>
<tr>
<td>6–7</td>
<td>3.0 (39.5)</td>
<td>3.9 (1.0)</td>
<td>142 (13.0)</td>
<td>0.5 (51.0)</td>
</tr>
</tbody>
</table>
The ATP content, which reflects both biomass and metabolic state of the microorganisms (Contin et al., 2000), was responding to the tracer addition in a more systematic and repeatable way than the nitrogen transformations, generally with an increase 1 day after an addition (Fig. 3).

That pattern was superimposed upon the trend of a reduction of the ATP content with the incubation time, but because of the 1–2 day displacement between peaks in ATP content and mineralization and immobilization rates, the ATP content could not explain the ceasing mineralization and immobilization rates after 3 days of incubation. Tsai et al. (1997) demonstrated that soils amended with glucose often had the largest amounts of ATP 1–2 days before the largest peak in numbers of bacteria and the frequency of dividing cells appeared (Bloem et al., 1992) after rewetting of an air-dried soil, which may be analogous to glucose addition in a dried soil, which may be analogous to glucose addition in the environment, e.g., low concentrations of ATP and organic carbon. Soil II also had several times lower NH₄⁺ content than Soil I and III throughout the experiment, but when calculated on basis of soil water content, the concentrations were in the same millimolar range in the three soils (Fig. 4). Since all known NH₄⁺ transporters isolated from bacteria, e.g., the NH₄⁺ transporters isolated from bacteria have a K_m in the micromolar range (Sieve et al., 1996; Montesinos et al., 1998; Soupene et al., 1998), the NH₄⁺ concentration should be sufficiently high to allow NH₄⁺ immobilization at a maximum rate in all three soils, even if less than 5% of the extractable NH₄⁺ is considered to be present in solution.

Variations in gross mineralization/immobilization rates could not be connected with corresponding variations in microbial biomass in those few studies that addressed the relationship by ‘snapshot’ observations during longer experiments. The gross mineralization rate as well as microbial biomass C and N were significantly affected by forest soil moisture and temperature, but not on soil biomass N, which remained constant throughout a year. On the other hand, mathematical simulation models have predicted the size and activity of the microbial biomass to have an impact on N mineralization and immobilization rates (Hadas et al., 1992; Blagodatsky and Richter, 1998). This discrepancy between theoretical model predictions and results from incubations or long-term field experiments may reflect methodological constraints in microbial biomass C and N measurements. The commonly used chloroform fumigation method does not produce reliable biomass comparisons in situations of low spatial and temporal
Table 4
The dependency of the gross immobilization and nitrification rates, and the ratios between gross total immobilization/gross mineralization and gross nitrate immobilization/gross ammonium immobilization, on the different measured factors in each of the three soils (Stepwise multiple regression analysis, forward procedure, F to enter: 4.000, F to remove: 3.996, degrees of freedom = 4). No factors were entered into the model when mineralization was tested as dependent.

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Soil I</th>
<th></th>
<th></th>
<th>Soil II</th>
<th></th>
<th></th>
<th>Soil III</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factors not entered in model</td>
<td>Factors entered in model</td>
<td>Coefficient</td>
<td>$R^2$</td>
<td>Sig.</td>
<td>Factors not entered in model</td>
<td>Factors entered in model</td>
<td>Coefficient</td>
<td>$R^2$</td>
</tr>
<tr>
<td>NH$_4$ immobilization</td>
<td>Respiration</td>
<td>ATP content</td>
<td>ATP production</td>
<td>Mineralization</td>
<td>0.658</td>
<td>0.915</td>
<td>NS</td>
<td>Respiration</td>
<td>ATP content</td>
</tr>
<tr>
<td>NO$_3$ immobilization</td>
<td>Respiration</td>
<td>ATP content</td>
<td>ATP production</td>
<td>Mineralization</td>
<td>0.046</td>
<td>0.016</td>
<td>NS</td>
<td>Respiration</td>
<td>ATP content</td>
</tr>
<tr>
<td>Nitrification</td>
<td>Respiration</td>
<td>ATP content</td>
<td>ATP production</td>
<td>Mineralization</td>
<td>0.098</td>
<td>0.651</td>
<td>0.242</td>
<td>Respiration</td>
<td>ATP content</td>
</tr>
<tr>
<td>Immobilization/Mineralization</td>
<td>ATP content</td>
<td>ATP production</td>
<td>Mineralization</td>
<td>Nitrification</td>
<td>1.525</td>
<td>0.966</td>
<td>&lt;0.05</td>
<td>Respiration</td>
<td>ATP content</td>
</tr>
<tr>
<td>NO$_3$/NH$_4$ immobilization</td>
<td>Respiration</td>
<td>ATP content</td>
<td>ATP production</td>
<td>Mineralization</td>
<td>None</td>
<td>–</td>
<td>–</td>
<td>Respiration</td>
<td>Mineralization</td>
</tr>
</tbody>
</table>
variation (Wardle and Ghani, 1995) and extracts the total microbial biomass C rather than the active part of the soil microbial community (van de Werf and Verstraete, 1987; Ross, 1991). For instance, the chloroform fumigation method would fail to discriminate between two bacterial populations with a similar biomass but varying 10 fold in growth rate (Bloem et al., 1989), and yet, the population with a higher proportion of actively growing bacteria could be expected to have a higher turnover rate of carbon and nitrogen. Microbial biomass C and N measured by the fumigation method may therefore be poorly related to nitrogen mineralization rates.

The gross N immobilization rate was found to be highly dependent on the mineralization rate in all three soils (Tables 3 and 4). The high immobilization rates during the first few days of incubation (Table 2) agree with those found in other drying-rewetting experiments. For example, Pulleman and Tietema (1999) found immobilization rates of 18–119 mg N kg soil $^{-1}$ d $^{-1}$ in dried and rewetted F horizon material at respiration rates similar to those in our experiment, and air dried and water logged mineral soils immobilized between 10 and 120 mg N kg soil $^{-1}$ d $^{-1}$ (Wang et al., 2001). The rates are much lower at field moisture conditions and similar to those we found in the late part of the incubation. As a comparison, Puri and Ashman (1999) found immobilization rates to vary between 0.5 and 1.7 mg N kg $^{-1}$ d $^{-1}$ in a sandy loam from a mature oak woodland, and 7–14 mg N kg $^{-1}$ d $^{-1}$ were immobilized in the organic horizon of six northern hardwood forests (Fisk and Fahey, 2001). Podzols in the NITREX study, which are located in a nitrogen deposition gradient in northwestern and central Europe, have immobilization rates of between 9 and 18 mg N kg soil $^{-1}$ d $^{-1}$ (Tietema, 1998). Immobilization rates as high as 70–500 mg N kg $^{-1}$ d $^{-1}$ at NH$_4^+$ concentrations of between 0.05 and 5.0 mM have been found in soil slurries of organic horizon material from a coniferous forest (Schimel and Firestone, 1989).

The pattern of the variations of the gross nitrification rates was consistent with the hypotheses on negative relationships between the rates and the C/N ratio (Fig. 1) and the ATP content (Table 3). It is also in agreement with earlier findings of negative relationships between the net nitrification rates and the C/N ratio of the forest floor (Gundersen et al., 1998b). This information taken together with the observation that the amount of NH$_4^+$ immobilized was far exceeding the amount that was nitrified suggests that high heterotrophic activity in a forest soil coincides with low nitrification activity, possibly because nitrifiers are poor competitors for NH$_4^+$ (Verhagen and Laanbroek, 1991; Verhagen et al., 1995). However, while the immobilization rate ceased after a few days of incubation, at least in Soil I and III, the nitrification rate remained constant and even increased (Table 2). This supports the idea that heterotrophs assimilate most of the available NH$_4^+$ when heterotrophic demand for N are high due to rapid growth (Hart et al.,

![Fig. 3. The average content of ATP (mg kg $^{-1}$ soil dry weight) present during a day in the three soils. The content differed significantly among soils ($p < 0.001$) and among days within each soil ($p < 0.001$). Error bars represent the standard error of the mean.](image)

![Fig. 4. The average concentration of NH$_4^+$-N in (top) mg kg $^{-1}$ soil dry weight, and (bottom) mM in the three soils during the different days of the experiment. Values are averages of the ‘inorganic paired’ treatments.](image)
1994), but it may also reflect a slow growth of nitrifiers compared to heterotrophs, and a possibility that nitrification and NO\textsubscript{3}\textsuperscript{-} immobilization may be underestimated in short-term laboratory incubations. The nitrification may also be underestimated because the FLUAZ model only considers autotrophic nitrification, which declines markedly below pH 6 and becomes negligible below pH 4.5 (Paul and Clark, 1996). NO\textsubscript{3}\textsuperscript{-} production in acid forest soils has been attributed to heterotrophic nitrification (Schimel et al., 1984; Duggin et al., 1991; Pedersen et al., 1999), but the evidence is equivocal. Acidophilic autotrophic nitrifiers may contribute with more than 90\% of the NO\textsubscript{3}\textsuperscript{-} production in acid forest soils (De Boer et al., 1992; Barradough and Puri, 1995; Stark and Hart, 1997), and Killham (1986) suggested that the character and availability of substrates are more important than soil pH to control the extent of heterotrophic nitrification.

The comparison of gross and net nitrification rates adds another piece of evidence to the concern about net nitrification measurements giving poor predictions of gross nitrification rates in soils (Stark and Hart, 1997). Very low in situ concentrations of NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} in combination with a high ATP content, such as in Soil III, are a good point of departure for a tight coupling of gross nitrification and immobilization, resulting in a negligible or negative net nitrification. Soils with a low C/N ratio, low ATP content, and high in situ concentrations of NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+}, such as Soil II, are good candidates for a positive net nitrification and subsequent leaching of excess NO\textsubscript{3}\textsuperscript{-}.

The correlation between the ATP content and net nitrification rate is not the only environmental implication arising from the attention given to the soil heterotrophic microbial activity in this work. Generally, it demonstrates a tight connection between nitrogen turnover and retention and the microbial activity and a co-variation from one forest soil to another between mineralization, immobilization, ATP content, and respiration. The property of a forest soil as a source of nitrogen for plants and of excess nitrogen ending up in ground water and surface water may thus be more variable with and dependent on its microbial biomass and activity than on its C/N ratio.


Relieving substrate limitation - soil moisture and temperature determine gross N transformation rates

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Abstract
A field experiment was designed with the objective to reveal the interactions between soil moisture, temperature, total, dissolved, and phosphate buffer extractable C and N, and microbial activity in the control of in situ gross N mineralization and immobilization rates in a deciduous forest. We set up three alternative hypotheses to explain variations of the gross N transformations: 1. microorganisms are C limited, 2. microorganisms are N limited, or 3. neither C nor N limit the microorganisms but moisture and temperature conditions. Each hypothesis had specific criteria to be fulfilled for its acceptance. The results demonstrated that neither C nor N were limiting respiration and gross N transformation rates, but the rates were more dependent on and variable with soil moisture and temperature than the size of the different C and N pools. The immobilization of N was dependent on the gross mineralization rate, suggesting that if the intracellular N content of the microorganisms is sufficiently high no additional N is immobilized from the surrounding soil and no enzymes are produced to mineralize organic N. If the microorganisms are starved for N, enzyme systems involved in both the assimilation and mineralization of N are activated. The concentrations of C and N in different forms therefore seem to be a result of microbial activity rather than causing it.

1. Introduction
Mineralization and immobilization by heterotrophic microorganisms are the quantitatively most important N transformation processes in many forest soils (Wessel and Tietema, 1992; Hart et al., 1997; Fisk and Fahey, 2001; Bengtsson et al., 2003). The mineralization process produces substrate for nitrifiers and plants, but heterotrophic microorganisms are also successful competitors for NH$_4^+$, and considerable amounts of NH$_4^+$ are immobilized in the heterotrophic biomass (Zak et al., 1990; Verhagen and Laanbroek, 1991; Verhagen et al., 1995). A number of soil characteristics, including various C and N pools (Iritani and Arnold, 1960; De Neve et al., 1994; Fisk and Schmidt, 1995; Bending et al., 1998), influence the N mineralization rate. The same characteristics, such as available C (Jackson et al., 1988, 1990; Barrett and Burke, 2000), the amount of inorganic N (Bengtsson and Bergwall, 2000), and the soil C/N ratio (Tietema, 1998), seem to control the rate of immobilization. Apart from abiotic soil characteristics, the microbial biomass and activity, viz., respiration rate, ATP content, and microbial C and N content, also influence mineralization and immobilization rates (Alef et al., 1988; Hart et al., 1994; Tietema, 1998; Barrett and Burke, 2000; Bengtsson et al., 2003). However, it seems counter-productive to consider any of the factors above in isolation if the aim is to understand the mechanisms that determine the rates of N mineralization and immobilization in situ, since the amount of C and N in different pools of organic matter not only influence the microbial activity but are also a result of it. Therefore, we designed a field experiment in which immobilization and mineralization rates, microbial activity, and a number of abiotic factors were simultaneously quantified.

Our previous observation (Bengtsson et al., 2003), that variations in the microbial biomass and activity among three different forest soils were reflected in corresponding variations in N transformation rates, was the basis for the conceptual idea that microorganisms act as catalytic converters of environmental conditions into mineralization and immobilization of N. The dependence of this conversion activity on individual environmental conditions has been frequently
studied, and there is a general agreement that the soil moisture content and temperature have a profound influence on the microbial activity (Swift et al, 1979; Paul and Clark, 1989; Killham, 1994). Drying and rewetting of soils induce significant microbial growth and activity (Lund and Goksøyr, 1980; Bottner, 1985; West et al., 1986; Bloem et al., 1992), but the effect of small day-to-day fluctuations in temperature and moisture content under field conditions is largely unknown. The amount of C is also thought to have a large impact on the microbial activity, and microorganisms in temperate forest soil are often considered to be C limited, in spite of the fact that they are surrounded by large amounts of C. However, the total C and N content in a soil are not necessarily correlated to the amount of C and N that is available to and utilized by the microorganisms. For instance, Matsumoto et al. (2000) showed that the activity of N mineralizing microorganisms was quantitatively related to protein-like compounds that make up most of a phosphate buffer extractable N fraction (PON). Likewise, root exudates, which consist mainly of watersoluble organic acids (Graystone et al., 1996; Uren, 2001), also have stimulatory effects on the microbial activity (Graystone et al., 1996).

Our objective was to reveal the interaction between moisture, temperature, total, dissolved, and phosphate buffer extractable C and N, and microbial activity in the control of mineralization and immobilization of N under natural conditions in a deciduous forest soil. The study was conducted in the field for five days. We set up three alternative hypotheses, each one with specific criteria to be fulfilled for its acceptance. Our first hypothesis, that microorganisms are C limited, had three criteria: 1) The microbial activity (respiration rate) is positively dependent on the amount of C in at least one of the three quantified C pools; 2) The N mineralization rate is independent of the amount of N; and 3) The N immobilization rate is not dependent on the amount of C in the pool that has a positive impact on the microbial activity. The second hypothesis, that microorganisms are N limited, had three criteria: 1) The microbial activity (respiration rate) is independent of the amount of C; 2) The N mineralization rate is positively dependent on the amount of N in at least one of the three quantified organic N pools; 3) The N immobilization rate is positively dependent on the gross N mineralization rate and on the amount of N in the pool that has a positive impact on the mineralization rate, but not on the amount of C. Finally, for the third hypothesis, that the microbial activity determine the N mineralization and immobilization rates, and that neither C nor N limit the microorganisms but rather moisture and temperature conditions two criteria were defined: 1) Small daily fluctuations in temperature and moisture content are translated into corresponding fluctuations in the microbial activity; 2) The microbial respiration rate is independent of the amount of C, and N immobilization and mineralization rates are independent of the concentration of N.

2. Material and methods

The experiment was conducted in a mixed beech/oak stand in Torup in southwestern Scania, the southernmost province in Sweden (55°33'N, 13°12'E), on 5-9 May 2003. One hundred and ten positions, separated by 1 m and forming a 1×1 m grid superimposed on a 10×11 m square, were marked. During day 1-3 and day 5 of the experiment, the microbial respiration rate was measured in soil samples taken at 20 of those positions. The same 20 positions were sampled each day. Samples were taken by inserting a 6 ml scintillation vial into the soil and then carefully withdrawing the vial. The respiration rate was estimated by adding the samples to 20 ml headspace vials, which were then sealed with rubber septa and kept at ambient temperature in the field. After 24 hours, the vials were transported to the laboratory and weighed. The amount of CO₂ in the headspace was measured in a Hewlett Packard 6890 gas chromatograph, equipped with a thermal conductivity detector, and connected to a Perkin Elmer HS 40XL headspace sampler. Helium was used as a
carrier gas at 6 ml min⁻¹ and CO₂ was separated from N₂O at 30°C on a HP-PLOT Q column (15 m, I.D. 0.53 mm). The injector temperature was 250°C and the filament temperature 300°C. Samples were injected at a split ratio of 3:1. The amount of water in the samples was determined by weighing the vials before and after drying at 105°C.

The microbial respiration rate during the fourth day was estimated at 108 of the marked positions (two positions were excluded due to obstacles) using the same sampling procedures as above. However, after the incubation of the headspace vials, 12 ml of gas (atmospheric pressure) from each vial were transferred with a gastight syringe to a set of evacuated 12 ml pressure) from each vial were transferred with a gastight syringe to a set of evacuated 12 ml exetainers. Exetainers that did not retrieve at least 11 ml of gas from the syringe were discarded. The amount of CO₂ in the headspace of these exetainers was measured in a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer (CF-IRMS) connected to a gas/solid/liquid preparation module equipped with a Europa Scientific gas autosampler (ANCA-GSL, PDZ Europa Scientific Instruments, Crewe, UK). The exetainers were flushed with a double holed needle so that all the headspace gas was transferred first through a water trap containing magnesium perchlorate, and then onto a GC column kept at 120°C where the inert gases were separated. The CO₂ was then quantified by IRMS.

On the fourth day of the experiment, 108 PVC tubes (10 cm in length and 11 cm in diameter) were inserted into the soil at the marked positions (two positions were excluded due to obstacles) after removal of the litter. Plants present within the periphery of the cylinders were cut at the soil surface with a pair of scissors and removed. Gross N mineralization and immobilization rates were estimated by the pool dilution/enrichment technique. Twenty ml of a solution containing 2.4 µg ¹⁵NH₄⁺-N ml⁻¹ (as ¹⁵NH₄Cl, 98% ¹⁵N, Cambridge Isotopic Laboratories) were added to each cylinder with a syringe. To ensure an even distribution of the label, the needle was inserted at least at 10 positions in the cylinder to a depth of five centimetres and slowly drawn towards the surface while the ¹⁵NH₄Cl solution was injected. Within two hours, two soil samples were taken from each cylinder as described above. The two soil samples (totally ~10 g dry weight) were combined in a pre-weighed 50 ml test tube and put on ice. In the lab, the tubes were weighed a second time and the soil extracted with 35 ml 1.0 M KCl over night on an orbital shaker (Tabulator Teknik AB) kept at 11 °C. The tubes were then centrifuged at 1200 rpm for 20 min on a Hermle Z510 centrifuge and the supernatant transferred to a 100 ml serum flask. Two more soil samples were taken from the cylinders 24 h after the first sampling, combined and extracted as described above.

NH₄⁺ and NO₃⁻ were isolated from the soil KCl extract by using standard IAEA diffusion procedures (IAEA, 2001), with minor modifications. Briefly, a standard office paper punch was used to cut quartz filter discs that were then placed on a strip of TFE tape and prepared with 10 µl of 2.5 M KHSO₄. A second strip of tape was placed on top of the filter and the two tape strips were sealed by pressing the open end of a test tube, in a rocking circular motion around the filter, against the tape. The trap was added to the serum bottle containing the KCl extract, 0.4 g of MgO was added, the serum bottle sealed and left at ambient temperature with periodic shaking by hand. After five days, the trap was removed and the filter was placed in a 5×8 mm tin cup and left to dry in a dessicator. The serum bottle was left open for 48 hours to release residues of ammonia. A new trap was added followed by 0.4 g of Devarda’s alloy and 0.4 g of MgO, and the incubation was repeated. ¹⁵N and ¹⁴N in the filters were determined using CF-IRMS. The filters were oxidized in an ANCA-GSL elemental analyzer and NOₓ reduced to N₂, which was passed to a 20-20 IRMS (PDZ Europa UK). The amount of NH₄⁺-N and ¹⁵NH₄⁺-N, NO₃⁻-N and ¹⁵NO₃⁻-N was quantified after subtraction of blank values and calibration against filter discs which had received 50, 100, or 150 µg N as glycine (calibrated against an IAEA KNO₃ standard) dissolved in 5 µl of water. The accuracy of the quantification was determined by calculating the average value and confidence interval of
Table 1. The equations used to calculate the gross N mineralization, immobilization, and nitrification rates, and the definition of the symbols in the equations.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Symbol</th>
<th>Meaning</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p = \frac{\ln \frac{c_0 - k}{f_0 - k}}{\ln \frac{W}{W_0}} \times \frac{W_0 - W}{t}$</td>
<td>$p$</td>
<td>Gross NH$_4^+$ consumption</td>
<td>mg N kg$^{-1}$ day$^{-1}$</td>
</tr>
<tr>
<td>$c = \frac{\ln \frac{c_0 - k}{f_0 - k}}{\ln \frac{W}{W_0}} \times \frac{W_0 - W}{t}$</td>
<td>$c$</td>
<td>$^{15}$N abundance of NH$_4^+$</td>
<td>atom %</td>
</tr>
<tr>
<td>$g = \frac{\ln \frac{c_0 - k}{f_0 - k}}{\ln \frac{W}{W_0}} \times \frac{W_0 - W}{t}$</td>
<td>$g$</td>
<td>$^{15}$N abundance of NO$_3^-$</td>
<td>atom %</td>
</tr>
<tr>
<td>$p = c = \frac{W}{t} \times \ln \frac{c_0 - k}{f_0 - k}$</td>
<td>$p$</td>
<td>Gross NO$_3^-$ production</td>
<td>mg N kg$^{-1}$ day$^{-1}$</td>
</tr>
<tr>
<td>$r = \frac{(g - \bar{g})Z_r - (g_0 - \bar{g})Z_0}{(k - \bar{k})t + \frac{f_0 - k}{c}W_0 - (f_0 - k)W}{c}$</td>
<td>$r$</td>
<td>Gross NO$_3^-$ production</td>
<td>mg N kg$^{-1}$ day$^{-1}$</td>
</tr>
<tr>
<td>$W = \frac{(g - \bar{g})Z_r - (g_0 - \bar{g})Z_0}{(k - \bar{k})t + \frac{f_0 - k}{c}W_0 - (f_0 - k)W}{c}$</td>
<td>$W$</td>
<td>Concentration of $^{14}$N plus $^{15}$N in NH$_4^+$</td>
<td>mg N kg$^{-1}$</td>
</tr>
<tr>
<td>$Z = \frac{(g - \bar{g})Z_r - (g_0 - \bar{g})Z_0}{(k - \bar{k})t + \frac{f_0 - k}{c}W_0 - (f_0 - k)W}{c}$</td>
<td>$Z$</td>
<td>Concentration of $^{14}$N plus $^{15}$N in NO$_3^-$</td>
<td>mg N kg$^{-1}$</td>
</tr>
</tbody>
</table>
eight filter discs (for each concentration of N), prepared with glycine as above. At all three N concentrations, the 95% confidence interval equaled the average value ±3%. The precision of the isotopic determinations was 0.2‰.

The gross N transformation rates were calculated by the $^{15}$N pool dilution/pool enrichment technique according to Blackburn (1979), Nishio et al. (1985), and Wessel and Tietema (1992). The equations are given in Table 1. For the calculations of gross NH$_4^+$ production (mineralization) and consumption rates we distinguished between two different situations, where the NH$_4^+$ concentration either increased (Equations 1 and 2) or remained constant (Equation 3). The NH$_4^+$ concentration was said to be constant if the values at the beginning and end of the 24-hour period differed by less than the internal precision of the instrument. This was determined by calculating the average value, $\bar{W}$, of $W_0$ and $W_t$ and forming the 95% CI of it. If the NH$_4^+$ concentration was outside the 95% CI, equations 1 and 2 were used, and if it was inside equation 3 was used and $W$ was set to $\bar{W}$.

The gross NO$_3^-$ production (nitrification) was calculated by Equation 4 (Nishio et al., 1985; Wessel and Tietema, 1992). The validity of equation 4 was justified by re-calculating it once with $g_0$ and once with $g_t$ substituted for $g$ ($g = (g_0 + g_t) / 2$). An $r$ calculated for $g$ had to take a value between $r$’s calculated for $g_0$ and $g_t$ (Wessel and Tietema, 1992). The gross NH$_4^+$-N immobilization was then calculated by subtracting the gross NO$_3^-$ production ($r$) from the gross NH$_4^+$ consumption ($c$). These calculations assume that the gross transformation rates remained constant and that no $^{15}$N was recycled to the enriched pool during the measurement period. The short (24 h) assay period was an effort to meet this assumption.

Field moist soil was transferred to 0.2 μm Z-spin filter units and centrifuged at 26000×g for 20 min at 4°C on a Hermle Z252MK. This recovered essentially all soil water since there was no additional weight loss after drying. The soil water in the receiver tube was transferred to a 5×8 mm tin cup, evaporated to dryness at 35°C, and analyzed by IRMS for dissolved organic C (DOC) and dissolved N (DN).

Phosphate buffer extractable C and N (POC and PON) were determined according to Matzumoto et al. (2000), with some modifications. Field moist soil (3.75 g) and 15 ml of 1/15 M phosphate buffer (pH 7), consisting of Na$_2$HPO$_4$ · 2H$_2$O (7.3 g l$^{-1}$) and KH$_2$PO$_4$ (3.5 g l$^{-1}$), was added to a 50 ml test tube. The soil was extracted on a homemade rotary shaker (~27 rpm) for one hour and centrifuged at 700×g for 15 min on a Hermle Z510. Then 50 μl of the supernatant were transferred to a 5×8 mm tin cup and evaporated to dryness in a Termaks oven at 35°C before analysis by IRMS. The total amount of C and N per gram soil was determined by grinding dried soil samples in a Retch MM200 ball mill, transferring a portion of the soil to a 5×8 mm tin cup and analyzing it in the IRMS.

The day-to-day variation in soil moisture ($\Delta$H$_2$O) at each of the 20 positions used for respiration measurements was calculated by subtracting the soil water content day $t$ from the soil water content day $t +1$. Changes in the respiration rate ($\Delta$CO$_2$) from day-to-day were calculated in the same way. Temperature and precipitation data for the five days of the experiment were taken from a weather station in Lund, ~20 km north of the site (SMHI, 2003).

3. Results

The temporal variation of the average microbial respiration rate was strongly dependent on the average daily temperature (Figure 1). The 4.0°C temperature difference between day 1 and 3 resulted in a difference of 15.8% in respiration rates. Similarly, the rate of moisture changes in each individual sampling point corresponded to a decrease/increase in the microbial respiration rate (Figure 2). The slightly overestimated $\Delta$CO$_2$ between day 5 and 4, and the slight underestimation between day 4 and 3 supports the suspicion that a small loss of CO$_2$ occurred in the transfer from the
Figure 1. Relationship between the daily average air temperature and the microbial respiration rate. The low respiration rate day 4 can be explained by small systematic losses of CO$_2$ when it was transferred from the headspace vials to the exetainers. Two different regressions were therefore made, one including day 4 (dotted line), and one excluding it (solid line).

Figure 2. Relationship between the rate of change in soil moisture and the rate of change in microbial respiration from one day to another. Number one represents the difference between day 2 and day 1, number two represents the difference between day 3 and day 2, and so on. See Material and Methods section for details.

headspace vials to the exetainers during day 4. Had the gas losses not occurred, the relationship between soil drying/wetting rates and microbial respiration rates would have been stronger. The rate of change in respiration from one day to another was in some cases very high (Figure 2). The spatial variation of the respiration rates was also associated with the water content of the soil, with higher respiration rates at spatial locations with high soil water content (Figure 3). Each cylinder received 20 ml of water day 4 when the $^{15}$NH$_4$Cl solution was injected, corresponding to a rainfall of 2.1 mm. With average soil water content of 25% this corresponds to an increase in water content of approximately six percentage points. Also day 1 and 2 of the experiment had incidents of light rainfall (<1 mm).

The C concentrations varied substantially among the soil samples (Table 2), with coefficients of variation in the order of 25-55%. However, the respiration rate was not dependent on the concentration of DOC, POC, or total C (Table 3), which would be sufficient to reject the first hypothesis that the soil microorganisms were C limited. Moisture (Table 3, Figure 2 and 3) and temperature (Figure 1) conditions alone therefore seemed to be able to explain both spatial and temporal variations of the respiration rate.

The gross N mineralization rate was positively related to the soil water content (Table 3), with high mineralization rates in soils with high water content, but also weakly to the microbial activity as determined by the respiration rate (Figure 4). Thus, it was difficult to distinguish a direct effect of soil water content on N mineralization rates from an indirect via the microbial activity. In addition to the microbial activity and the soil water content, the concentration of PON also had a positive influence on the gross mineralization rate (Table 3). The concentration of total N, DN, and NH$_4^+$ had no significant influence on the gross mineralization rate (Table 3). Thus, the first two criteria of the second hypothesis, that the microbial activity (respiration rate) was not dependent on the amount of C, and that the N mineralization rate was positively dependent on the amount of N in at least one of the quantified N pools, were satisfied.
<table>
<thead>
<tr>
<th>Statistic</th>
<th>$\text{NH}_4^+\text{-N}$ (mg kg$^{-1}$)</th>
<th>$\text{NO}_3^-$ (mg kg$^{-1}$)</th>
<th>Total C (g kg$^{-1}$)</th>
<th>Total N (g kg$^{-1}$)</th>
<th>C/N ratio (soil)</th>
<th>POC (g kg$^{-1}$)</th>
<th>PON (mg kg$^{-1}$)</th>
<th>C/N ratio (POC/PON)</th>
<th>DOC (mg kg$^{-1}$)</th>
<th>DN (mg kg$^{-1}$)</th>
<th>C/N ratio (DOC/DN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>10.9</td>
<td>8.8</td>
<td>40.4</td>
<td>2.9</td>
<td>17.3</td>
<td>3.0</td>
<td>251.5</td>
<td>12.3</td>
<td>37.1</td>
<td>12.6</td>
<td>4.2</td>
</tr>
<tr>
<td>SD</td>
<td>6.7</td>
<td>3.2</td>
<td>12.0</td>
<td>2.1</td>
<td>7.3</td>
<td>0.8</td>
<td>72.7</td>
<td>2.0</td>
<td>20.2</td>
<td>6.6</td>
<td>4.9</td>
</tr>
<tr>
<td>CV (%)</td>
<td>61.1</td>
<td>36.7</td>
<td>29.8</td>
<td>71.3</td>
<td>42.3</td>
<td>26.9</td>
<td>28.9</td>
<td>16.3</td>
<td>54.5</td>
<td>52.8</td>
<td>115.7</td>
</tr>
<tr>
<td>Skewness</td>
<td>1.2</td>
<td>0.8</td>
<td>1.1</td>
<td>3.1</td>
<td>1.1</td>
<td>0.4</td>
<td>0.8</td>
<td>0.4</td>
<td>1.2</td>
<td>0.8</td>
<td>4.0</td>
</tr>
<tr>
<td>10th Percentile</td>
<td>4.3</td>
<td>4.7</td>
<td>27.7</td>
<td>1.4</td>
<td>9.5</td>
<td>2.1</td>
<td>1592</td>
<td>9.6</td>
<td>16.2</td>
<td>5.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Median</td>
<td>9.6</td>
<td>8.6</td>
<td>38.8</td>
<td>2.4</td>
<td>16.5</td>
<td>2.9</td>
<td>2389</td>
<td>12.1</td>
<td>30.8</td>
<td>12.0</td>
<td>2.5</td>
</tr>
<tr>
<td>90th Percentile</td>
<td>20.4</td>
<td>12.6</td>
<td>57.3</td>
<td>4.8</td>
<td>28.6</td>
<td>4.0</td>
<td>3559</td>
<td>15.2</td>
<td>62.0</td>
<td>20.3</td>
<td>8.5</td>
</tr>
</tbody>
</table>
Table 3. The dependency of the gross mineralization, immobilization, and respiration rates on the different abiotic soil properties. (Stepwise multiple regression analysis, forward procedure, F to enter = 4.000, F to remove = 3.996). All factors except mg DOC kg\(^{-1}\), mg DN kg\(^{-1}\), mg DN l\(^{-1}\), and POC/PON were ln-transformed before analysis.

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Factors not in model</th>
<th>Factors in model</th>
<th>Coefficient</th>
<th>Model (R^2)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration</td>
<td>mg DOC l(^{-1})</td>
<td>H(_2)O (%)</td>
<td>3.22</td>
<td>0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>mg DOC kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg POC kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g C kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineralization</td>
<td>mg NH(_4)-N kg(^{-1})</td>
<td>H(_2)O (%)</td>
<td>2.99</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>mg DN l(^{-1})</td>
<td></td>
<td>0.99</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg DN kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g N kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH(_4)(^+))()) immobilization</td>
<td>mg NH(_4)-N kg(^{-1})</td>
<td>Mineralization</td>
<td>0.99</td>
<td>0.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>mg DOC kg(^{-1})</td>
<td>mg DN l(^{-1})</td>
<td>0.02</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg DOC kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>DOC/DON</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg PON kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg POC kg(^{-1})</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>POC/PON</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g N kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g C kg(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/N ratio</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>H(_2)O (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On average, 11.2 mg NH\(_4\)-N kg\(^{-1}\) d\(^{-1}\) were immobilized, slightly less than the amount that was mineralized (Table 4). The immobilization rate was not dependent on the concentration of DOC, POC, or total soil C (Table 3), suggesting, again, that microorganisms were not C limited. This conclusion was reinforced by the lack of influence of varying C/N ratios on the gross immobilization rate (Table 3). Neither was there any dependence of the N immobilization rate on the concentration of NH\(_4\)-N, PON or the total N content of the soil, ruling out N limitation of microorganisms as a controlling factor of immobilization (Table 3).

On the other hand, there was a positive relationship between gross mineralization and immobilization rates (Table 3). High DN concentrations also had an additional positive influence on the immobilization rate (Table 3), but the main factor controlling immobilization was the gross mineralization rate. A doubling of this rate would also double the immobilization rate (Table 3). However, since the immobilization rate, in contrast to the mineralization rate, was not dependent on the amount of PON, which was the third criteria of the hypothesis that microorganisms were N limited, we had to reject this hypothesis as well. The third hypothesis, that moisture and temperature conditions alone determine the microbial activity and hence the N mineralization and immobilization rates, was the only one that could not be rejected on the basis of not fulfilling the defined criteria.

4. Discussion

The close connection between temporal variations of temperature and respiration rates and the high degree of explanation of spatial and temporal variation in respiration by the soil water content indicate that temperature and water have the greatest influence on the microbial activity. The water content and its co-variable microbial activity also explained most of the variation of the gross N mineralization rate, reinforcing observations by
rate corresponded to a smaller change in the that a certain relative change of the respiration forest soil was 3-4 times steeper, suggesting that Hart et al. (1994) found in an Oregon 1998; Barret and Burke, 2000), while the slope Ashman, 1998; Scott et al., 1998; Tietema, similar to ours (Alef et al., 1988; Puri and mineralization rates with a slope that was variation in microbial respiration and N (1999). Several studies have observed a co- Puri and Ashman (1998) and Thomsen et al. 2. In soils with high competition for NH4 among heterotrophic microorganisms, nitrifiers, and plants, the heterotrophs would need to mineralize more N to meet their metabolic demands since plants and nitrifiers would use some of the produced NH4+, resulting in a steep slope. 3. In non- or slow growing microbial populations, essentially all utilized C is diverted towards catabolic needs, resulting in a shallow slope since little or no N is assimilated. 4. Differences in microbial growth efficiency among soils may arise from differences in the chemical composition of the soil organic C.

We tried to address the chemical heterogeneity and bioavailability of the organic C by dividing it into three fractions, total C, POC, and DOC. DOC was assumed to be best correlated with respiration for a number of reasons: 1. DOC is by definition dissolved in the soil water and can easily diffuse to the cells. 2. The C containing molecule must pass the cell membrane, and 3. Smaller molecules (DOC) are more likely to pass the cell membrane than larger (POC). The absence of a significant relationship between the respiration rate and the DOC concentration, or any other C pool, shows that organic C was not a main factor determining the microbial activity and the rate of turnover. In contrast, Hart et al. (1994) found the respiration rate and the concentration of K2SO4 extractable organic C to be inversely related to chloroform fumigated microbial C during an initial phase of high microbial growth in soil samples that were incubated under constant temperature and soil moisture conditions, suggesting that extractable organic C was a major C source for microorganisms. Soil organic carbon and microbial biomass are in general well

Fig. 3. The relationship between the gravimetric soil water content and the microbial respiration rate during day 4 of the experiment.

Fig. 4. The relationship between the microbial respiration rate and the gross mineralization rate. The maximum and minimum derivative of the function describing back-transformed respiration and mineralization values were 0.09 and 0.05.

Puri and Ashman (1998) and Thomsen et al. (1999). Several studies have observed a co-variation in microbial respiration and N mineralization rates with a slope that was similar to ours (Alef et al., 1988; Puri and Ashman, 1998; Scott et al., 1998; Tietema, 1998; Barret and Burke, 2000), while the slope that Hart et al. (1994) found in an Oregon forest soil was 3-4 times steeper, suggesting that a certain relative change of the respiration rate corresponded to a smaller change in the gross mineralization rate in the Torup soil than in the Oregon soil. This variation from one soil to another of the slope of the relationship between mineralization and respiration rates is also evident from the soils survey of Schimel (1986). The difference among soils may be related to several soil and microbial community characteristics: 1. Microorganisms with a high C/N ratio assimilate less N per assimilated C than microorganisms with a low C/N ratio, resulting in a shallow slope. 2. In soils with high competition for NH4+ among heterotrophic microorganisms, nitrifiers, and plants, the heterotrophs would need to mineralize more N to meet their metabolic demands since plants and nitrifiers would use some of the produced NH4+, resulting in a steep slope. 3. In non- or slow growing microbial populations, essentially all utilized C is diverted towards catabolic needs, resulting in a shallow slope since little or no N is assimilated. 4. Differences in microbial growth efficiency among soils may arise from differences in the chemical composition of the soil organic C.

We tried to address the chemical heterogeneity and bioavailability of the organic C by dividing it into three fractions, total C, POC, and DOC. DOC was assumed to be best correlated with respiration for a number of reasons: 1. DOC is by definition dissolved in the soil water and can easily diffuse to the cells. 2. The C containing molecule must pass the cell membrane, and 3. Smaller molecules (DOC) are more likely to pass the cell membrane than larger (POC). The absence of a significant relationship between the respiration rate and the DOC concentration, or any other C pool, shows that organic C was not a main factor determining the microbial activity and the rate of turnover. In contrast, Hart et al. (1994) found the respiration rate and the concentration of K2SO4 extractable organic C to be inversely related to chloroform fumigated microbial C during an initial phase of high microbial growth in soil samples that were incubated under constant temperature and soil moisture conditions, suggesting that extractable organic C was a major C source for microorganisms. Soil organic carbon and microbial biomass are in general well
correlated (Zak et al., 1990; Zhang and Zhang, 2003; Milne and Haynes, 2004), but as Bloem et al. (1989) have demonstrated, the microbial activity can vary up to tenfold in soils with the same microbial biomass.

Since the majority of laboratory experiments on C and N turnover have been made at constant temperature and constant and non-limiting soil water conditions (references), the variations in activity per biomass unit are restrained and the importance of soil organic matter in controlling the C and N transformation is overestimated. Heterogeneity in soil physical properties cause varying spatio-temporal patterns in soil water status at the metre scale (Dekker et al., 1999; Wendroth et al., 1999), and species-specific transpiration behavior of trees results in a high spatio-temporal variation in drying rates in mixed forest stands (Schume et al. 2003). When this spatial and temporal variability of soil moisture content and temperature is included in N transformation assays, the dependence on soil organic matter seems to be relieved.

The change in respiration rates from one day to another in response to soil drying/wetting was sometimes large but of the same magnitude as observed by Pulleman and Tietema (1999). They found respiration rates to increase by 250% four hours after wetting field moist forest floor material, and by ~4300% when the same material had been air dried for 11 days before rewetting. Similarly, Fierer and Schimel (2003) observed respiration rates in dried forest and grassland soils to increase by 400-500% compared with field moist soils when the soils were rewetted.

In addition to the microbial activity, the concentration of PON was positively related to the gross N mineralization rate, in agreement with Matsumoto et al. (2000). PON is a homogeneous, bacterially derived group of protein-like compounds with a size of 8-9 kDa, which seems to be the source of the mineralized N (Matsumoto et al. 2000). However, it is also possible that PON is not mainly a source of N in itself, but that it represents residues of exoenzymes that become abundant and released to degrade other organic N compounds during high microbial activity. Indeed, Zaman et al. (1999) found correlations between extracellular enzyme activities and the gross mineralization rate. This interpretation is also supported by the results of Matsumoto et al. (2000), who observed the concentration of the phosphate buffer extractable protein-like compounds to increase in response to addition of various C sources known to increase the microbial activity and N mineralization, and possibly also the production and release of PON. Such a positive relationship between the concentration of PON and the respiration rate was found in our study ($R^2 = 0.16, p < 0.01$). On the other hand, the concentration of POC had no influence on the respiration rate, possibly for the same reason as for DOC. In addition, the phosphate buffer also extracts some more refractory humic substances (Matsumoto et al., 2000), which may obscure a quantitative relationship between the amount of POC and the respiration rate.

The dependence of the gross immobilization rate on the gross mineralization rate (Table 3) is in agreement with several laboratory studies with both sieved soils and intact soil cores (Davidson et al., 1992; Hart et al., 1997; Verchot et al., 2001; Bengtsson et al., 2003). This seems to be a consistent

### Table 4. Summary statistics for gross N mineralization, immobilization, and microbial respiration rate.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Gross mineralization (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>Gross NH$_4^+$ immobilization (mg N kg$^{-1}$ d$^{-1}$)</th>
<th>Microbial respiration (mg C kg$^{-1}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>15.3</td>
<td>11.2</td>
<td>149.2</td>
</tr>
<tr>
<td>SD</td>
<td>11.6</td>
<td>8.6</td>
<td>118.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>76.0</td>
<td>76.9</td>
<td>79.4</td>
</tr>
<tr>
<td>Skewness</td>
<td>1.1</td>
<td>0.9</td>
<td>2.5</td>
</tr>
<tr>
<td>10th Percentile</td>
<td>2.8</td>
<td>1.4</td>
<td>56.3</td>
</tr>
<tr>
<td>Median</td>
<td>13.8</td>
<td>9.8</td>
<td>115.7</td>
</tr>
<tr>
<td>90th Percentile</td>
<td>33.6</td>
<td>23.5</td>
<td>306.0</td>
</tr>
</tbody>
</table>
relationship, based on a diversity of soils and incubation conditions, probably because the same microorganisms participate in both processes. The synthesis and/or activation of proteins involved in the uptake and utilization of nitrogenous compounds is closely regulated in concert with the availability of their substrates (Merrick and Edwards, 1995). That is, if the intracellular N content of the microorganisms is sufficient, no additional N is immobilized from the surrounding soil, and no enzymes are produced to mineralize organic N. On the other hand, if the microorganisms are starved for N, a number of enzyme systems involved in both the assimilation of inorganic N and mineralization of organic N are activated.

The small but positive influence of the DN concentration on the immobilization rate should not be interpreted as an evidence for N limitation, for several reasons. First, if microorganisms were N limited the immobilization rate should be more dependent on the total amount of DN per gram of soil than on the concentration in the soil water. Second, the concentration of ammonium and the gross immobilization rate were not correlated. Third, if the concentration of DN was limiting the immobilization rate, it should also limit the mineralization rate, since the DN would have to be mineralized to ammonium before immobilized. However, that dependence was not observed (Table 3). Alternatively, the small increase in immobilization rates with increasing DN concentrations may be an effect of uptake of N for osmoprotection. During osmotic up-shift caused by increased concentration of solutes in the soil water as a soil dries, microorganisms maintain turgor by accumulating osmotically active solutes in the cytoplasm, of which many contain N, e.g., free amino acids and derivates thereof, the dipeptide N-acetylglutaminylglutamine, and quaternary amines (Kempf and Bremer, 1998).

One objection to the statement that microorganisms are not N limited is the limited N leakage from N fertilization experiments (Emmett et al., 1995; Gundersen et al., 1998; Kjønaas et al., 1998). This is difficult to explain unless microorganisms are N limited, since there is no intracellular storage compounds for N and it is most likely actively taken up by microorganisms. However, if we recalculate our mean gross N mineralization rate to g m$^{-2}$ day (using a soil density of 1 g cm$^{-3}$), we end up with a gross N mineralization of 0.75 g m$^{-2}$ day in the top 5 cm of the soil. The natural N deposition in southwestern Scania, 0.0054 g m$^{-2}$ day (20 kg ha$^{-1}$, Lagner et al., 1996), and the N addition in the NITREX experiments, 0.0096 g m$^{-2}$ day (Gundersen et al., 1998), represents 0.7-1.3% of the ammonium that was naturally produced. That is, a reduction in the gross N mineralization rate of about 1% would be enough to compensate for the addition of inorganic N. This decrease would hardly be detectable given the great spatial and temporal variability of N transformation rates (Paper III).

Taken together, the positive relation between microbial activity and N transformation rates and the negligible importance of the size of different C and N pools suggest that, at least in temperate forest soils rich in organic matter, neither C nor N is limiting the microbial activity. Other factors, such as moisture content, drying rate, and temperature may be more important in determining the microbial activity and the N transformation rates. Numerous laboratory studies have also shown that both respiration and N mineralization rates increase with increasing moisture and temperature (e.g. Puri and Ashman, 1998; Thomsen et al., 1999; Subke et al., 2003; Templner et al., 2003). Our study demonstrates that those results are applicable under field conditions, although the range of variation in temperature and soil moisture content from day to day is several times lower than in most laboratory experiments. By relieving the limitation of water and temperature on the microbial activity, as is often done in laboratory experiments, conditions are created at which the rates of C and N assimilation and mineralization are determined by the quality and bioavailability of the soil organic matter.
Acknowledgements

This work was sponsored by grants from the Foundation for Strategic Environmental Research in Sweden. Niklas Törneman and Therese Nilsson are greatly acknowledged for their assistance in the field and laboratory.

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sites with different N status. Canadian Journal of Forest Research 28, 967-976.
Spatial distribution of gross N transformation rates and plants 
– A reciprocal relationship

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Abstract
A geostatistical analysis was performed to reveal the reciprocal dependence between the spatial variation of in situ gross N mineralization, NH$_4^+$ immobilization, and nitrification rates and the abundance, distribution, and species composition of understory plants in a mixed beach-oak forest. Variations in N transformation rates explained the spatial variation of understory plants within the forest, even at scales as small as a few metres. NO$_3^-$ preferring plants were more common in areas with low heterotrophic activity, while the total cover was higher in areas with high N transformation rates. Beech and oak trees also had an effect on the spatial variation of the understory. Beech trees created conditions that were suitable for NO$_3^-$ preferring plants, whereas the plant cover was higher under oak trees, probably because of less light interception. Oak generally had a positive impact on the N transformation rates and beech a negative, probably in response to the litter quality of the two species. The influence of trees alone could not explain the full magnitude of the variation, or the presence of overlapping hotspots with high mineralization and immobilization rates. These were probably caused by other factors, such as soil moisture content.

1. Introduction
Large-scale spatial heterogeneity in topography, bedrock geology, and hydrology has a major influence on variations in habitat characteristics, such as water and nutrient availability, light exposure, and temperature in a forest (Hutchinson et al., 1999; Hook and Burke, 2000). These variations are reflected in the species composition of plant communities. At smaller scales, within-habitat spatial heterogeneity maintains diversity and facilitates the co-existence of species (Chesson, 1986; Tilman and Kareiva, 1997). For instance, the heterogeneity of ammonium and nitrate concentrations at a scale of 2 m (Lechowicz and Bell, 1991; Jackson and Caldwell, 1993) is likely to be recognized by roots of trees and shrubs and affect species distributions differently depending on differences in uptake affinity. Spatial variations at a scale of 0.2 m (Schlesinger et al., 1996; Farley and Fitter, 1999) appear to be relevant for herbaceous plants. The plants themselves are active in creating and maintaining this mosaic of fertility due to e.g. differences in transpiration, throughfall, and litter deposition (Ferrari, 1999; Schume et al., 2003).

Microbial biomass and composition exhibit spatial heterogeneity at the same scales as plants (Ettema and Wardle, 2002). Their patch sizes are structured by topography, soil carbon, and moisture at scales from tens to hundreds of metres (Fromm et al., 1993; Robertson et al., 1997), but by plant size and spacing at centimetres to metres scale (Bruckner et al., 1999; Klironomos et al., 1999; Saetre, 1999; Saetre and Bláth, 2000). Nitrifying bacteria can perceive heterogeneity even at a scale of some few millimetres and below, perhaps in response to micro-sized pores and soil aggregates and fine roots (Grundmann and Dehouzie, 2000; Grundmann et al. 2001). Microbial activity, such as net N transformations and respiration, also seems to be structured by the spatial patterning of plants (Robertson et al., 1988; Saetre, 1999; Stoyan et al., 2000).

Primary production in forest ecosystems is often considered to be N limited (Tamm, 1991; Knops and Tilman, 2000), and N availability is an important factor in determining the distribution and species composition of plants (Diekmann and Falkengren-Grarup, 1998; Hutchinson et al., 1999; Falkengren-Grarup and Diekmann, 2003). N availability cannot
simply be defined as the concentration of inorganic N, since this N pool often is very small and may have a turnover time of less than a day (Hart et al., 1994; Scott et al., 1998; Verchot et al., 2001; Bengtson et al., 2003). The use of net mineralization and nitrification rates in defining N availability to plants may be misleading, since it is not uncommon to find high gross mineralization rates in soils with low or negative net mineralization (Davidson et al., 1992; Hart et al., 1994; Verchot et al., 2001; Bengtsson et al., 2003). Similarly, several cases of rapid NO\textsubscript{3}\^- turnover in soils without net nitrification have been documented (Davidson et al., 1992; Stark and Hart, 1994; Scott et al., 1998; Verchot et al. 2001).

However, floristic variation among deciduous forests can partly be explained by differences in net N mineralization and nitrification rates (Diekmann and Falkengren-Grerup, 1998; Hutchinson et al., 1999; Falkengren-Grerup and Diekmann, 2003). This does not necessarily reflect a link between plants and microbial activities but may be an indirect effect of the same soil properties affecting both the plant community composition and N transformation rates. On the other hand, plant litter provides the necessary carbon for supporting N immobilization by microorganisms (Watkins and Barraclough, 1996; Andersen and Jensen, 2001; Knops et al., 2002). It is also a source of N for heterotrophic microorganisms in undisturbed soils (Muller and Bormann, 1976; Jandl, 1997; Tardiff, 1998). Spatial heterogeneity in plant litter distribution and composition may cause the observed differences in net N transformation rates since the quality of the litter differs between plants (Ferrari, 1999; Sartiyildiz and Anderson, 2003a and 2003b; Chapman et al., 2003; Schweitzer et al., 2004), and since different compounds in plant litter might either increase or decrease the microbial activity (Northup, 1995; Henriksen & Breland, 1999; Hongye et al., 2000; Kraus et al., 2004). Furthermore, some plant species may be better at competing with microorganisms for N due to differences in N demand and uptake capacity. This may also explain the observed differences in net mineralization and nitrification rates among plant communities.

We therefore designed a field experiment to reveal the reciprocal dependence between the spatial variation of in situ gross N transformation rates and understory plant abundance, distribution, and species composition, and the influence of individual beach and oak trees on the same variation.

2. Material and Methods

2.1. Site description

The experiment was conducted in a mixed beech/oak stand (Fagus sylvatica L. and Quercus robur L.) in Torup in southwestern Scania, the southernmost province in Sweden (55°33’N, 13°12’E) on May 5-9, 2003. It had a relatively varying understory layer of plant species with different N demand (Table 1). No bottom layer was present. The soil can be described as a Dystric Cambisol (FAO system) with a pH of 4.3.

2.2. 15\textsubscript{N} pool dilution experiment

Gross N mineralization, immobilization and nitrification rates were estimated by the pool dilution/enrichment technique. At 108 positions, separated by 1 m and forming a 1×1 m grid superimposed on a 10×11 m square (two positions were excluded due to obstacles), PVC cylinders (10 cm in length and 11 cm in diameter) were inserted into the soil after removal of the litter. Plants present within the periphery of the cylinders were cut at the soil surface with a pair of scissors and removed. Twenty ml of a solution containing 2.4 µg 15\textsubscript{NH}\textsubscript{4}-N ml\textsuperscript{-1} (as 15\textsubscript{NH}\textsubscript{4}Cl, 98% 15\textsubscript{N}, Cambridge Isotopic Laboratories) were added to each cylinder with a syringe. The needle was inserted to a depth of five centimetres in at least 10 positions in the cylinder and slowly withdrawn to the surface while the 15\textsubscript{NH}\textsubscript{4}Cl solution was injected. Within two hours, two soil samples were taken from the top 5 cm of the soil in each cylinder by inserting a 6 ml scintillation vial into the soil and then carefully withdrawing it. The two samples
(approximately 10 g dry weight) were combined in a pre-weighed 50 ml test tube and put on ice. In the lab, the tubes were weighed a second time and the soil extracted with 35 ml 1.0 M KCl overnight on an orbital shaker (Tabulator Teknik AB) at 11 °C. The tubes were then centrifuged at 1200 rpm for 20 min on a Hermle Z510, and the supernatant transferred to a 100 ml serum flask. Two more soil samples were taken from the cylinders 24 h after the first sampling, combined, and extracted as described above.

NH\textsubscript{4}+ and NO\textsubscript{3}- were isolated from the soil KCl extract by using standard IAEA diffusion procedures (IAEA, 2001) with minor modifications. Briefly, a standard office paper punch was used to cut out quartz filter discs that were then placed on a strip of PTFE tape and prepared with 10 µl of 2.5 M KHSO\textsubscript{4}. A second strip of tape was placed on top of the filter and the two tape strips were sealed by pressing the open end of a test tube, in a rocking circular motion around the filter, against the tape to create a NH\textsubscript{3}-trap. The trap was added to the serum bottle containing the KCl extract, 0.4 g of MgO was added, the serum bottle sealed and left at ambient temperature with periodic shaking by hand. After five days, the trap was removed and the filter was placed in a 5×8 mm tin cup and left to dry in a dessicator. The serum bottle was left open for 48 hours to release residues of ammonia. A new trap was added to the bottle followed by 0.4 g of Devarda’s alloy and 0.4 g of MgO, and the incubation was repeated.

15N and 14N in the filters were determined using continuous flow isotope ratio mass spectrometry (CF-IRMS). The filters were oxidized in an ANCA-GSL elemental analyzer and NO\textsubscript{3} reduced to N\textsubscript{2}, which was passed to a 20-20 IRMS (PDZ Europa UK). The amount of NH\textsubscript{4}-N and 15NH\textsubscript{4}-N, NO\textsubscript{3}-N and 15NO\textsubscript{3}-N was quantified after subtraction of blank values and calibration against filter discs which had received 50, 100, or 150 µg N as glycine (calibrated against an IAEA KNO\textsubscript{3} standard) dissolved in 5 µl of water. The accuracy of the quantification was determined by calculating the average value and confidence interval of eight filter discs (for each concentration of N), prepared with glycine as above. At all three N concentrations, the 95% confidence interval was within ±3% of the average value. The precision of the isotopic determinations was 0.2‰.

2.3. Survey of field layer

The abundance of each vascular plant species in the 90 1x1 m plots was estimated as the horizontal cover and expressed in percent as <1%, 1-3%, 3-5%, 5-10%, 10-15%, 15-20%, 20-30%, 30-40%, 40-50%, 50-60%, and 60-70%. No species had a higher cover than 70% in any of the plots. A principal component analysis (PCA) was made to assess differences in community composition among plots. The cover of the different plant species in each of the 90 plots was used as input data. The analysis was performed with the MVSP 3.01 statistical package (Kovach computing services, UK). The effect of dominating species was reduced by transforming the cover percentages to classes ranging from 1 (<1%) to 11 (60-70%) and using the software’s standardize function. The score on the first principal axis for each plot was recorded and used in the geostatistical analysis.

An index of nitrogen availability was calculated for each plot by combining the functional N index (FNIS, Equation 1, from Diekmann and Falkengren-Grerup, 1998) and cover of the species in that plot (equations 2 and 3). The FNIS indices are derived from detrended correspondence analysis of the distributions of plant species in hundreds of deciduous forests in relation to the rates of net ammonification and nitrification and depend on the concept that the presence of a particular species is a function of these processes (Diekmann and Falkengren-Grerup, 1998).

\[
\text{FNIS} = 3.40 - 0.065 \text{NH}_4^+ \text{index} + 0.019 \text{NO}_3^- \text{index} \quad (1)
\]

A high NH\textsubscript{4}+ index means that the species tends to occur at locations with high net ammonification rates, and a high NO\textsubscript{3}- index that it tends to occur at locations with high net nitrification rates. Two different N availability indices were calculated for each plot; in the
Table 1. Understory plant species found in the experimental plot in the beech/oak forest, their FNIS values (from Diekmann and Falkengren-Grerup, 1998), the number of 1×1 m squares they were found in, and the average cover in these squares.

<table>
<thead>
<tr>
<th>Species</th>
<th>FNIS value</th>
<th>Number of occurrences</th>
<th>Average cover (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemone nemorosa</td>
<td>1.9</td>
<td>86</td>
<td>22.8</td>
</tr>
<tr>
<td>Carex pilulifera</td>
<td>-0.46</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Dactylis glomerata</td>
<td>2.82</td>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td>Deschampsia cespitosa</td>
<td>3.47</td>
<td>3</td>
<td>4.5</td>
</tr>
<tr>
<td>Lamiastrumum galeobdolon</td>
<td>2.83</td>
<td>31</td>
<td>1.1</td>
</tr>
<tr>
<td>Maianthemum bifolium</td>
<td>1.71</td>
<td>9</td>
<td>1.2</td>
</tr>
<tr>
<td>Melica uniflora</td>
<td>2.86</td>
<td>68</td>
<td>2.4</td>
</tr>
<tr>
<td>Milium effusum</td>
<td>3.44</td>
<td>56</td>
<td>1.6</td>
</tr>
<tr>
<td>Oxalis acetosella</td>
<td>2.56</td>
<td>83</td>
<td>4.3</td>
</tr>
<tr>
<td>Poa nemoralis</td>
<td>2.75</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td>Rubus idaeus</td>
<td>1.8</td>
<td>25</td>
<td>1.5</td>
</tr>
<tr>
<td>Stellaria holostea</td>
<td>2.65</td>
<td>70</td>
<td>2.8</td>
</tr>
<tr>
<td>Stellaria nemorum</td>
<td>4.1</td>
<td>6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Since Anemone nemorosa do not have any preference for NH₄⁺ or NO₃⁻ it was considered to belong to FNIS class 5 (on a scale from 1-9). It was given the average FNIS value of the plants in FNIS class 5, 1.9 (calculated from Diekmann and Falkengren-Grerup, 1998).*

2.4. Calculations of tree influence

The influence potential of beech and oak on the gross N mineralization and immobilization rates was determined according to Saetre (1999). The influence of an individual tree was defined to be largest at its base and then decrease exponentially from it. Large trees were considered to have a larger influence potential than small trees and the influence was additive so that at a spatial location, p, the combined influence (IP) of all trees was expressed as:

\[ IP(p) = \sum_k DBH_k \cdot \exp(-d_k) \]  

where DBHₖ is the diameter at breast height of tree k and dₖ is the distance between tree k and point p. The IP values were calculated at each node on the 9x10 m grid and used in the geostatistical analysis. IP at distances beyond 5 m was considered to be negligible. In total, 3 oaks and 5 beeches in and around the experimental plot were included in the calculations of IP. The coefficient of influence (CoI) for each tree species on a certain dependent variable, Y, could then be calculated by means of multiple regression (Saetre 1999):

\[ Y = a \cdot IP_{beech} + b \cdot IP_{oak} + c \]

where a is the regression coefficient for beech influence (CoI_{beech}), b is the regression coefficient for oak influence (CoI_{oak}), and c is the model intercept.
Table 2. The equations used to calculate the gross N mineralization, immobilization, and nitrification rates, and the definition of the symbols in those equations.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Symbol</th>
<th>Meaning</th>
<th>Unit</th>
</tr>
</thead>
</table>
| \[
p = \frac{\ln \frac{f_0 - k}{f_0 - k} \times W_0 - W_t}{W_t/W_0} \times \frac{W_0 - W_t}{t}
\] | \( c \) | Gross \( \text{NH}_4^+ \) consumption | mg N kg\(^{-1}\) day\(^{-1}\) |
| \[
\bar{g} = 1 + \left[ \frac{\ln \frac{f_0 - k}{f_0 - k} \times W_0 - W_t}{W_t/W_0} \right] \times \frac{W_0 - W_t}{t}
\] | \( g \) | \( ^{15} \text{N} \) abundance of \( \text{NO}_3^- \) | atom % |
| \[
p = c - \frac{W}{t} \ln \frac{f_0 - k}{f_0 - k}
\] | \( p \) | Gross \( \text{NH}_4^+ \) production | mg N kg\(^{-1}\) day\(^{-1}\) |
| \[
r = \frac{(g - \bar{g})Z_g - (g_0 - \bar{g})Z_0}{(k - g)\bar{g} + (f_0 - k)W_0 - (f_0 - k)W_t/c}
\] | \( r \) | Gross \( \text{NO}_3^- \) production | mg N kg\(^{-1}\) day\(^{-1}\) |
| \[
W = \frac{(g - \bar{g})Z_g - (g_0 - \bar{g})Z_0}{(k - g)\bar{g} + (f_0 - k)W_0 - (f_0 - k)W_t/c}
\] | \( W \) | Concentration of \( ^{14} \text{N} \) plus \( ^{15} \text{N} \) in \( \text{NH}_4^+ \) | mg N kg\(^{-1}\) |
| \[
Z = \frac{(g - \bar{g})Z_g - (g_0 - \bar{g})Z_0}{(k - g)\bar{g} + (f_0 - k)W_0 - (f_0 - k)W_t/c}
\] | \( Z \) | Concentration of \( ^{14} \text{N} \) plus \( ^{15} \text{N} \) in \( \text{NO}_3^- \) | mg N kg\(^{-1}\) |
2.5. Calculation of gross N transformation rates

The gross N transformation rates were calculated by the $^{15}$N pool dilution/pool enrichment technique according to Blackburn (1979), Nishio et al. (1985), and Wessel and Tietema (1992). The equations are in Table 2. For the calculations of gross NH$_4^+$ production (mineralization) and consumption rates we distinguished between two different situations, where the NH$_4^+$ concentration either increased (Equations 6 and 7) or remained constant (Equation 8). The NH$_4^+$ concentration was said to be constant if the values at the beginning and end of the 24-hour period differed by less than the internal precision of the instrument. This was determined by calculating the average value, $\overline{W}$, of $W_0$ and $W_t$ and forming the 95% CI of it. If the NH$_4^+$ concentration was outside the 95% CI, equations 6 and 7 were used, and if it was inside, equation 8 was used and $W$ was set to $W_t$.

The gross NO$_3^-$ production (nitrification) was calculated by equation 9 (Nishio et al., 1985; Wessel and Tietema, 1992). The validity of equation 9 was justified by re-calculation it once with $g_0$ and once with $g_t$ substituted for $g$ ($\overline{g} = (g_0 + g_t) / 2$). An $r$ calculated for $\overline{g}$ had to take a value between $r$’s calculated for $g_0$ and $g_t$ (Wessel and Tietema, 1992). The gross NH$_4^+$-N immobilization was then calculated by subtracting the gross NO$_3^-$ production ($r$) from the gross NH$_4^+$ consumption ($c$). These calculations assume that the gross transformation rates remained constant and that no $^{15}$N was recycled to the enriched pool during the measurement period. The short (24 h) assay period was an effort to meet this assumption.

2.6. Geostatistics

Variogram model fitting, kriging, and mapping were performed with the geostatistical software GS$^2$TM Version 5 (Gamma Design Software, USA). The active lag distance (the range over which the semivariance was calculated) was set to 7.64 m for gross N mineralization, immobilization, TW$_{FNIS}$, and PC1 since the variograms tended to be erratic at greater lags. However, the active lag distance was set to 8.64 m for IP$_{beech}$ and IP$_{oak}$, 9.64 m for WA$_{FNIS}$, and 10.64 m for gross nitrification since the semivariance did not reach the sill at shorter distances. The semivariance was determined for lag classes (maximum distance) 1, 1.42, 2, 2.25, 3.17, 4.25, 5.4, 6.41, 7.63, 8.61, and 9.50 m. The lag classes were chosen to be separated by approximately 1 m, except at 1-2 m distances, where the number of pairs was sufficiently high for a finer resolution. Exponential, spherical or gaussian model variograms were fitted to the calculated semivariances using a least squares technique. Ordinary kriging was used for interpolation of values between sampling points. The interpolation estimates were placed on a uniformly spaced grid with 0.25 m intervals and used in the different statistical analyses.

3. Results

The N transformation rates alone could not explain the spatial pattern of the distribution of different plant species or vice versa. The gross mineralization and immobilization rates were spatially autocorrelated within a range of 3.5 and 2.7 m, respectively (Fig. 1, Table 3), and varied between 0.4 and 52.8 mg NH$_4^+$-N kg$^{-1}$ day$^{-1}$ (median 13.8), and 0.3 and 34.6 mg NH$_4^+$-N kg$^{-1}$ day$^{-1}$ (median 10.9). The frequency distributions were positively skewed due to the presence of hotspots with high mineralization and immobilization rates (Fig. 2). Those hotspots overlapped, indicating a tight connection between gross N mineralization and immobilization rates. WA$_{FNIS}$ was spatially autocorrelated at a different and much larger scale (Fig. 1, Table 3), just as the nitrification rate, but its spatial pattern differed from that of WA$_{FNIS}$ (Fig. 1 and 2, Table 3). On the other hand, TW$_{FNIS}$ exhibited spatial dependence at a similar scale as the mineralization and immobilization rates, with an effective range of 4.2 m (Fig. 1, Table 3). Similarly, analysis of the spatial variation of the plant community composition by the PCA

6
Fig. 1. Semivariograms for the gross N mineralization, immobilization, and nitrification rates, as well as the different estimates of the species composition of the understory plant community (WA<sub>FNIS</sub>, TW<sub>FNIS</sub>, and PC1). The lines represent the models fitted to the estimated semivariance (open squares); see Table 5 for parameter descriptions.

revealed that it was autocorrelated within a spatial scale of <3.7 m (PC1, Fig. 1, Table 3). The spatial patterns of TW<sub>FNIS</sub> and PC1 loadings were remarkably similar (Fig. 2; Spearman Rank Correlation, Rho = 0.92, p<0.0001), and both were correlated to the total cover of the understory (Spearman Rank Correlation, Rho = 0.99 (TW<sub>FNIS</sub>) and 0.87 (PC1), p<0.0001). Thus, the spatial patterns of N transformation rates seemed to be more related to the spatial pattern of the cover of plants than to the species composition.

WA<sub>FNIS</sub> was negatively related to the gross nitrification as well as the gross mineralization rate (Table 4), indicating that NO<sub>3</sub>- preferring species were less common at sites with high gross mineralization and nitrification rates. Although there was a slight difference in regression coefficients, similar results were produced by the principal component analysis.
Table 3. Model parameters for the semivariograms in Figure 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model</th>
<th>Nugget variance ( (C_0) )</th>
<th>Sill variance ( (C_0 + C) )</th>
<th>( C/C_0 + C ) (%)</th>
<th>Range (m)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross N mineralization</td>
<td>Exponential</td>
<td>20.9</td>
<td>138.1</td>
<td>84.9</td>
<td>3.5</td>
<td>0.70</td>
</tr>
<tr>
<td>Gross N immobilization</td>
<td>Spherical</td>
<td>9.9</td>
<td>74.9</td>
<td>86.8</td>
<td>2.7</td>
<td>0.71</td>
</tr>
<tr>
<td>Gross nitrification</td>
<td>Exponential</td>
<td>14.3</td>
<td>33.0</td>
<td>56.7</td>
<td>12.0</td>
<td>0.89</td>
</tr>
<tr>
<td>PC 1</td>
<td>Spherical</td>
<td>0.0039</td>
<td>0.0193</td>
<td>79.8</td>
<td>3.7</td>
<td>0.82</td>
</tr>
<tr>
<td>WA(_{FNIS})</td>
<td>Gaussian</td>
<td>0.018</td>
<td>0.237</td>
<td>92.4</td>
<td>30.9</td>
<td>0.99</td>
</tr>
<tr>
<td>TW(_{FNIS})</td>
<td>Exponential</td>
<td>0.0001</td>
<td>0.1522</td>
<td>99.9</td>
<td>4.2</td>
<td>0.75</td>
</tr>
</tbody>
</table>

The first principal component (PC1), which explained 59.1% of the floristic variation, was negatively related to both the gross mineralization and the gross nitrification rate (Table 4). If the total instead of the relative cover was used in the calculation of the N index (TW\(_{FNIS}\)), it was positively related to the gross N mineralization rate and negatively to the gross nitrification rate (Table 4).

There was also a weak positive correlation between the gross mineralization and nitrification rates (Spearman Rank Correlation, Rho = 0.187, \( p<0.0001 \)), which made the relationships between the plant community composition and gross mineralization and nitrification rates hard to interpret. We therefore introduced the ratio between gross nitrification and gross mineralization as an interaction term in the multiple regression analysis. This nitrification ratio had a higher explanatory power than the individual rates, and was positively related to WA\(_{FNIS}\) and negatively to TW\(_{FNIS}\) and PC1 (Table 4). It was also negatively correlated to the gross N mineralization rate, such that a smaller fraction of the mineralized N was nitrified at sites with high mineralization and nitrification rates (results not shown). The species composition of the understory therefore seemed to be more dependent on the relative fraction of the mineralized N that is nitrified than on the rates themselves, while the total cover seemed to be dependent on the gross mineralization rate.

Since the tree influence potential was defined to decrease exponentially with the distance from a tree and be negligible at distances above 5 m, the range of spatial autocorrelation of N mineralization and immobilization rates fitted well into the range of tree influence (Fig. 1, Table 3), as did variations in the cover and species composition of the understory (TW\(_{FNIS}\) and PC1, Fig. 1, Table 3). Oak had a positive influence on the gross mineralization rate, while the opposite was true for beech (Table 6). Given that the total cover of the understory was the major source of variation of TW\(_{FNIS}\) and PC1 (Spearman Rank Correlation, Rho = 0.99 (TW\(_{FNIS}\)) and 0.87 (PC1), \( p<0.0001 \)), the negative influence of beech on them (Table 6) was probably caused by reduced light input, resulting in a low cover under the canopy. Beech trees seemed to create favourable conditions to NO\(_3^-\) preferring plants, since they had a positive influence on WA\(_{FNIS}\) (Table 6). This was opposite to the finding that both the gross nitrification and mineralization rate were lower when beech influence was high (Table 6), indicating that the gross nitrification rate was poorly related to NO\(_3^-\) availability.

4. Discussion

A possible explanation to the low prevalence of NO\(_3^-\) preferring plants, i.e. plants with high FNIS values, at sites with high gross nitrification rates may be a lack of correlation between gross and net rates (Davidson et al., 1992; Hart et al., 1994; Stark and Hart, 1997; Verchot et al.; 2001), especially in soils with a high heterotrophic activity, where high gross mineralization and immobilization rates coincide with a tight microbial recycling of NH\(_4^+\) and NO\(_3^-\) (Davidson et al., 1992; Hart et al., 1994; Verchot et al., 2001). Plants are
Fig. 2. Kriged contour plots for the influence potential of oak and beech (IP_{oak} and IP_{beech}), the gross N mineralization, immobilization, and nitrification rates (mg kg^{-1} d.w.^{-1}), and the different estimates of the species composition of the understory plant community (WA_{FNI}, TW_{FNI}, and PC1).
The dependency of the species composition of the understory plant community on the gross N transformation rates. The gross mineralization was ln-transformed and the gross nitrification (ln+1)-transformed before analysis to ensure normal distribution of the residuals. Since the input values of the dependent variables were interpolated from spatially autocorrelated data (n = 1221), a conservative value of p=0.001 was chosen as the significance level.

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Independent</th>
<th>Coefficient</th>
<th>Significance</th>
<th>Model R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA_FNIS</td>
<td>Intercept</td>
<td>2.523</td>
<td>&lt;0.0001</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Gross mineralization</td>
<td>-0.144</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gross nitrification</td>
<td>-0.043</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>TW_FNIS</td>
<td>Intercept</td>
<td>0.317</td>
<td>&lt;0.0001</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Gross mineralization</td>
<td>0.263</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gross nitrification</td>
<td>-0.194</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>Intercept</td>
<td>0.366</td>
<td>&lt;0.0001</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Gross mineralization</td>
<td>-0.077</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gross nitrification</td>
<td>-0.075</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

generally considered to be inferior competitors to heterotrophic microorganisms for N (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990; Kaye and Hart, 1997), and may take up less NH\(_4^+\) and NO\(_3^-\) in soils with high microbial activity than in soils with low microbial activity (Paper IV). Similarly, nitrifiers are inferior competitors to heterotrophic microorganisms for NH\(_4^+\) (Zak et al., 1990; Verhagen and Laanbroek, 1991; Verhagen et al., 1995), which may explain the decreased ratio of nitrification to mineralization with increasing mineralization rates. A larger fraction of the mineralized N became available to nitrifiers when mineralization rates and heterotrophic activity were low, as in Hart et al. (1994) and Zak et al. (1990), and high residual NO\(_3^-\) concentrations are typically found in soils with low heterotrophic activity and gross N mineralization rates (Hart et al., 1994; Zaman et al., 1999; Bengtsson et al., 2003). Since FNIS values are based on potential net ammonification and nitrification rates, plant species that occur at sites with a large fraction of inorganic N present as NO\(_3^-\) get a high FNIS value (Diekmann and Falkengren-Grerup, 1998). This may explain why NO\(_3^-\) preferring plants were less abundant at sites with high gross nitrification rates and more dependent on the relative fraction of the mineralized N that was nitrified than on the rates themselves.

The positive influence of beech on WA_FNIS indicates that the trees create conditions that are more favorable to NO\(_3^-\) preferring plants than to others, e.g. by decreasing the heterotrophic activity and gross mineralization rates. This interpretation is also supported by the observation of Verchot et al. (2001) that beech stands have higher net nitrification rates and in situ concentrations of NO\(_3^-\) compared to oak stands. The question whether trees had a direct effect on the mineralization rate or indirect via the understory was not intended to be resolved by the experimental design. However, beech litter has a higher lignin content compared to oak (Saryildiz and Anderson, 2003a and 2003b), and the lignin concentration and lignin/N ratio of the litter has a negative influence on the decomposition rate (Saryildiz and Anderson, 2003a and 2003b) and net N transformation rates (Ferrari, 1999). Polyphenolic compounds also have a negative effect on N mineralization rates (Schweitzer et al., 2004), probably by forming protein-polyphenol complexes that are inaccessible to soil bacteria (Northup, 1995). Thus, the evidence, so far, are inclined towards a direct effect of canopy trees on the N mineralization rate, caused by differences in litter quality.

The positive influence of oak on the gross N mineralization and immobilization rates may be a result of oak litter providing carbon to the soil microorganisms, supporting high N transformation rates (Watkins and Barraclough, 1996; Andersen and Jensen, 2001; Knops et al., 2002). In addition, more light penetrates
Table 5. The dependency of the species composition of the understory plant community on the gross nitrification and gross mineralization rates and on the ratio between those rates. The gross mineralization was ln-transformed and the gross nitrification (ln+1)-transformed before analysis to ensure normal distribution of the residuals. Since the input values of the dependent variables were interpolated from spatially autocorrelated data (n = 1221), a conservative value of $p = 0.001$ was chosen as the significance level.

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Independent</th>
<th>Coefficient</th>
<th>Significance</th>
<th>Model $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA_FNIS</td>
<td>Intercept</td>
<td>2.420</td>
<td>&lt;0.0001</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Gross mineralization</td>
<td>-0.088</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gross nitrification</td>
<td>-0.022</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitrification/Mineralization</td>
<td>0.220</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>TW_FNIS</td>
<td>Intercept</td>
<td>0.707</td>
<td>&lt;0.0001</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Gross mineralization</td>
<td>0.054</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gross nitrification</td>
<td>0.050</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitrification/Mineralization</td>
<td>-0.827</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>Intercept</td>
<td>0.485</td>
<td>&lt;0.0001</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Gross mineralization</td>
<td>-0.004</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gross nitrification</td>
<td>-0.016</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitrification/Mineralization</td>
<td>-0.315</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

through the oak canopy than through the beech canopy, facilitating a higher cover of vascular plants under oaks. The “vernal dam hypothesis” (Muller and Bormann, 1976) suggests that those plants may act as a temporary sink for N and be the source of seasonal variations in N flows (Jandl, 1997; Tardiff, 1998; Olsson and Falkengren-Grerup 2003). The importance of understory plants in determining N flows has been questioned on the grounds that soil microorganisms immobilize up to an order of magnitude more N even during spring (Zak et al., 1990; Rothstein, 2000), and that neither removal of spring ephemeral plants nor addition of fresh litter of those plants has any effect on soil NO$_3^-$ concentrations during spring or N mineralization in the summer (Rothstein, 2000). The contrasting influence of oak and beech on the N transformation rates may add additional uncertainty to the analysis of the tree influence, since the positive influence of oak on the gross mineralization rate may follow from the absence of beech influence in the vicinity of oaks, and vice versa.

The two independent ways of characterizing the spatial variation of the understory, TW_FNIS and PC1, ranked the total cover of the understory as more variable than the species composition. This is reasonable since most of the species have a vegetative reproduction and individuals may extend their underground range and appear in many of the plots with one or more shoots, independent of plot-to-plot variations of N transformation rates. Unlike the presence/absence of aboveground parts, their frequency (cover) and the gross N transformation rates should covary from one plot to another if plant available N is dependent on gross rates of N transformations. The presence of individuals that cover large areas would also explain the longer range of spatial autocorrelation for WA_FNIS compared with TW_FNIS, since samples are almost certain to be spatially autocorrelated at least over the area that the same individuals cover. The understory cover therefore seems to give a better estimation of gross N transformation rates than the species composition, at least in sites with a relatively narrow FNIS-range.

Many soil properties, including net N transformation rates, have been shown to vary by one to several orders of magnitude at scales <1-40 m in a number of ecosystems (Robertson et al., 1988; Smith et al., 1994; Gallardo et al., 2000). As in this study, more or less pronounced “hotspots” are often found, resulting in positively skewed frequency
Table 6. Influence of beech and oak (Equation 5) on the gross N mineralization and immobilization rates, and on the species composition of the understory plant community (see Material and Methods section for details). The gross N mineralization and immobilization rates were ln-transformed to ensure normal distribution of the residuals.

<table>
<thead>
<tr>
<th>Dependent (Y)</th>
<th>Beech CoI (a)</th>
<th>Oak CoI (b)</th>
<th>Full model (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross mineralization</td>
<td>-1.54**</td>
<td>0.82**</td>
<td>0.30**</td>
</tr>
<tr>
<td>Gross immobilization</td>
<td>0.53</td>
<td>0.94**</td>
<td>0.22**</td>
</tr>
<tr>
<td>PC 1</td>
<td>-0.57**</td>
<td>-0.09**</td>
<td>0.39**</td>
</tr>
<tr>
<td>WA_FNIS</td>
<td>0.77**</td>
<td>-0.20**</td>
<td>0.47**</td>
</tr>
<tr>
<td>TW_FNIS</td>
<td>-1.57**</td>
<td>0.04</td>
<td>0.34**</td>
</tr>
</tbody>
</table>

* significant at the 0.001 level, ** significant at the 0.0001 level

distributions (Robertson et al., 1988; Smith et al., 1994). Although trees influenced gross mineralization and immobilization rates, there was no evidence from the geostatistical analysis for their contribution to the N transformation hotspots. Instead, other factors affecting the microbial activity, such as soil moisture and small-scale variations in temperature, probably caused these variations (Paper II).

The extent of spatial variation in gross N mineralization and immobilization rates found here has implications for the interpretation of experiments such as NITREX and EXMAN, where nitrogen was added to or removed from the ambient deposition at eight sites spanning a gradient of N deposition across Europe (Wright and van Breemen, 1995; Wright and Rasmussen, 1998). Unfortunately, none of 36 NITREX studies available in the BIOSIS BIOL database examined the difference in gross mineralization or immobilization rates between treatment and control plots, but only among sites. The data on net N transformation rates and NO$_3^-$ leakage are inconclusive (e.g. Emmett et al., 1995; Gundersen et al., 1998; Kjønaas et al., 1998), perhaps because several samples from each treatment were pooled in the laboratory incubations, and because sample independence has been assumed in field incubations. It is possible that treatment effects get hidden if samples from treatment plots are pooled and when ordinary parametric statistics are used to evaluate the data. Furthermore, the use of average values may underestimate rates expressed per area unit if N transformation rates are positively skewed and appear in “hotspots”. This emphasizes the importance of considering the scale of spatial heterogeneity and dependence when field experiments are designed (Jonsson and Moen, 1998; Aubry and Debouzie, 2000).

In conclusion, our results suggest that variations in N transformation rates not only explain differences in the floristic variation between ecosystems and regions (Hutchinson et al., 1999; Falkengren-Grerup and Diekmann, 2003), but also the spatial variation of the ground vegetation within ecosystems, even at scales as small as a few metres. The influence of oak and beech on the N transformation rates supports previous observations that individual trees affect the magnitude and spatial patterns of different soil properties and processes (Bruckner et al., 1999; Sætre, 1999; Stoyan et al., 2000) Influence of trees alone could not explain the full magnitude of the variation or the presence of hotspots. These were probably caused by other factors such as soil moisture content and small-scale variations in temperature (Puri and Ashman, 1998; Thomsen et al., 1999; Subke et al., 2003; Templer et al., 2003). Taken together, the observations imply that a better knowledge about the causes of spatial variations in gross N transformation rates may hold the key to understand not only the factors affecting the below ground N dynamics, but also small-scale variations in the distribution and composition of above ground plant biota.

Acknowledgements

This work was sponsored by grants from the Foundation for Strategic Environmental Research in Sweden. Niklas Törneman and
Therese Nilsson are greatly acknowledged for their assistance in the field and laboratory.

References


Competition for nitrogen – litter leachate favours microorganisms at the expense of plants

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Abstract
A greenhouse experiment was designed to test the idea that the C:N ratio of the soil determines if the heterotrophic soil microorganisms are N or C limited and consequently whether they compete with plants for N or not. The short-term (24 hours) $^{15}$N-uptake uptake by plants and microorganisms in planted and unplanted soils was determined and bacterial activity was measured by the $^3$H-thymidine incorporation technique. Two deciduous forest soils with C:N-ratios of 20 and 31 were used and one litter amended soil with a C:N-ratio of 34. A novel and important part of the experimental design was to use an unplanted control soil that had been planted (with plants having the roots enclosed in nylon bags in the pots) until $^{15}$N was added and was therefore similar to the planted soil (these plants were also grown in nylon bags until 3 days before $^{15}$N-additions when they were allowed to grow free in the soil). Our data suggest that plants and soil microorganisms compete for inorganic N but under influence of other factors than the C:N ratio. In contrast to previous studies, we found differences between planted and unplanted control soils in both microbial $^{15}$N uptake (soil with C:N 20) and bacterial activity (litter amended soil, C:N 34), which emphasizes the significance of the character of the unplanted control soil. The results also illustrate the importance of considering the different characteristics of e.g. fungi and bacteria when discussing plant-microbial competition.

1. Introduction
For a long time the superiority of plants or soil microorganisms in competition for nitrogen (N) has been debated, and the heterotrophic microorganisms have often been considered as winners (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990). Unequivocal demonstration of competition is, however, difficult if competition is defined as the simultaneous demand by two or more organisms for a common limited resource so that both are inhibited by the interaction (Tilman, 1982). Whereas the first part of the definition calls for reasonably straightforward experiments, the second part may be a challenge for very asymmetric interactions in which the dominant competitor may be so little affected by the subordinate that negative effects cannot be detected (Keddy, 1989). Uptake of $^{15}$N labelled inorganic nitrogen was measured to test the potential competition between plants and microorganisms. If the microorganisms were limited by and competing for N with plants the microbial $^{15}$N uptake and activity should increase when the plants were removed.

Plant growth can be limited by N even in relatively fertile soils, with high N turn-over rates, (Tamm, 1991), while carbon availability and decomposability is often considered to be the major factor limiting microbial growth and N assimilation (Tate, 1995). However, as discussed by e.g. Kaye and Hart (1997), this generalisation depends on the C:N ratio of the substrate used by the heterotrophic microorganisms. Fungi and bacteria are considered to be N limited at substrate C:N ratios above 30, but the ratio varies in the literature between 13 and 30 depending on the carbon use efficiency and the biomass C:N ratio of the microorganisms, (e.g. Kaye and Hart, 1997; Killham, 1994). Thus, C-rich substrates with C:N ratios exceeding 30 are likely to be N-limited to microorganisms and result in a net microbial immobilization of N and potential competition between plants and microorganisms. The main aim of the present study was to test the importance of the C:N ratio of the soil for competition for N between plants and soil microorganisms in two forest soils that were similar in pH, dominating tree species, soil type and climate.
Plants are the major source of soil organic matter and the high C:N ratios of plant litter, often above 30, suggests that litter decomposition is N limited and a potential source of plant-microbial competition. Indeed, microorganisms are known to immobilise large amounts of inorganic N from surrounding soil during early stages of plant litter decomposition (Downs et al., 1996; Bremer and Kuikman, 1997; Jingguo and Bakken, 1997). Adding fresh litter with high C content to the soil may temporarily increase amount of C with high turnover rate and C:N ratio of that organic matter fraction and shift the balance between N mineralization/immobilization towards immobilization. To test the importance of C quality in relation to soil C:N ratio we used a soil-litter mixture with the same C-content and similar C:N ratio and pH as a natural soil with high C:N ratio. Heterotrophic microorganisms are known to use both \( \text{NH}_4^+ \) and \( \text{NO}_3^- \), with a preference for \( \text{NH}_4^+ \) (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990; Norton and Firestone, 1996). However, recent studies have shown that soil microorganisms can also immobilise \( \text{NO}_3^- \) when N is limiting (Zak et al., 1990; Davidson et al. 1992; Stark and Hart, 1997). Since the assimilative reduction of \( \text{NO}_3^- \) to \( \text{NH}_4^+ \) requires energy, which is often limiting to heterotrophic microorganisms (Tate, 1995), and since it is repressed by \( \text{NH}_4^+ \) (Paul and Clark, 1996), microbial assimilation of \( \text{NO}_3^- \) is thought to occur in places with \( \text{NH}_4^+ \) deficit (Schimel et al., 1989). Plants seem to grow best on a combination of \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) (Falkengren-Gerup and Lakkenborg-Kristensen, 1994), but various species have different preferences both within and between habitats (Hågbom and Ohlson, 1991; Falkengren-Gerup, 1995; Falkengren-Gerup et al., in press). Thus, plants and soil microorganisms are able to use the same forms of N and may compete for them as long as N is the growth limiting resource.

Short-term (hours to a few days) experiments are most appropriate to demonstrate specific plant-microbe competition events because of the short turn-over times of soil microorganisms. Most of these studies show that microorganisms take up a greater part of added \( ^{15}\text{NH}_4^+ \) and \( ^{15}\text{NO}_3^- \) than plants (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990; Norton and Firestone, 1996). However, this do not prove plant-microbial competition for N and so far increased metabolic activity and N uptake in microorganisms in absence of plants has not been demonstrated.

We hypothesise that

1. soil micro-organisms and plants compete for \( \text{NH}_4^+ \) and \( \text{NO}_3^- \), at higher soil C:N ratios but not at lower because theoretically, N is more likely to be a limiting element for heterotrophic microorganisms at high soil C:N ratios,
2. microorganisms take up more \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) than plants in all soils but the magnitude of the difference will be inversely related to the soil C:N ratio,
3. the difference between microbial and plant uptake will be greater for \( \text{NH}_4^+ \) than \( \text{NO}_3^- \) since \( \text{NO}_3^- \) is more readily mobile in soil and microorganisms seem to prefer \( \text{NH}_4^+ \) over \( \text{NO}_3^- \).

2. Materials and Methods

2.1. Soil description

The two soils with high (31) and low (20) C:N ratios, respectively, were classified as Dystric Cambisol (FAO system) and located 500 m apart at Torup, southern Sweden (55°33′N, 13°37′E). Oak and beech were the dominating tree species and the understory vegetation was sparse. At the end of October 2002 litter was removed within a 20 by 20 m square and five samples of the top five cm of the soil were collected, sieved through a 4 mm mesh and then analysed to determine the C:N ratio. The heterogeneity of the soil limited us to pool only two of the five samples from each site to get appropriate C:N. After determining pH and water holding capacity (WHC), the soil was stored at 2°C until planting two weeks after sampling. The soil-litter mixture (20L) was produced by mixing the oak-beech litter.
fragments (≤ 1 mm, C:N ratio 42) with the soil with C:N ratio 20 to obtain a soil with the same C content and a similar C:N ratio as the soil with C:N ratio 31 (0.21 litter g⁻¹ dw soil). The litter was collected at the site with low C:N ratio and at the same time as the soils. Properties of the three soil materials are given in Table 1.

2.2. Plant material

Festuca gigantea (L) was used in the experiment because it is relatively common in the type of oak-beech forest where the soils were sampled. F. gigantea has a rather high N demand and NO₃⁻ uptake with FNIS value of 8 (Diekmann and Falkengren-Grerup, unpublished) and Ellenberg N-value of 6 (Ellenberg, 1991). The plants were grown from seeds in > 99% pure silica sand supplied with a nutrient solution (mineral N concentration was 150 µM NO₃⁻ and 100 µM NH₄⁺) for one to two weeks after germination and then planted in the experimental soils. Seedlings that qualified for the experiment had a shoot height of 7-10 cm and root length of 5-8 cm.

2.3. Experimental design and ¹⁵N additions

The soils were wetted with deionized water to obtain 60% WHC before planting. Transparent plastic pots with a diameter of 27 mm and a height of 85 mm were wrapped in tin-foil to exclude sunlight from the soil core. They were filled with soil from the samples with low and high C:N ratios and with the soil-litter mixture to obtain a weight of 40, 35 and 30 g f.w soil respectively to get the same volume. Each pot was planted with three specimens of F. gigantea. The roots of each plant were enclosed in nylon bags (width 15 mm, height 50 mm and mesh size 25 µm) to keep the roots out of the bulk soil but allow N uptake from the surrounding soil. The pots were placed in a greenhouse with 16°C night/20°C day temperature and with 16 hours of day light radiation (with additional light of 160 µmol m⁻² s⁻¹). Sprinklers adjusted the relative air humidity to about 50%. The pots were used in the ¹⁵N uptake experiment three weeks after planting and the soils were kept at 60% of WHC by addition of deionised water. Pots used for the treatment with plants had the grasses replanted without nylon bags three days before ¹⁵N additions so that the roots were free to explore the soil. In the treatment without plants the grasses were kept in the root bags until 1 hour before ¹⁵N additions. In this way unplanted and planted soils were similar during the competition assay. When replanting, the plants with root bags were removed with minimal disturbance to the soil and the plants were carefully taken out of the bags. The soil in the root bags were emptied in the hole left from the bag and the grass were replanted in the same hole.

The pots received either distilled water (control), or distilled water spiked with 50 nmol ¹⁵N per g dw soil ¹⁵NH₄Cl or Na¹⁵NO₃ (Cambridge Isotopic Laboratories, >98% ¹⁵N). The ¹⁵N additions resulted in < 5% of added ¹⁵NH₄⁺ to intrinsic NH₄⁺ (extractable amounts in 0.4M KCl measured by flow injection analysis at the start of the experiment) in the two natural soils and 52% in the soil-litter mixture, and 82 and 13% of added ¹⁵NO₃⁻ to intrinsic NO₃⁻ in soil 20 and 31 respectively. The ¹⁵NO₃⁻ addition to the soil-litter mixture was 100 times the intrinsic. Four 0.5 mL injections per pot were made with a syringe, which was gradually withdrawn from the bottom to the top of the pot to distribute the solution evenly. The soils were dried by ~2 g (this was achieved by avoid watering the day before ¹⁵N additions) before injections to avoid over-watering. One hour after injection, half of the pots were harvested to determine the concentration of extractable ¹⁴N/¹⁵N and NO₃⁻ and NH₄⁺. Plants were gently removed, the soil was thoroughly mixed and 10 g was extracted with 50 ml of 0.4 M KCl for one hour by shaking. The extracts were filtered through acid washed Munktell analytical filter papers (00K). The extracts were then analysed by flow injection analysis (FIASstar, Tecator ASN 50-05/90, 110-01/92) to determine the NH₄⁺ and NO₃⁻ concentrations. Five mL of the extracts were vacuum centrifuged without heat in a Savant AES 1000 and the residue analysed to determine ¹⁵N and total N concentration. As the
Table 1. The pH (0.2 M KCl), soil-C and -N (mg g\(^{-1}\) dw soil), soil C:N ratio, microbial biomass N (µg N g\(^{-1}\) dw soil).

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH</th>
<th>Soil C</th>
<th>Soil N</th>
<th>Soil C:N</th>
<th>Microbial biomass N</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3.5</td>
<td>37</td>
<td>2.9</td>
<td>20</td>
<td>78.5</td>
</tr>
<tr>
<td>31</td>
<td>3.8</td>
<td>164</td>
<td>5.2</td>
<td>31</td>
<td>147.6</td>
</tr>
<tr>
<td>20L</td>
<td>3.6</td>
<td>164</td>
<td>4.0</td>
<td>34</td>
<td>82.0</td>
</tr>
</tbody>
</table>

pH of the soil water extracts was below 4.5, loss of ammonia through volatization was < 2% (data not shown). The \(^{15}\)N, \(\text{NO}_3^-\) and \(\text{NH}_4^+\) concentrations one hour after \(^{15}\)N addition were then used as starting values for the calculation of the N-uptake. The remaining pots were placed for 24 h in a climate chamber (20°C/15°C day-night temperature) with 16 hours per day of radiation of 400 µmol m\(^{-2}\) s\(^{-1}\) and an air humidity of 50%. When harvested, the plants were gently removed from the soil and rinsed in 1.0 mM KCl and 0.5 mM CaCl\(_2\) for 5 minutes to remove residual label. Soil samples were taken for mass spectrometry analysis. Samples of 10 g of soil where taken for chloroform fumigation-extraction (Brookes et al., 1985; our samples was extracted by 0.4 M KCl instead of 0.5 M K\(_2\)SO\(_4\)) to determine the microbial \(^{15}\)N uptake and biomass-N. Plants were dried at 70°C for 24 hours. Five mL of the KCl soil extracts from fumigated and unfumigated samples were vacuum centrifuged without heat in a Savant AES 1000 to remove water and the residue analysed to determine \(^{15}\)N and total N concentration. Dried plant material was weighed and ground to a fine powder using a ball mill (Retsch, Mixer Mill 200), while salt from dried soil extracts were ground by using a mortar and pestle.

2.4. Analysis of \(^{15}\)N by mass spectrometry

Plant tissues, soil and salt from dried soil extracts were weighed in tin capsules and analysed for \(^{15}\)N by continuous flow isotope mass spectrometry. The samples were oxidized in an ANCA-GSL elemental analyzer, NO\(_x\) reduced to N\(_2\), which was passed to a 20-20 IRMS (PDZ Europa UK). The amount of \(^{15}\)N and total N were quantified after subtraction of blank values and calibration against tin capsules with absorbent which had received 5, 50, or 100 µg N as glycine (calibrated against and IAEA KNO\(_3\) standard) dissolved in 5 µl of water. The precision of the isotopic determinations was 0.2‰. The plant and microbial uptake of NH\(_4^+\) or NO\(_3^-\) was estimated by multiplying the fraction of \(^{15}\)N immobilised by plants and microorganisms (data from fumigation-extraction) by the \(^{14}\N/^{15}\N\) ratio in the NH\(_4^+\) and NO\(_3^-\) pools one hour after \(^{15}\)N additions, assuming that the uptake of \(^{15}\)N and \(^{14}\N\) was proportional to their relative abundance:

\[ N_i = (N_{i0} - N_t/15N_t) \]

where \(N_i\) is the gross immobilization of NH\(_4^+\) or NO\(_3^-\), \(N_t\) is the \(^{15}\)N taken up by the microorganisms or the plants and \(N_{i0}/15N_{t0}\) is the ratio of total N and the added \(^{15}\)N in the NH\(_4^+\)- and NO\(_3^-\)-pools 1 hour after \(^{15}\)N additions. The \(N_t\) values are calculated by subtracting the natural abundance of \(^{15}\)N from the data we received from the \(^{15}\)N treatments.

2.5. \(^3\)H-thymidine incorporation

The \(^3\)H-thymidine incorporation technique (Bååth et al., 2001) estimates the bacterial growth rate as the rate of DNA synthesis. A 50 mL centrifuge tube containing two g of soil and 30 ml of distilled water was put in a shaker for 15 minutes and then centrifuged at 3000 rpm. Methyl\(^{3}\)H]thymidine (Amersham, 25 Ci mmol\(^{-1}\), 925 GBq mmol\(^{-1}\)) and 1.5 mL bacterial suspension (the supernatant) were added to 2 mL eppendorf-vials that were incubated at 20°C for 2 hours (final \(^3\)H-thymidine concentration of 100nM). Incorporation was stopped by adding 75µL cold 100% trichloroacetic acid (TCA) to each vial and the vials were shaken on a vortex and then stored in the refrigerator for 30 minutes. The vials
were then centrifuged at 15 000 g for 10 minutes and the radioactive pellets were washed with 1.5 mL cold 5% TCA (to precipitate macro molecules) and 1.5 mL 80% ice cold ethanol (to remove thymidine bound lipids). Then 0.2 mL 1 M NaOH was added and heated to 90ºC for 1 hour to solubilise the macromolecules. After cooling, 1.25 mL scintillation cocktail (Ultima Gold, Packard) was added and the radioactivity was measured on a Beckman LS 1801 liquid scintillation spectrometer.

2.6. Statistical analyses

The effect of plants on bacterial activity (\(^3\)H-thymidine incorporation rate) was tested in a 3-way ANOVA showing a significant effect of plants, and it was followed by multiple comparisons (LSD test) testing all treatment separately. The effect of plants on microbial \(\text{NH}_4^+\) and \(\text{NO}_3^-\) uptake was tested using a 3-way ANOVA followed by multiple comparisons (LSD test) testing all treatment separately. Plant \(^{15}\text{NH}_4^+\) and \(^{15}\text{NO}_3^-\) uptake was analysed in a 2-way ANOVA followed by multiple comparisons testing all treatments separately. The relationship between bacterial activity (\(^3\)H-thymidine incorporation) and microbial \(^{15}\text{N}\) uptake was tested in an ANCOVA with \(^{15}\text{N}\)-form and with-without plant as fixed factors. All statistical analyses were performed in SPSS 10.0.5 and Statistica 6.1).

3. Result

3.1. Microbial and plant N uptake

The presence of the plant reduced microbial uptake of \(\text{NH}_4^+\) and \(\text{NO}_3^-\) (ANOVA; \(p<0.05\), \(n=5\)) in the soil with the lowest C:N ratio (20) but had no significant effect in the other two soils (Fig. 1). The plant N uptake was significantly reduced by the litter amendment of the soil with low C:N ratio (20L) but independent of the C:N ratio of the natural soils. The \(\text{NH}_4^+\) uptake was about one order of magnitude larger than the \(\text{NO}_3^-\) uptake in both plants and microorganisms. The plant \(\text{NH}_4^+\) uptake was similar in the two natural soils but significantly lower in the soil-litter mixture (ANOVA; \(p=0.05\)), while there was no difference in uptake of \(\text{NO}_3^-\) (Fig 1).

The plants took up 1.3 – 2 times more \(\text{NH}_4^+\) and \(\text{NO}_3^-\) than the microorganisms in the two natural soils, while microorganisms took up an order of magnitude more N in the litter amended soil (Table 2). The ratio between microbial and plant N uptake increased with increasing C:N ratio, especially in comparison between the natural soils and the litter amended and the ratio was lower for \(\text{NO}_3^-\) than for \(\text{NH}_4^+\) (Table 2).

3.2. Bacterial activity

The bacterial activity (\(^3\)H-thymidine incorporation rates measured in DPM) and the microbial N uptake were positively related across the soils (ANOVA; \(p< 0.001\), \(R^2=0.764\)). The ratio of the activity to \(^{15}\text{N}\) uptake was calculated with the plant uptake included or excluded in order to get an additional estimate of the effect of plant \(^{15}\text{N}\) uptake on microbial uptake. In the litter amended soil the ratio of the activity to microbial \(^{15}\text{N}\) uptake was higher in the planted (9.68\(\cdot\)10\(^6\) and 17.7\(\cdot\)10\(^6\) for \(\text{NH}_4^+\) and \(\text{NO}_3^-\) respectively) than in the unplanted (8.42\(\cdot\)10\(^6\) and 16.6\(\cdot\)10\(^6\) for \(\text{NH}_4^+\) and \(\text{NO}_3^-\) respectively). When plant uptake of \(^{15}\text{N}\) was included in the ratio (8.70\(\cdot\)10\(^6\) and 16.0\(\cdot\)10\(^6\) for \(\text{NH}_4^+\) and \(\text{NO}_3^-\) respectively) the planted and unplanted treatments got similar ratios.

The activity was significantly lower in soil 20 compared to the other soils on a soil dry weight basis (Fig. 2, ANOVA; \(p<0.001\), \(n=5\)), but significantly higher on a soil C basis (ANOVA; \(p<0.001\), \(n=5\)), implying a higher C quality in soil 20.

The plants had an over-all positive effect on the bacterial activity (ANOVA \(p=0.01\), \(n=5\)), but multiple comparisons between treatments showed that the significance was attributed to the litter amended soil (Fig. 2). Plants seemed to stimulate the bacterial activity in the natural soils as well, especially soil 31 (\(p=0.068\), \(n=5\)), but only when \(^{15}\text{NH}_4^+\) was added. The impact
Figure 1. Uptake of (a) NH$_4^+$ and (b) NO$_3^-$ (µg N per pot) by plants and soils microorganisms in the three soils, for the planted and unplanted treatments. The bars represent means and standard error (n=5).

of the plants in the litter amended soil was probably associated with the root morphology. The roots were thinner, more branched, and with a higher frequency of rot hairs than in the other soils (observed during harvest). That would enhance the surface to volume ratio and most likely increase rhizodeposition, stimulating rhizosphere bacteria.

3.3. Ammonium and nitrate concentrations

The NH$_4^+$ and NO$_3^-$ concentrations in the natural soils increased by a factor two or slightly more during the 24 hour assay (Table 3). The effect may be related to the enclosure of roots in nylon bags, indicating that the soil microorganisms were not N limited, at least not in the bulk soil, or to the exclusion of ectomycorrhiza.

Soil 20 and 31 had significantly higher NH$_4^+$ concentrations than soil 20L and the NO$_3^-$ concentration was significantly higher in soil 31 compared to the other two soils (Fig. 3 and Table 3). The NH$_4^+$ concentrations were slightly higher (ANOVA; p=0.118, n=15) and the NO$_3^-$ concentrations lower (p<0.05, n=15) in the planted soils than in the unplanted (Table 3).

3.4. Soil effects on F. gigantean

The root dry weight was similar in the soils (ANOVA; p=0.985, n=20), with means ranging from 19.07 to 19.39 mg, while the shoot dry weight varied with the soil type. Soil 20 and 20L had the lowest shoot weights, 26.44 and 23.64 mg, respectively, and soil 31 the highest of 35.03 mg, significantly higher than the other soils (ANOVA; p=0.01, n=20). The shoots in soil 20 were short and dark green while the shoots in soil 20L showed signs of chlorosis. After NH$_4$NO$_3$ (six times 5 ml 500µM) was added to the litter amended soil the plants shifted in colour toward darker green. The plants in soil 31 showed no signs of nutrient deficiency.

4. Discussion

The data on the uptake of NH$_4^+$ and NO$_3^-$ by microorganisms and plants (Fig. 1) in the planted and unplanted pots can be used to reject the first hypothesis, that competition for N is expected in soils with high rather than low C:N ratios. The microorganisms were less successful in acquiring inorganic N from soil 20 in presence of active plant roots than in their absence but unaffected by the plants in the soils with the higher C:N ratio. However, only 50% of the decreased microbial NH$_4^+$ uptake in soil 20 could be explained by plant uptake indicating that it may be other factors limiting microbial uptake. One of them may be plant control of microbial phosphorous (P) uptake, subsequently limiting the N uptake. Soil 20 had a high fraction of mineral soil and a low pH (3.5 in KCl), at which inorganic P tend to be fixed in unavailable forms that involves Al-, Fe- or Mn-ions, oxides or hydrous oxides (Schlesinger, 1991; Gallardo and Schlesinger, 1994). Indeed, the plants in soil 20 did show symptoms of P limitation, such as short shoots with a dark-green colour. Phosphate supply has
Table 2. Microbial and plant uptake of $^{15}$N in percent of added $^{15}$N.

<table>
<thead>
<tr>
<th>Soils</th>
<th>$^{15}$N-form</th>
<th>Ratio Micro:Plant $^{15}$N-uptake</th>
<th>Plant $^{15}$N uptake (%)</th>
<th>Microbial $^{15}$N uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>NO$_3^-$</td>
<td>0</td>
<td>10.03</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NH$_4^+$</td>
<td>0.42</td>
<td>9.02</td>
<td>3.27</td>
</tr>
<tr>
<td></td>
<td>NO$_3^-$</td>
<td>0.49</td>
<td>5.58</td>
<td>2.74</td>
</tr>
<tr>
<td></td>
<td>NH$_4^+$</td>
<td>0.74</td>
<td>8.90</td>
<td>8.82</td>
</tr>
<tr>
<td>31</td>
<td>NO$_3^-$</td>
<td>9.5</td>
<td>2.57</td>
<td>24.29</td>
</tr>
<tr>
<td></td>
<td>NH$_4^+$</td>
<td>13.4</td>
<td>4.59</td>
<td>40.10</td>
</tr>
<tr>
<td>20L</td>
<td>NO$_3^-$</td>
<td>10.5</td>
<td>24.29</td>
<td>19.21</td>
</tr>
<tr>
<td></td>
<td>NH$_4^+$</td>
<td>13.4</td>
<td>4.59</td>
<td>40.10</td>
</tr>
</tbody>
</table>

also been shown to control nitrification in different forest soils (Pastor et al., 1984), which may explain the low nitrate concentrations in soil 20 despite high NH$_4^+$ supply.

However, the plants had no significant effect on the bacterial activity in soil 20, suggesting that they specifically inhibited fungal N uptake and not bacterial uptake. It seems reasonable to assume that bacteria with their higher surface to volume ratio and high growth rates will be superior to the plants at taking up NH$_4^+$ at low concentrations. However, mycelia forming fungi may compete with plants on more equal terms than bacteria because of their lower turn-over rates and higher surface to volume ratios as compared to bacteria and unicellular fast-growing fungi.

One of the key assumptions of the first hypothesis was the limited availability of inorganic N that follows with a high C:N ratio. It is true that the NH$_4^+$ concentration was lower in soil 31 than in soil 20, but the NO$_3^-$ concentration was more than 5 times as high (Fig. 3, Table 3). However, NH$_4^+$ and NO$_3^-$ concentrations and plant uptake of NH$_4^+$ were significantly lower in soil 20L than in the natural soils, suggesting the litter amended soil as the best candidate to fulfill the assumption on N limitation in the experiment. The plants in this soil had characteristics of nutrient limitations, such as development of a high frequency of finer roots (Badalucco & Kuikman, 2001) and signs of chlorosis in the shoots, which was reversed when the soil was fertilised with NH$_4$NO$_3$. The insensitivity of microbial N uptake to the presence of plant roots in that soil (Fig. 1) is a strong argument to reject the first hypothesis.

The data on the microbial relative plant uptake of $^{15}$N labelled NH$_4^+$ and NO$_3^-$ seem to be more in favour of acceptance than rejection of the second hypothesis suggesting that plants will be more efficient at low C:N ratios and microorganisms at high. Whereas the plant uptake was inversely related to the C:N ratio, the microorganisms increased the uptake of added ions in the planted soil as the C:N ratio became larger (Table 2). One is given the impression that the plants acquire a higher proportion of added N in soil 20 and that microorganisms acquire a higher proportion in soil 20L, as expressed by the uptake ratio in Table 2. The difference between plant and microbial N uptake was largest in soil 20L, and the low plant $^{15}$N uptake compared to microbial, despite the apparent N deficiency in the plants, suggests a one-sided competitive interaction were the microorganisms were superior.

Apart from soil 20, the bacterial activity was the main factor determining microbial N uptake, while plants had no effect. Plants stimulated the bacterial activity only when fragmented litter had been added to soil 20, resulting in low inorganic N concentrations. A fungal fraction in soil 20L as well as in soil 20 may explain both the discrepancies between microbial N uptake and bacterial activity in response to plants in soil 20, and the higher microbial N uptake in soil 20L compared to soil 31 despite the similar bacterial activity. It is possible that the structural litter components in soil 20L promoted N uptake by fungi decomposing these compounds, adding to the N taken up by bacteria growing on litter leachates and plant exudates (Suberkropp and
Figure 2. The bacterial activity measured by $^{3}H$-thymidine incorporation [Disintegration’s Per Minute (DPM) g$^{-1}$ dw soil] in the three soils, for the planted and unplanted treatments. Bars represent means and standard error (n=5). (ANOVA; ns= $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Klug, 1976; Swift et al., 1979; Henriksen and Breland, 1999). It is interesting to notice that the microorganisms had a higher ratio of activity to $^{15}N$ uptake in the planted 20L soil than the unplanted. With everything else in the planted and unplanted soil being equal, the difference should be attributable to the presence of plants, that is, an expression of competition. Indeed, if the number for the $^{15}N$ uptake by the plants is added to the number for the $^{15}N$ uptake by the microorganisms in the planted soil, the ratios of microbial activity to $^{15}N$ uptake in the planted and unplanted soils become similar both for NH$_4^+$ and for NO$_3^-$.

Thus, the bacterial activity was positively affected by plants but the microbial uptake of NH$_4^+$ and NO$_3^-$ did not increase in proportion to the activity, which might be explained by competition between plants and fungi for N while bacterial N uptake was relatively unaffected. This explanation could account for the increase in bacterial activity in planted soils, an increase that most likely is a response to high C:N rhizodeposition which is difficult to explain in a soil that in many respects seem to be N limited. That is, bacterial acquisition of N may be efficient enough for bacterial growth not to be N limited, while plants and fungi are potentially N limited and compete for this nutrient. An alternative explanation could be that N limited microorganisms are stimulated by N containing compounds, such as, NH$_4^+$, NO$_3^-$, amino acids, vitamins, enzymes and nucleotides (Uren, 2001) in the rhizodeposition. The extra N input from roots, though small in relation to C, could be important to N limited microorganisms (Brimecombe et al., 2001).

The first assumption in the second hypothesis, suggesting that microbial N uptake would be higher than plant N uptake in all soils, had to be rejected since the plant uptake of NH$_4^+$ and NO$_3^-$ exceeded the uptake by microorganisms, except in soil 20L. This was unexpected since microbial uptake that was 2 to 10 times as high as plant uptake in previous short-term competition studies (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990).
Norton and Firestone (1996) compared microbial and plant (Pinus ponderosa seedlings with ectomycorrhiza) uptake of NH$_4^+$ and NO$_3^-$ in a sandy loam soil from a mixed conifer site. They found that plants accounted for 30% of the total NH$_4^+$ consumption and 70% of the NO$_3^-$ consumption, which is more consistent with our results. The studies that reported a higher microbial to plant $^{15}$N uptake were field studies (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990) or laboratory experiment (Norton and Firestone, 1996) where mycorrhizal N uptake probably was included in the microbial uptake. There was no ectomycorrhiza present in our experiment and the arbuscular mycorrhizal N uptake is not likely to have been significant because the roots were enclosed in root bags, thus giving a possible explanation to the lower microbial N uptake in the natural soils. The third hypothesis was supported by the ratio of microbial to plant uptake, which was lower for NO$_3^-$ than for NH$_4^+$This was expected from previous studies (Jackson et al., 1989; Schimel et al., 1989; Zak et al., 1990; Norton and Firestone, 1996) and from the higher diffusion rates of NO$_3^-$ compared to NH$_4^+$, which would favour plant roots with their more limited cover of the soil volume.

Our data suggest that plants and soil microorganisms compete for inorganic N but under influence of other factors than the C:N ratio. In contrast to previous studies (Schimel et al., 1989; Zak et al. 1990; Norton and Firestone, 1996), we found differences between planted and unplanted control soils in both microbial $^{15}$N uptake (soil with C:N 20) and bacterial activity (litter amended soil, C:N 34), which emphasizes the significance of the character of the unplanted control soil. The results also illustrate the importance of considering the different characteristics of e.g. fungi and bacteria when discussing plant-microbial competition.

References


Rapid turnover of DOC in temperate forests accounts for increased CO₂ production at elevated temperature

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Climatic models predict a global-mean temperature rise of 1.2-6.9 °C by the end of the century. The effect of the temperature increase on the mineralization of organic carbon is debated and has implications for the interpretation of temperate forest soils as carbon sinks. Whereas CO₂ enrichment experiments have failed to show an effect of elevated CO₂ levels on the mass of carbon in the mineral soil, carbon is accumulated in soil organic matter fractions with relatively short turnover times, suggesting a limited potential of long-term carbon sequestration in forest soils. Here we present a simple model, supported by experimental data, that accounts for temperature dependence of the CO₂ production through a tight microbial control of the flow of carbon between soil organic matter (SOM), DOC, and the microbial biomass.

Increased tree growth in response to elevated CO₂ levels and global warming are thought to mitigate the current rise in atmospheric CO₂ concentrations. But then, increased temperature can also be expected to accelerate the decomposition of soil organic matter and the release of CO₂ to the atmosphere. This assumption is supported by short-term degradation experiments, but results from forest warming experiments and analysis of decomposition data from soils spanning the global range of mean annual temperature have called it into question. One of the reasons for the opposing conclusions may be that turnover times of soil C are sometimes estimated with the assumption that C exists as a single homogenous pool, possibly masking the effect of temperature on a small, active soil C fraction with temperature dependent turnover rates. Additional problems to accurately predict C mineralization rates may arise from the ubiquitous use of first order decomposition kinetics when modelling soil organic matter breakdown. First order approximations may be useful in describing C mineralization rates but depend on the assumption that the maximum concentration of the substrate is much lower than the half-saturation constant. To our knowledge, the assumption has not been validated, probably since the combination of high microbial diversity, the ability of microorganisms to utilize several different substrates, and the presence of numerous possible substrates makes it virtually impossible.

To circumvent these problems we designed an experiment in which the concentration and δ¹³C of total C (SOM), phosphate buffer extractable C (POC), and DOC in a temperate forest soil were estimated. DOC was enriched in ¹³C by 1.5‰ compared to CO₂, and the other two C pools were depleted by 1.4‰ (Fig. 1). The comparison suggested that DOC was the source of the respired CO₂, since CO₂ is normally depleted in the order of 1-2‰ compared to the substrate. Given that C mineralization is described by first order kinetics, and that DOC was the source of the respired CO₂, the respiration rate should be dependent on the concentration of DOC. However, the CO₂ production during a 24 hour incubation of 108 soil samples from the top 5 cm of the mineral soil at field temperature was not related to the concentration of C in any of the three C pools, indicating that first order approximations would be inappropriate to estimate C mineralization rates. Furthermore, the quantity of the respired C exceeded the DOC pool 3-4 times, suggesting a turnover of DOC several times per day (Figure 2). The validity of this observation was elucidated by an independent calculation of the flow of C between the microbial biomass and the DOC pool.

Since gross C assimilation in soil microorganisms is difficult to estimate directly, it was calculated from the gross NH₄⁺-N assimilation found in a ¹⁵N pool dilution experiment. The NH₄⁺-N assimilation in the
108 soil samples was $11.2 \pm 1.2$ mg N kg$^{-1}$ d$^{-1}$ (Mean $\pm$ SE). This would correspond to an assimilation of 112 mg C kg$^{-1}$ d$^{-1}$, given a microbial C/N ratio of 10 ($C_{\text{assimilated}} = N_{\text{assimilated}} \times C/N_{\text{microorganisms}}$). With a respiration rate of $149.2 \pm 11.4$ mg C kg$^{-1}$ d$^{-1}$ (Fig. 2), the calculated C use efficiency became 42.9% ($C_{\text{use efficiency}} = (C_{\text{assimilated}} / (C_{\text{assimilated}} + C_{\text{respired}})) \times 100$, which is within the range normally found$^{19}$.

In situ variations in microbial biomass or numbers in soils are hardly detected from day to day or even week to week, although the microbial activity can vary ten-fold$^{20,22}$. Therefore, we assumed a constant microbial biomass, and that 112 mg C kg$^{-1}$ d$^{-1}$ was mobilized and returned to the DOC pool. The $^{13}$C enrichment of the microorganisms utilizing DOC, corresponding to the depletion of $^{13}$C in CO$_2$, was calculated as: $f_{\text{required}} \times (\delta^{13}C_{\text{CO}_2} - \delta^{13}C_{\text{DOC}}) - f_{\text{assimilated}} \times (\delta^{13}C_{\text{DOC}} - \delta^{13}C_{\text{microorganisms}}) = 0$, where $f_{\text{required}}$ and $f_{\text{assimilated}}$ represent the fraction of the substrate that was respired and assimilated, respectively. With a C use efficiency of 42.9%, $^{13}$C of the microorganisms was -22.7‰, equivalent to a $^{13}$C enrichment by 2.0‰ compared to their C source (DOC). This magnitude of enrichment is in agreement with literature data$^{3,24}$.

Two insights emerge from the calculations. First, with an import of 112 $\mu$g C kg$^{-1}$ and an export of 261 $\mu$g C kg$^{-1}$ d$^{-1}$ ($C_{\text{assimilated}} + C_{\text{respired}}$) the DOC pool would rapidly be depleted. Second, with a $^{13}$C enrichment by 2.0‰ of the microorganisms and their waste products, $\delta^{13}$C of DOC would rapidly increase. The ambiguity can be resolved by considering soil organic matter (SOM) as another source of DOC, since neither litter nor roots could supply enough DOC. We calculated the fraction of DOC that would originate from SOM ($f_{\text{SOM}}$) in order to balance the import of enriched C from the bacteria and maintain $\delta^{13}$C of DOC at -24.7‰:

$$f_{\text{SOM}} = (\delta^{13}C_{\text{DOC}} - \delta^{13}C_{\text{SOM}}) / (\delta^{13}C_{\text{microorganisms}} - \delta^{13}C_{\text{SOM}}),$$

where $\delta^{13}C_{\text{microorganisms}}$ was -22.7‰ (calculated above) and $\delta^{13}C_{\text{SOM}}$ -27.6‰ (Fig. 1). SOM was found to contribute with 59.1% of the DOC, leaving a 40.9% contribution with the microorganisms, and the total DOC production became 112 / 0.409 = 274 mg C kg$^{-1}$ d$^{-1}$, or almost exactly the same quantity as was exported. The calculations reaffirm the rapid turnover of DOC by microorganisms, in accordance with the observation that DOC leached from soil is old because of extensive recycling in the microbial biomass$^{25}$. The findings emphasize the importance of microbial activity in controlling the flow of C to and from the DOC pool.

The enzymatic breakdown of SOM to DOC is obviously a rate-limiting step for the CO$_2$ production, and since enzymatic activity is dependent on temperature, increased temperatures would tend to enhance the breakdown of SOM and the release of CO$_2$. The mechanism for the control of CO$_2$ production suggested is independent of the extent to which some of the DOC is refractory$^{26}$, since the flow in and out of the DOC pool is dependent on the microbial activity rather than on the amount of DOC. Our analysis of the transient fate of DOC draws the attention to microbial activity as the link between increased CO$_2$ production from temperate forest soils and increased temperature and precipitation, causing what has been referred to as a “runaway greenhouse effect”$^{22}$.

**Methods**

The experiment was conducted in a mixed beech/oak stand in Torup in southwestern
The amount of DOC and its $\delta^{13}C_{\text{PDB}}$ was determined by transferring field moist soil samples to 0.2 µm Z-spin filter units, which were centrifuged at 26000×g for 20 min at 4°C. The soil water in the receiver tube was transferred to a 5×8 mm tin cup, evaporated to dryness at 35°C, and analysed by IRMS as described above. The total amount of C in SOM and its $\delta^{13}C_{\text{PDB}}$ was determined by grinding dried (70°C) soil samples in a Retch MM200 ball mill, transferring a portion of the soil to a 5×8 mm tin cup and analysing by IRMS.

18. Wessel and Tietema
27. IAEA Training Course Series No. 2. *Use of Isotope and Radiation Methods in Soil and..."
Acknowledgements. This work was sponsored by grants from the Foundation for Strategic Environmental Research in Sweden. Niklas Törneman and Therese Nilsson are greatly acknowledged for their assistance in the field and laboratory, respectively.
Bacterial immobilization and remineralization of N at different growth rates and N concentrations

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Abstract
An experiment was designed with the ambition to resolve two largely unaddressed questions about the turnover of N in soils. One is the influence of microbial growth rate on mobilization and remineralization of cellular N. The other is to what extent heterotrophic immobilization of NO$_3^-$ is controlled by the soil concentration of NH$_4^+$. Bacteria were extracted from soil and inoculated into an aqueous medium at a low density. Various pool dilution/enrichment experiments were then carried out to [1] calculate the gross N immobilization and remineralization rates, [2] their dependence on NH$_4^+$ and NO$_3^-$ concentrations, and [3] the microbial preference for NH$_4^+$ and NO$_3^-$ depending on the NH$_4^+/NO_3^-$ concentration ratio. Remineralization of microbial N occurred mainly at high growth rates and NH$_4^+$ concentrations, while intracellular recycling of N seemed to be an efficient way for bacteria to withstand low inorganic N concentrations. Thus, mobilization and remineralization of microbial N is likely to occur only when conditions promote high growth rates, i.e. when microorganisms are not substrate limited and temperature and moisture conditions are favourable. The results supported previous observations of high NO$_3^-$ immobilization rates, especially at low NH$_4^+$ concentrations, but NO$_3^-$ was also immobilized at high NH$_4^+$ concentrations. The latter can be understood if part of the microbial community has a preference for NO$_3^-$ over NH$_4^+$.

1. Introduction

Soil microorganisms are efficient scavengers of inorganic N, with immobilization rates sometimes even exceeding mineralization rates (Nadelhoffer et al., 1984; Giblin et al., 1991; Wagener and Schimel, 1998). Bacteria have the capacity to utilize both NH$_4^+$ and NO$_3^-$, but NO$_3^-$ needs to be reduced to NH$_4^+$ before it can be incorporated into amino acids (Jansson, 1958; Broadbent and Tyler, 1962; Myrold and Tiedje, 1986). The reduction of NO$_3^-$ to NH$_4^+$ requires energy, which is often limiting heterotrophic microorganisms in soils (Tate, 1995), making NH$_4^+$ the preferred N source for heterotrophic bacteria. However, the use of isotopic techniques to estimate gross rates of N turnover has illustrated the capacity of soil microorganisms to immobilize NO$_3^-$ even in the presence of a measurable NH$_4^+$ pool (Zak et al., 1990; Davidson et al. 1992; Stark and Hart, 1997; Kaye and Hart, 1998). Depletion of NH$_4^+$ at micro-sites within a heterogeneous soil has been proposed as an explanation (Schimel et al., 1989), but the conditions under which NO$_3^-$ immobilization occurs remain uncertain. Whereas the quantitative importance of the immobilization process is well recognized, the factors determining the rate of remineralization of microbial N are largely unknown. Since the bacterial biomass often has short turnover times (Joergensen et al., 1990; Hart et al., 1994; Blåth, 1998; Vance and Chapin, 2001), it is reasonable to expect that mobilization of N contained within the bacterial biomass is quantitatively as important as immobilization of inorganic N. Several mechanisms may be responsible for the mobilization. Bacteria accumulate solutes, many of which contain N, in the cytoplasm during osmotic upshift due to soil drying. When the soil is rewetted, rapid and extensive solute efflux occurs as a result of osmotic downshift (Wood, 1999) This gives a mechanistic explanation to the well-known phenomenon that rewetting of an air-dried soil results in a flush of C and N mineralization (Birch, 1958; Agarwal et al., 1971; van Gestel et al., 1991; Pulleman and Tietema, 1999; Fierer and Schimel, 2003). Predation may also be important in controlling remineralization of microbially bound N. For instance, high protozoan activity coincides with high mineralization and immobilization rates and
short turnover times of the microbial biomass (Christensen et al., 1996, Rønn et al., 2002). Similarly, the abundance of nematodes and the microbial activity are correlated (Mamilov and Dilly, 2002), and addition of earthworms to a soil decreases the total microbial biomass but increases its activity (Zhang et al., 2000).

The immobilization of N is largely dependent on the microbial biomass and activity. Accordingly, a high production and breakdown of RNA (Mason and Egli, 1993) and proteins accompanies bacterial growth, especially during exponential growth (Norris and Koch, 1972), and Therkildsen et al. (1997) showed that urea production in marine bacteria depended on the bacterial state of growth. Therefore, one may anticipate that the growth rate control of the intracellular turnover of N compounds is extended to the mobilization rates, either as a result of leakage of NH₃ or by excretion of metabolic waste products, such as urea.

We made a series of bioassays with soil bacteria suspended in an aqueous medium to test the influence of microbial growth rate on the remineralization of cellular N (hypothesis: remineralization positively dependent on growth rate), and the influence of different NH₄⁺ concentrations on NO₃⁻ immobilization (hypothesis: no NO₃⁻ is immobilized at high NH₄⁺ concentrations). The experiments were made in an aqueous environment to eliminate the influence of predation and osmotic variations on the mobilization rate, and the effect of micro-sites free of NH₄⁺ on the NO₃⁻ immobilization rate, which may have occurred in a soil.

2. Material and Methods

2.1. Extraction of bacteria

Bacteria were extracted from a deciduous forest soil (Dystric Cambisol) collected at Torup in southwestern Scania, the southernmost province in Sweden (55°33'N, 13°12'E), using the homogenization-extraction method described by Bååth (1992). A portion of soil (2.5 g wet weight) was homogenized with 200 ml of distilled water in a Sorvall Omnimixer at 80% of max speed for 1 min. The soil suspension was then centrifuged at 750 × g for 10 min at 5°C, after which the supernatant containing the bacteria was decanted through glass wool and then filtered through a 0.8 µm filter to remove predators. The filtrate was transferred to centrifuge tubes and centrifuged at 10000 × g for 10 min at 4°C on a Sorvall RC5B Plus. The supernatant was discarded and the pellet washed twice with the culture medium (see below).

2.2. Isolation and ¹⁵N enrichment of the bacteria

A bacterial culture was established in 1000 ml of artificial lake water (Lehman 1980). The lake water was enriched with glucose (3.0 µg C ml⁻¹, final concentration), phosphate (KH₂PO₄, 1.7 µg ml⁻¹, final concentration), and ¹⁵N-nitrogen (¹⁵NH₄Cl, 0.3 µg N ml⁻¹, final concentration), and the extracted bacteria inoculated to a density of 1.0×10⁵ bacteria ml⁻¹. The bacteria were then left to grow in the dark at 20°C on an orbital shaker. At the same time, another bacterial culture was prepared in the same way as above, but with ¹⁵N replaced by ¹⁴N. When the bacterial density in the cultures had reached 1.0×10⁷ bacteria ml⁻¹, 25 ml aliquots from the ¹⁴N and ¹⁵N cultures were transferred to centrifuge tubes and centrifuged at 10000 × g for 10 min at 4°C. The supernatant was discarded, the pellet washed twice with the lake water and resuspended in 25 ml of lake water.

2.3. Incubation and sampling

The suspended bacteria were inoculated to a density of 6.6×10³ bacteria ml⁻¹ into 500 ml polypropylene flasks containing 225 ml of lake water. One third of the flasks received ¹⁵N-enriched bacteria and the others bacteria grown on ¹⁴N. The cultures growing on ¹⁴N were divided into two subgroups, one receiving ¹⁵NH₄⁺ and ¹⁴NO₃⁻, and the other ¹⁴NH₄⁺ and ¹⁵NO₃⁻. The cultures with ¹⁵N-enriched bacteria received ¹⁴NH₄⁺ and ¹⁴NO₃⁻. Each of the three treatments (¹⁵N-enriched bacteria (87.7 atom%
15N excess), 15N-enriched NH4+ (10% 15N excess), and 15N-enriched NO3- (10%15N excess) was further divided into three subgroups, receiving 50, 100, or 250 ng NH4+-N ml−1, respectively. Nitrate was added as 100 ng NO3−-N ml−1, carbon as glucose at 3.0 µg C ml−1, and phosphate as K2HPO4 at 1.7 µg ml−1 to all groups. The cultures were left to grow in the dark at room temperature for 4 days. Samples for measurements of bacterial density were taken at the start of the experiment and every 24 hours. Four ml samples were fixed with formaldehyde at a final concentration of 2%.

2.4. Determination of 14N + 15N in NH4+, NO3−, and bacteria

Samples for measurements of 14N + 15N in NH4+ and NO3− were taken at the start of the experiment and after 48 and 96 hours. Bacteria were collected after 96 h for determination of bacranal 15N enrichment. Aliquots of 20 ml were centrifuged at 10000 × g for 10 min at 4°C. The supernatant was transferred to 20 ml scintillation vials and frozen, the pellet washed once with sterile media and dissolved in 10 ml of MilliQ water. The samples were kept frozen until analyzed.

The frozen supernatant was thawed in a refrigerator over night, and then 18 ml were filtered through a 0.2 µm polycarbonate filter to remove bacteria still remaining in the supernatant. The filtrate was collected in a 20 ml headspace vial. NH4+ and NO3− were diffused according standard IAEA procedures (IAEA, 2001) with minor modifications. Briefly, a standard office paper punch was used to cut out quartz filter discs that were then placed on a strip of PTFE tape and prepared with 10 µl of 2.5 M HCl. A second strip of tape was placed on top of the filter and the two tape strips were sealed by pressing the open end of a test tube, in a rocking circular motion around the filter, against the tape to create an NH4+-trap. The trap was then added to the headspace vial containing the supernatant. To prevent swelling of the trap due to inward diffusion of water vapor, KCl was added to a final concentration of 1.0 M, followed by 0.7 g of MgO. The headspace vials were sealed and left at 35 °C with periodic shaking by hand. After 48 hours, the trap was removed, and the filter was placed in a 5×8 mm tin cup and left to dry in a desiccator. The serum bottle was left open for 48 hours to release residues of ammonia. A new trap was added to the bottle followed by 0.7 g of Devarda’s alloy and 0.7 g of MgO, and the incubation was repeated. The 15N isotopic excess in bacteria after 96 h was determined by filtering the dissolved bacteria through a GF/F filter (diameter 25 mm). Five smaller discs were then cut out from the filter with a punch (diameter 5 mm), placed in a 5×8 mm tin cup, and left to dry in the desiccator.

15N and 14N in the filters were determined using continuous flow isotope ratio mass spectrometry (CF-IRMS). The filters were oxidized in an ANCA-GSL elemental analyzer and NO3− reduced to N2 which was passed to a 20-20 IRMS (PDZ Europa UK). The amount of NH4+-N and 15NH4+-N, NO3−-N and 15NO3−-N was quantified after subtraction of blank values and calibration against filter discs which had received known amounts of N as NH4Cl (calibrated against an IAEA KNO3 standard) dissolved in 5 µl of water.

2.5. Estimation of N transformation rates

Nitrogen transformation rates were calculated with the FLUAZ-model (Mary et al., 1998). The calculations are based on the isotopic dilution and isotopic enrichment principles (Monaghan and Barraclough, 1995). It uses a numerical 4th order Runge-Kutta algorithm with a variable time-step to solve the differential system, and a non-linear fitting program (based on Marquardt’s algorithm) to calculate the unknown N transformation rates between the NH4+, NO3−, organic N and biomass N pools. The gross remineralization rate and the gross NH4+ and NO3− immobilization rates were calculated from the measurements of the amounts and 15N isotopic excesses of NH4+ and NO3−. The FLUAZ model minimizes the quadratic weighted error rather than the sum of squares and therefore requires the average value plus the coefficient of variation for the measured variables as
Table 1. The generation time (days), NH$_4^+$ and NO$_3^-$ immobilization, remineralization, and nitrification rates during the experiment at the three different initial NH$_4^+$ concentrations (ng N ml$^{-1}$). All rates are expressed as ng N ml$^{-1}$ d$^{-1}$.

<table>
<thead>
<tr>
<th>Days</th>
<th>NH$_4^+$N</th>
<th>Generation time</th>
<th>NH$_4^+$ immobilization</th>
<th>NO$_3^-$ immobilization</th>
<th>Remineralization</th>
<th>Nitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>250</td>
<td>0.247</td>
<td>95.7</td>
<td>4.28</td>
<td>14.3</td>
<td>20.15</td>
</tr>
<tr>
<td>3-4</td>
<td>1.266</td>
<td>65.6</td>
<td>33.3</td>
<td>7.8</td>
<td>3.53</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>100</td>
<td>0.251</td>
<td>34.6</td>
<td>3.22</td>
<td>0.31</td>
<td>12.26</td>
</tr>
<tr>
<td>3-4</td>
<td>1.417</td>
<td>0.11</td>
<td>37.4</td>
<td>0.02</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>50</td>
<td>0.263</td>
<td>18.87</td>
<td>0.00</td>
<td>0.06</td>
<td>11.46</td>
</tr>
<tr>
<td>3-4</td>
<td>2.129</td>
<td>0.75</td>
<td>67.0</td>
<td>0.24</td>
<td>3.57</td>
<td></td>
</tr>
</tbody>
</table>

The estimated rates from the $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$ enrichment experiments were validated by simulating the $^{15}$N isotopic excess in NH$_4^+$, NO$_3^-$, and biomass pools for given N transformation rates (Mary et al., 1998). The initial $^{15}$N isotopic excess in NH$_4^+$, NO$_3^-$, and bacteria was taken from the treatment with $^{15}$N labelled bacteria, and the dilution of bacterial $^{15}$N during the experiment was simulated from the estimated gross remineralization rate and the gross NH$_4^+$ and NO$_3^-$ immobilization rates in the $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$ enrichment experiments. The simulated $^{15}$N isotopic excess in bacteria day 4 was then compared to the measured $^{15}$N isotopic excess day 4.

3. Results

The remineralization rates of immobilized N were low, especially at the two lowest initial NH$_4^+$ concentrations (Table 1), indicating an efficient intracellular recycling of N in the bacteria. Remineralization and NH$_4^+$ immobilization were positively correlated (Table 2), supporting our hypothesis, but the fraction of the immobilized N that was remineralized was independent on the NH$_4^+$ immobilization rate (Table 2), probably...
because of the low ratio of remineralization to NH$_4^+$ immobilization day 1-2 at the lowest initial NH$_4^+$ concentration. The remineralization rate was positively related to the growth rate day 1-2 (Table 1). It was also highest in the culture with the highest growth rate day 3-4. The combination of a positive correlation between NH$_4^+$ immobilization and remineralization and the influence of the growth rate on remineralization day 1-2 supports our first hypothesis, that the remineralization rate would be dependent on the growth rate.

The immobilization of NH$_4^+$ and NO$_3^-$ followed the same general pattern at all three initial NH$_4^+$ concentrations, with high rates of NH$_4^+$ immobilization between the first two days of the incubation, followed by increased NO$_3^-$ immobilization between day 3 and 4 as the NH$_4^+$ concentrations decreased (Table 1). The NH$_4^+$ immobilization rate was linearly related to the initial NH$_4^+$ concentration during the exponential growth phase (day 1-2, Fig. 1), with the highest rates at the highest initial concentration (Fig. 2). Similarly, the NO$_3^-$ immobilization rate during day 1-2 was also highest at the highest initial NH$_4^+$ concentration (Table 1), contradictory to our hypothesis that NO$_3^-$ immobilization would not occur at high NH$_4^+$ concentrations. However, the NO$_3^-$ immobilization rate day 1-2 was low compared to day 3-4, when NH$_4^+$ became depleted.

Two main patterns of nitrification emerged from the data. First, the highest nitrification rates coincided with the highest initial NH$_4^+$ concentrations and the exponential growth phase (Fig. 1, Table 1). The nitrification rates decreased during day 3-4 (Table 1), at the same time as the rate of NO$_3^-$ immobilization became quantitatively more important than the NH$_4^+$ immobilization, that is, the bacteria seemed to replace NH$_4^+$ with NO$_3^-$ as a N source (Table 1). Second, the ratio of nitrification to NH$_4^+$ immobilization was negatively correlated to the NH$_4^+$ immobilization (Table 2), indicating that the relative fate of NH$_4^+$ shifted from nitrification to NH$_4^+$ immobilization with increasing bacterial growth and NH$_4^+$ immobilization rates.

The FLUAZ model accurately simulated the dilution of $^{15}$N in labelled cells during the experiment for the given NH$_4^+$ immobilization, NO$_3^-$ immobilization, and remineralization rates from the $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$ treatments. The predicted $^{15}$N in the labelled cells day 4 was inside the 95% confidence interval of the measured $^{15}$N enrichment in all treatments, indicating that the FLUAZ model calculated the rates accurately (Fig. 3). The simulations were less precise at low initial NH$_4^+$ concentrations, probably since the precision of the quantification and isotopic determination by IRMS decreased at low N concentrations.

4. Discussion

Remineralization of microbial N appears to occur mainly at high growth rates and NH$_4^+$ concentrations. Two different mechanisms may account for the high remineralization rates on...
Fig. 1. The growth of bacteria at the three different initial NH\textsubscript{4}\textsuperscript{+} concentrations. Error bars represent the standard error of the mean.

Fig. 2. The NH\textsubscript{4}\textsuperscript{+} immobilization rates day 1-2 at the three different initial NH\textsubscript{4}\textsuperscript{+} concentrations.

day 1-2 and day 3-4, respectively, in the treatment with the highest initial NH\textsubscript{4}\textsuperscript{+} concentration. The turnover rate of intracellular mRNA is high during exponential growth (Norris and Koch 1972), but most of the C and N are used to synthesize new RNA and proteins and the excretion and remineralization of N compounds become low, as in the cultures with low initial NH\textsubscript{4}\textsuperscript{+} concentrations. An unbalanced C to N supply enhances mRNA degradation (Mason and Egli, 1993), and while mRNA C is recycled, the excess N is excreted. The dependence of remineralization on the carbon supply was evident from the substrate C:N ratio in the treatments. The treatment with the highest initial NH\textsubscript{4}\textsuperscript{+} concentrations and the high remineralization rate had the lowest initial C:N ratio, <10, while the two treatments with lower remineralization had initial C:N ratios of 15 and 20. Bacteria with an average C:N ratio of 6 and a C use efficiency of 50% are assumed to be C limited at C:N ratios <12 (Tate, 1995).

The mechanism responsible for the high remineralization rate day 3-4 may be that bacterial strains with a high growth rate and increased efficiency of translation proliferated in the rich environment. The payoff for high rates of reproduction is a reduced ability to withstand starvation (Kurland and Mikkola, 1993). When substrates became depleted and the fast growing strains growth-arrested, oxidation of proteins may have lead to proteolysis (Nyström, 2002).

Our results add another piece of evidence to the observations that soil microorganisms immobilize NO\textsubscript{3}\textsuperscript{-} even in the presence of a measurable NH\textsubscript{4}\textsuperscript{+} pool (Zak et al., 1990; Davidson et al. 1992; Stark and Hart, 1997), and that NH\textsubscript{4}\textsuperscript{+} depletion at micro-sites within a soil is not a prerequisite for NO\textsubscript{3}\textsuperscript{-} immobilization (Schimel et al., 1989). These results may also have implications for the interpretation of a potential competition for N between plants and microorganisms (Kaye and Hart, 1997). One requirement for competition is that the competitors use the same sources of N. Plants seem to have different preferences for NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} both within and between habitats (Högblom and Ohlson, 1991; Falkengren-Grerup and Lakkenborg-Kristensen, 1994; Falkengren-Grerup, 1995), while heterotrophic microorganisms are considered to prefer NH\textsubscript{4}\textsuperscript{+} and immobilize NO\textsubscript{3}\textsuperscript{-} only under N limited conditions (Jansson, 1958; Broadbent and Tyler, 1962; Myrold and Tiedje, 1986). Our results suggest that this may not be the case. Some strains seem to express phenotypic plasticity in the uptake of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}, depending on the concentration, but others immobilize NO\textsubscript{3}\textsuperscript{-} even at high NH\textsubscript{4}\textsuperscript{+} concentrations. This may follow from a selection for strains with high capacity and affinity for NO\textsubscript{3}\textsuperscript{-}, independent of NH\textsubscript{4}\textsuperscript{+}.
concentrations, and those strains may compete with plants preferring NO$_3^-$ even at relatively high NH$_4^+$ concentrations.

The observation that the ratio between nitrification and NH$_4^+$ immobilization decreased with increasing NH$_4^+$ immobilization rates supports our results from in situ estimations of nitrification and NH$_4^+$ immobilization rates (Paper III). A larger fraction of NH$_4^+$ was available to nitrifiers when the heterotrophic activity was low, as in Hart et al. (1994) and Zak et al. (1990), probably since nitrifiers are inferior competitors to heterotrophic microorganisms for NH$_4^+$ (Zak et al., 1990; Verhagen and Laanbroek, 1991; Verhagen et al., 1995). Substantial amounts of NH$_4^+$ were nitrified at the beginning of the incubation at all three NH$_4^+$ concentrations. The efficient intracellular recycling of N in the treatments with low initial NH$_4^+$ concentrations meant that nitrification as well as NH$_4^+$ immobilization ceased as NH$_4^+$ was depleted. However, while NH$_4^+$ was still immobilized during day 3 and 4 in the treatment with the highest initial NH$_4^+$ concentration, nitrification decreased, reflecting higher heterotrophic biomass yields compared with autotrophic. Considerable amounts of NH$_4^+$ were nitrified as long as the heterotrophic biomass was low and the NH$_4^+$ concentrations sufficiently high to support the N demand of both heterotrophs and the nitrifiers, but as the biomass of the heterotrophs increased and the NH$_4^+$ concentrations dropped, nitrification became insignificant.

In conclusion, bacteria seem to have a high ability to recycle N intracellularly, especially at low N concentrations and growth rates. Remineralization of microbial N by other processes than predation or soil drying/rewetting is likely to occur only when conditions promote high growth rates, i.e. when microorganisms are not substrate limited and temperature and moisture conditions are favourable. The results support previous observations of high NO$_3^-$ immobilization rates by soil microbial communities, especially at low NH$_4^+$ concentrations, and add new evidence for NO$_3^-$ immobilization carried out by a fraction of the microbial community even at high NH$_4^+$ concentrations.

Acknowledgements

This work was sponsored by grants from the Foundation for Strategic Research in Sweden.
References


The following is a list of Doctoral theses from the Department of Chemical Ecology and Ecotoxicology, Lund University, Sweden

9. PER WOIN, Xenobiotics in aquatic ecosystems: effects at different levels of organization. December 15, 1995.
10. GÖRAN EWALD, Role of lipids in the fate of organochlorine compounds in aquatic ecosystems. October 18, 1996.
18. HELENA BJÖRN, Uptake, turnover and distribution of chlorinated fatty acids in aquatic biota. October 1, 1999.