University of South Bohemia in České Budějovice

Faculty of Science

The effect of forest management and plant dominant on nitrogen transformation in soils of acidified mountain spruce forests in the Bohemian Forest National Park

Master thesis

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České Budějovice 2013

Staňková, P. 2013: The effect of forest management and plant dominant on nitrogen transformation in soils of acidified mountain spruce forests in the Bohemian Forest National Park. Mgr. Thesis, in English. – 74 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation: the aim of this study was to compare and contrast mineral and microbial nitrogen concentrations in soils of semi-natural and natural mountain spruce forests in the area of Březník, the Bohemian Forest National Park, under dead wood and four plant dominants with respect to different forest management after windstorm and bark beetle events applied in 1997.

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České Budějovice, prosinec 2013
Bc. Pavla Staňková

"Accept that some days you're the pigeon, and some days you're the statue."

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List of abbreviations

- AAs…………………….amino acids
- Bo………………….......bilberry *Vaccinium myrtillus* (dominant)
- C……………………….carbon
- CEC……………………cation-exchange capacity
- C_{ext}……………………..extractable carbon in soil
- CFE…………………….Chloroform Fumigation Extraction
- C_{mic}……………………..carbon in microbial biomass
- C_{tot}………………………total soil carbon
- D……………………..…dead wood (dominant)
- DON……………………dissolved organic nitrogen
- DW……………………..dry weight of the soil
- FIA……………………...Flow Injection Analyzer
- N…………………….…nitrogen
- N_{ext}…………………...…extractable nitrogen in soil
- N_{mic}……………………..nitrogen in microbial biomass
- N_{tot}………………………total soil nitrogen
- M……………………..…*Avenella flexuosa* (dominant)
- Mch……………………..…moss (dominant)
- P (P2, P3, P5)...………...clear-cut (P) plots; phosphorus
- S (S3, S5, S7)………….....non-intervention plots (S); sulphur
- T………………………...*Calamagrostis villosa* (dominant)

1 GENERAL INTRODUCTION

Since the development of our society, people keep on changing their environment to make the most of it. Through our activities, such as agriculture, industry, transport, extraction of different natural sources, urbanisation or forestry, we affect all components of the global ecosystem – water, air, soil and other living organisms. In the Central Europe it is almost impossible to find a place untouched by people that has preserved its unique plant and animal communities. However, there are still places that can be called natural or at least semi-natural. Usually they can be found in harsher conditions and in places that are not very accessible. In better cases, these areas are being protected and cherished for their biological and historical value. One of these places is the Bohemian Forest Mountain Range located at the border of the Czech Republic with Germany and Austria in the southwestern part of our country. This area is being protected as part of the Bohemian Forest National Park. The valuable parts can be found in higher or moist areas where semi-natural Norway spruce stands and peatbogs have evolved.

It is believed that natural disturbances (windstorms, pest attacks, fire) are an integral part of spruce forests and that they are necessary for restoration of the dynamic equlibrium forest (Jonášová and Prach, 2004; Jonášová and Matějková, 2007; Müller et al., 2008; Šantrůčková et al., 2010; Svoboda et al., 2010). Natural ecosystems are capable of facing these abrupt changes. Forest stands established and treated by people, on the other hand, tend to be more susceptible to the disturbances (Fanta, 1997; Dobrovolný and Brázdil, 2003; Wermelinger, 2004).

In the last two decades, spruce forests are subjected to large disturbances by wind and bark beetle attacks. The large-scale dieback of Norway spruce stands was triggered by a combination of more factors. Mild winters together with warm summers facilitated the reproduction of the bark beetle (*Ips typographus*) (Jonášová and Prach, 2004). The effects of the bark beetle outbreak have been enhanced by the fact that since the 1950s forest have been exposed to acid depositions of nitrogen and sulphur from industrial and agricultural activities and from transport (Kopáček et al., 2001). These deposition affected plant nutrition and soil chemistry and resulted in an increased flux of nitrates and sulphates to ground water, which was followed by soil acidification and increased base cations leaching (most importantly that of Ca and Mg) (Schulze, 1989; Jonard et al., 2012). Even after measures have been adopted to decrease these emissions, soils and waters still exhibit acidification. Acidification of already acid soils causes further loss of nutrients and mobilization of toxic

forms of aluminium, which is otherwise insoluble (Hruška and Cienciala, 2005; Šantrůčková et al., 2010). The occurrence of storm events completes this vicious circle of adverse factors contributing to dieback of the forest stands. The changes in soil properties affect the microbial communities and thus also biochemical transformations and availability of nutrients in soil. Among the most affected nutrients is nitrogen whose cycle is interconnected with other nutrients' cycles (Chapin et al., 2002).

The best and most appropriate way how to cope with bark beetle outbreaks and its damages in forests is still a subject of debate. After the bark beetle outbreak in 1990s, damaged forest stands were treated in two ways. Trees were cut down and removed following the traditional procedure in commercial forests. In the core zones, on the other hand, forest stands were left without any intervention. Since then an extensive research has been conducted in several parts of the Bohemian Forest Mountain Range, e.g. in the central part in the area of Březník (Jonášová and Prach, 2004, 2008) or in the watersheds of mountain glacier lakes - the Plešné, Čertovo and Černé Lake (Kopáček et al., 2002a, 2002b).

This diploma thesis deals with the effect of forest dieback and consequent management in damaged forest stands on biochemical processes in soil focusing on the nitrogen cycle. We assessed and compared the organic and inorganic pools and net processes of the nitrogen cycle in the area of Březník. The results can be used for future forest research and for decision making on the proper management practices.

2   REVIEW

2.1   General characteristics of the nitrogen cycle

Nitrogen (N) is, together with other biogenic macroelements (hydrogen, oxygen, carbon, phosphorus and sulfur), an essential structural component of all organisms on the Earth. It accounts for more than 6% of their dry mass on average (Bothe et al., 2007). Living organisms need nitrogen to sustain their growth and reproduction, for it is present in nucleic acids, amino acids and proteins. Moreover, for microorganisms nitrogen forms can serve as electron donors or acceptors in energy metabolism. Microorganisms are a driving force of N transformations on the Earth.

2.1.1   Forms and pools of nitrogen

Nitrogen can be present in many inorganic and organic forms and its cycle is very complex. This complexity results from the chemical features of nitrogen. Having five valence electrons, it can undergo a variety of oxidations and reductions. Its oxidation states range from –III (e.g., in ammonia NH_3) to +V (e.g., in nitrate NO_3^-). Thus N forms can be used in energy metabolism as well as in assimilation processes. The vast majority of the oxidation-reduction reactions are mediated by prokaryotic organisms (such as bacteria or archaea) (Schimel, 2001).

The largest pool of nitrogen is the *atmosphere* (Schlesinger, 1997). Nitrogen in the form of dinitrogen molecule (N_2) makes up about 79% of the Earth's atmosphere. This gaseous compound is very stable for the huge amount of energy is needed to break up the triple bond. Next to N_2 molecule, nitrogen oxides (NO_x – nitrous oxide N_2O, nitric oxide NO, and nitrogen dioxide NO_2) and reduced nitrogen (ammonia NH_3 or various organic compounds) are present in the air. NO_x can be either free or associated with liquid or solid particles. *Water* may contain all nitrogen-containing gases in solution, as well as low concentrations of urea, ammonia and organic compounds with low molecular mass (Sprent, 1987).

Most of the nitrogen in terrestrial ecosystems is found in the soil. *Soil*, as an interface of bio-, hydro-, and atmosphere, can contain all forms of nitrogen (organic, inorganic, reduced, oxidized, even N_2 bound in rocks). Some forms (ammonium NH_4^+) can be bound to soil particles, while the others (e. g., nitrates NO_3^-) are subjected to leaching (Sprent, 1987; Brady and Weil, 2002).

Soil organic nitrogen typically accounts for 5% of the soil organic matter (Brady and Weil, 2002). It includes a huge variety of compounds. Only about half of them can be isolated and identified (proteins, amino and nucleic acids, polymers of the cell wall, amino sugars, antibiotics, *etc*.), whereas the other half is of an unknown chemical composition. Therefore, soil organic nitrogen is often characterized and divided into several forms via fractionation. This procedure is based on acid hydrolysis and the forms of nitrogen include: acid insoluble-N, ammonia-N, amino acid-N, amino-sugar-N and hydrolyzable unknown-N. These forms typically account in soils for 10-20%, 20-35%, 30-45%, 5-10% and 10-20%, respectively (Myrold, 2005).

The term soluble organic nitrogen (SON) or dissolved organic nitrogen (DON) comprises organic nitrogen compounds of various chemical characteristics (hydrophobic and hydrophilic) that are soluble enough and thus can be taken up by plants or leached from the soil. DON is defined as difference between total dissolved nitrogen and dissolved inorganic nitrogen (nitrate-N, ammonia-N and nitrite-N). This pool accounts for approximately 0.3 to 1.5% of the total organic nitrogen in soils (Brady and Weil, 2002). Studies in unpolluted forest ecosystems show that DON can be the major form of nitrogen lost to groundwater and surface waters (Perakis and Hedin, 2002). The importance of DON lies in the fact that, next to ammonia and nitrates, it is now widely recognized as another source of N for plants (Brady and Weil, 2002). On the other hand, DON can be leached into water and contribute to environmental problems, such as eutrophication and acidification of streams (van Kessel et al., 2009) or estuaries (Seitzinger and Sanders, 1997). Moreover, DON can be of an anthropogenic origin and represent the dominant form of N lost to the waters (Kroeger et al., 2006).

On a global scale, inorganic N seldom accounts for more than 1 to 2% of the total nitrogen in soil (except the systems where chemical fertilizers are applied) (Brady and Weil, 2002). This pool is very dynamic and may turn over within a day. In general, smaller pools of nitrogen tend to have a short residence time and to turn over more quickly than the larger pools, such as highly stable atmospheric dinitrogen (Myrold, 2005).

2.1.2 *Processes of the nitrogen cycle*

Nitrogen cycling involves several processes where various microorganisms play an important role. Nitrogen is being used in assimilation processes as well as in energy metabolism. Processes connected with N assimilation are *nitrogen fixation*, *ammonification*,

and *assimilatory N transformation.* Processes connected with energy metabolism are *nitrification, denitrification* and *Anammox* (Bothe et al., 2007)*.*

About 60% of the fixed N comes from **biological fixation** (Newton, 2007). However, N fixation can occur also without the presence of microorganisms, e.g. during thunderstorms (nitrogen and oxygen are combined) or in industry in the Haber-Bosch process where ammonia is produced from N_2 and H_2 under pressure and at high temperatures (Sprent, 1987).

Nitrogen mineralization (ammonification and **nitrification)** stands for reactions that change organic-N compounds into mineral forms of available N. **Ammonification** and **nitrification** are two key processes in the global nitrogen cycle; they link the organic matter decomposition with other processes of N transformation as well as the nitrogen assimilation processes with energy metabolism. In the first step, organic compounds containing N are converted to ammonium. Complex polymers are broken down by extracellular enzymes (proteinases, proteases and deaminases) to monomers and ammonium. The smaller organic compounds can go through the cell membrane and are further metabolized within the microbial cells to ammonium. Several enzymes and processes are involved based on the compound being broken down (amino acids are deaminated by amino-acid dehydrogenases and oxidases, amino sugars are first phosphorylated and then deaminated, nucleotides hydrolyzed to nucleosides, dephosphorylated and hydrolyzed to purines and pyrimidines that are catabolized to ammonium) (Myrold, 2005). The excess of ammonium that is not used is then released from the cell.

Ammonium is then in nitrification (generally autotrophic) oxidized to nitrites in the first step, and then to nitrates. The number of species involved in chemoautotrophic nitrification is quite restricted to groups of proteobacteria. They comprise genera such as *Nitrosomonas, Nitrosospira* and *Nitrosococcus* (NH_3 oxidizers), and *Nitrobacter, Nitrococcus* and *Nitrospina* (NO_2^- oxidizers) (Myrold, 2005; Prosser, 2007). They thrive in alkaline or slightly acid soils. In acid soils, however, genera of heterotrophic nitrifiers are involved in the process (Tamm, 1991). They can either belong to bacteria (*Alcaligenes, Arthrobacter*, some actinomycetes) or to fungi (*Aspergillus*). Autotrophic nitrifiers gain energy from these processes (ammonium is an electron donor), heterotrophic nitrifiers don't (Myrold, 2005).

The process opposite to mineralization is **immobilization (assimilation).** Inorganic forms of N are converted into organic forms that are then incorporated (assimilated) in the microbial biomass (Brady and Weil, 2002). For instance, NO_3^- is incorporated into the cells

and further transformed and used as an N source for growth by many bacteria, fungi, algae and plants. For NO_3^- assimilation, specific uptake systems are needed. Bacteria possess two types of proteinaceous transporters – an ABC-type transporter or an MFS-permease, both located in the cytoplasmic membrane. Assimilation is usually regulated by two processes – by induction of NO_3^- and/or NO_2^-, or by repression of the assimilation in the presence of NH_4^+ (Moreno-Vivián and Flores, 2007). Nitrate is commonly taken up by plant roots, even by the plants that have never been in contact with it before. It can be stored in the vacuoles or transported to the shoots for reduction. Nitrate is reported to be involved in osmoregulation, gene regulation and very likely in cytokinin production (Tischner and Kaiser, 2007).

There are several reports on significant abiotic immobilization of N (Johnson et al. 2000; Dail et al., 2001, *etc*.). As a reaction on these reports, Davidson et al. (2003) proposed a mechanism called the Ferrous Wheel Hypothesis. Nitrate is reduced to nitrite (catalysed by iron or perhaps manganese), which further reacts with dissolved organic matter to DON. They claim that this chain of reactions may occur within seconds after nitrates enter the soil solution (Davidson et al., 2003). Conversely, results of Schmidt and Matzner (2009) suggest that the reaction of nitrite and soil organic matter is rather unlikely to occur and that the hypothesis needs revision (Schmidt and Matzner, 2009).

Nitrogen mineralization and immobilization occur simultaneously in the soil. The dynamics are dependent above all on the C/N ratio of the organic material (Brady and Weil, 2002). The critical ratio value (determining whether N is mineralized or immobilized) is estimated on around 20 or 25. Basically, higher values indicate that microorganisms have to take up more C to satisfy their nutrition demands and immobilization prevails. At values of C/N ratio lower than about 20 to 25, net mineralization exceeds immobilization and the surplus N may be released and then subjected to leaching (Paul and Clark, 1996; Hodge et al., 2000; Myrold, 2005).

Mineralization (ammonification and nitrification) can be measured as a change of ammonium and nitrate concentration over certain time period. This is called the *net mineralization*. It can be assessed with the use of traditional chemical methods based on extraction and spectrophotometric analysis of nitrates and ammonium. A disadvantage of this method is that during incubation part of mineral N produced by ammonification and nitrification is consumed (assimilated) by microbes. The proportion of consumed N is highly variable and does not depend on the rate of measured processes. To get information about N mineralization, gross rates of ammonification and nitrification must be determined. *Gross*

nitrogen ammonification and *gross nitrogen nitrification* give us information about the processes of biological transformation of organic N to ammonium and of ammonium to nitrate, respectively. Gross rates of both processes are determined using ^{15}N isotope dilution method (Myrold, 2005).

Nitrogen is also used in energetic metabolism of microorganisms. Next to nitrification, which was mentioned in connection to ammonification, other nitrogen transformations involved in energy metabolisms of microorganisms are denitrification and Annamox.

Denitrification is defined as the dissimilatory reduction of nitrate to N_2O and N_2 by microbes-mediated reactions. It is a form of respiration in which nitrates or nitrites are used as electron acceptors. It consists of four reactions catalyzed by nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase. All these enzymes function in the absence of oxygen. However, some bacteria are able to use oxygen and nitrate as an electron acceptor at the same time (Tamm, 1991; van Spanning et al., 2007). Denitrifying bacteria are quite a diverse group of organisms comprising organotrophs (*Alcaligenes, Azospirillum, Bacillus, Halobacterium, Pseudomonas, Rhizobium, etc.*), phototrophs (*Rhodopseudomonas*), and lithotrophs (*Bradyrhizobium, Nitrosomonas, Paracoccus, Thiobacillus, etc.*) (Myrold, 2005).

Anammox process is defined as anaerobic oxidation of ammonium to dinitrogen molecule. This reaction has been discovered quite recently. However, its importance grows with the fact that it can be applied in wastewater treatment (Strous et al., 1997; Fux et al., 2002; Op den Camp et al., 2007). Anammox bacteria are known to inhabit marine water column (Dalsgaard et al., 2005) as well as a wide range of different soil habitats (Humbert et al., 2010).

2.1.3 *Factors affecting the processes of the nitrogen cycle*

When considering the N cycling we have to keep in mind the complexity and interconnected nature of all processes and agents involved. Low concentrations or even absence of one form may be a result of high rate of immobilization on one hand, or of limited/restricted production on the other hand.

Booth et al. (2005) gathered and processed data on soil characteristics and gross rates of N ammonification and nitrification from about 100 studies and made a synthesis on the controlling factors of N cycling in terrestrial ecosystems. They highlight the role of the soil organic matter, particularly of both C and N concentrations in soil and the C/N ratio, as

indicators of substrate quantity and quality, respectively. These substrate properties affect not only N mineralization rates, which appear to be positively correlated with microbial biomass and soil C and N concentrations, but the whole fate of mineralized N (Booth et al., 2005). Part of the mineral N is consumed (incorporated into microbial biomass). The surplus of mineral N forms in soil can be thus either a result of high N mineralization rates or of low N assimilation rates. Systems rich in organic C have usually higher capacities in N assimilation and accumulation and thus less NO_3^- is released compared to those with low organic C pools (Evans et al., 2006). Similarly, Tahovská et al. (2013) give evidence of the importance of C availability in microbial nitrate immobilization in N saturated forests as a mechanism of nitrate leaching prevention. C limitation could be, thus, the possible controlling factor in explaining the observed differences in some N-saturated ecosystems and their susceptibility to nitrate leaching (Tahovská et al., 2013). In other words, nitrate concentration shows a consistent and negative nonlinear correlation with C availability (dissolved organic C). When C/N ratio decreases, C limitation (N saturation) of microbial metabolism occurs. This leads to enhanced NH_4^+ availability resulting in nitrification. Nitrates are in excess and heterotrophic microorganisms are not able to maintain low nitrate concentrations (Stark and Hart, 1997; Taylor and Townsend, 2011).

2.1.4 A new concept of the nitrogen cycle

According to the traditional concepts of N transformations in soil the key role is attributed to N mineralization with ammonium being the crucial form of N (Aber et al., 1989 and 1998). Schimel and Bennett (2004) present a new model of N cycling in soil and highlight the process of **depolymerization** being the main reaction driving the N cycle. Nitrogen-containing organic material in soils includes mostly plant and microbial residues that consist of peptides and proteins and of other structurally complex compounds. These N-containing polymers are converted to monomers that can be used by microbes and plants. The authors stress the role of micro-sites in soil, where different processes of the N cycle may dominate and thus affect the N dynamics in the soil. This model may also explain much of the observed variation in the N cycling (Schimel and Bennett, 2004).

The main differences between classical and new paradigm on N cycle are described in Figure 1 (Schimel and Bennett, 2004). They are reflected in the processes considered to be the crucial in the cycle – net mineralization in the case of the classical paradigm (Aber et al., 1998), depolymerization in the case of the new paradigm (Schimel and Bennett, 2004). The concepts also diverge in the forms of N the plants are capable of taking up. The classical

paradigm strictly distinguishes between microbial decomposition and plant uptake. Plants only take up N in mineral forms (Aber et al., 1989 and 1998). The new paradigm counts with the ability of plants to take up also organic forms of N (Schimel and Bennett, 2004).

According to the new paradigm, ecosystems exist along an N-availability gradient. N availability affects the ongoing processes and leads to shifts in the N form plants are dependent on. In low-N systems, where N-cycling and decomposition are slow, plants and microbes compete for
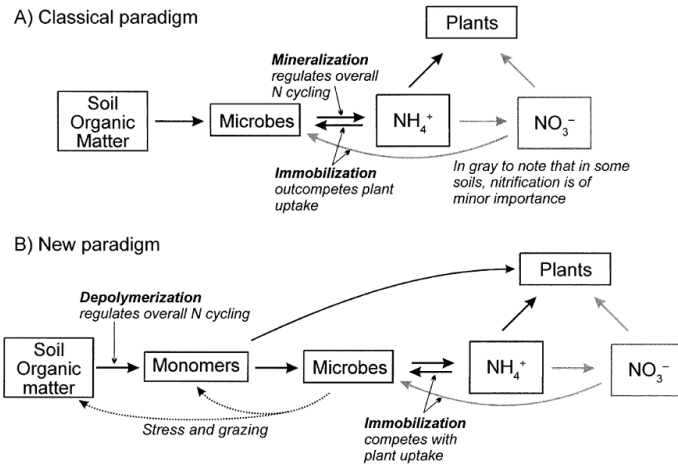
Figure 1. Comparison of the classical N saturation model (A) with the new paradigm (B). Taken from Schimel and Bennett (2004).

organic N-monomers. As decomposition increases, N becomes more available to organisms and microbes start to mineralize the soil organic matter to NH_4^+. Ammonia is transferred to N-limited micro-sites and is immobilized by plants and microbes. Further on, the competition between plants and microbes decreases, mineral N starts to dominate and the system is N-saturated (Schimel and Bennett, 2004). At this point, the classical view on the N cycle (as illustrated by Aber et al., 1989 and 1998) can be the case. However, even in N-saturated soils, plants are able to take up organic N (Schimel and Bennett, 2004). The concept of soil heterogeneity creating numerous micro-sites with different conditions was further used by Geisseler et al. (2010) in their conceptual model of two pathways of N utilization by microorganisms (Geisseler et al., 2010). This model is presented in the Chapter 2.1.6.2.

2.1.5 The concepts of nitrogen saturation

With connection to the increased N deposition to natural and semi-natural ecosystems, the concept of N saturation became increasingly important when interpreting N cycling and its changes.

For a long time the classical model of N saturation proposed by Aber and his colleagues (1989 and 1998) has been taken for valid. Nitrogen saturation was described as "the availability of ammonium and nitrate in excess of total combined plant and microbial nutritional demand" (Aber et al., 1989). In this model, ecosystem responds to higher N

inputs at several stages from N-limiting to N-saturated conditions. First, as a reaction to higher loads, N becomes more available to plants. They incorporate it into their biomass and thus decrease the C/N ratio of their litter. N mineralization and nitrification accelerates due to litter-N enrichment of the upper parts of the soil profile. When the demands of vegetation for N are met, nitrates are in excess, which leads to nitrate leaching in the last stage of ecosystem N saturation. The most important role in this model belongs to N mineralization and nitrification, whereas microbial immobilizati-on of nitrogen is of minor importance (Aber et al., 1998).

As a result of a long-term N addition experiment in an oak forest, Lovett and Goodale (2011) presented a new conceptual model of N saturation (see Figure 2). This model focuses on the mass balance which is characterized by N inputs (deposition and fertilization), internal sinks (vegetation and soil) and outputs (nitrate leaching and volatilization of N-containing gases). One of the key points of
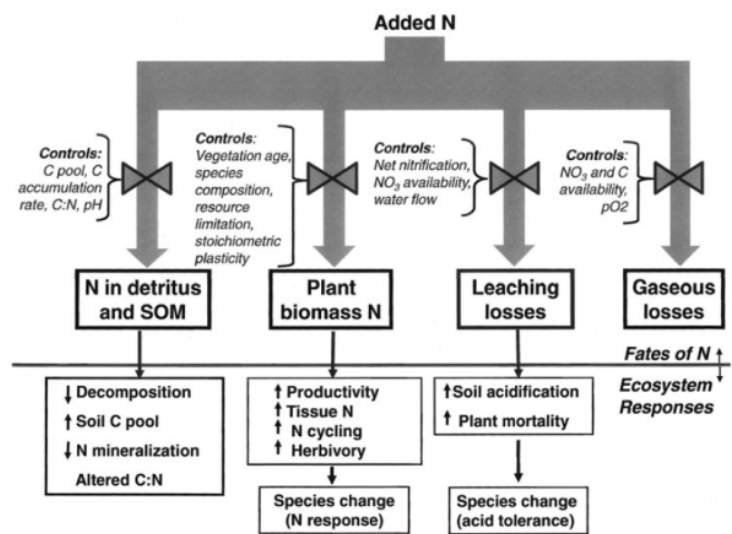
Figure 2. Conceptual model as presented by Lovett and Goodale (2011). The picture illustrates the N flow to all possible sinks, together with controlling factors and ecosystem responses to the flow of added N.

this model is that added N can flow to all sinks at the same time and that these sinks do not have to reach their saturation capacity. Further, the flow of N through these sinks and its final fate depends on the strength of the sinks, which in turn determines how the effects of N saturation are displayed in the ecosystem (Lovett and Goodale, 2011). The weakness of this model is that mineral N assimilation into microbial biomass is (like in Aber et al., 1998) neglected (Lovett and Goodale, 2011). However, this pathway of N immobilization was shown to be common in some ecosystems (Booth et al., 2005).

Stark and Hart (1997), on the other hand, drew attention to the importance of microbial assimilation of mineral N. They focused on 11 undisturbed forest ecosystems and found out that, despite the low soil pH, low N availability and depositions, the rates of nitrification were high. Surprisingly, nitrate concentrations were low in most cases. The isotopic measurements showed that the vast majority of nitrates produced were assimilated

into microbial biomass. The authors proved that soil microorganisms have the capacity to assimilate nitrates and thus prevent their leaching from the soil. They also suggest that a decrease in microbial assimilation of nitrates may result from greater availability of NH_4^+ and reduced inputs of plant C after some disturbances (Stark and Hart, 1997).

Kopáček et al. (2013) reviewed the above mentioned N cycling concept and incorporated also the sulphur (S) cycling into it reflecting the changes in nutrient cycling in soils after being affected by massive N and S depositions due to industrial activities during the second half of 20th century. They suggest that the shift in the microbial community linked to elevated N and S deposition and N saturation is manifested by decrease in the fungi/bacteria ratio and by a transition towards C limitation (Nilsson et al., 2012; Kopáček et al., 2013). Fungi are better competitors for N in N-poor environments and play an important role not only in plant nutrition (Smith and Read, 2008) but also in forest soil N retention (Nilsson et al., 2012). Many fungi live in a symbiotic association with plants (mycorrhizal symbiosis) and supply them with nutrients (mostly phosphorus and nitrogen) in exchange for assimilates produced in photosynthesis. Fungi further enhance plant resistance to pathogens and protect them against herbivores. They are also equipped with a variety of enzymes allowing them to decompose even complex organic substrate (Smith and Read, 2008). As a result of elevated N and S deposition fungal biomass decreases since plant can get their nitrogen "without paying their symbiotic partners for it" (Wallenda and Kottke, 1998; Schimel and Bennett, 2004). Similarly, lower amounts of ectomycorrhizal mycelia were accompanied by increased nitrate leaching, which may suggest that fungi play an important role in forest soil N retention (Nilsson et al., 2007). The decline in fungal biomass further affects litter decomposition and mineralization of soil nitrogen – decomposition of recalcitrant organic carbon such as conifer needles or woody biomass is reduced and more N is utilized by bacteria with lower C/N ratio of their biomass. This, in turn, leads further to decreasing of soil C/N ratio (Kopáček et al., 2013).

2.1.6 Organic nitrogen uptake by plants and microorganisms

A better understanding of plant N availability and consumption is crucial for modeling and interpreting the effects of N deposition on these ecosystems (Andersson and Berggren, 2005).

2.1.6.1 Organic nitrogen uptake by plants

The distribution of N forms and their importance in the N cycle depends not only on soil parameters, but also on plant species and on the soil microbial community. For a long

time, plants were considered to take up only inorganic forms of N (ammonia and nitrates) and also the research was focused on these forms. In the last decades, papers confirming that plants are able to utilize organic N and compete for it with microorganisms were published. The research done by Persson and Näsholm (2001) showed that it is common for boreal forest plants to take up amino acids (Persson and Näsholm, 2001). Others came up with similar conclusion on direct uptake of organic N in the form of amino acids, peptides, and proteins by roots without any mycorrhizal "helpers" (Lipson and Näsholm, 2001). A greenhouse experiment with additions of labeled amino acids (AAs) showed a significant effect of soil amino acid concentrations and their uptake. Surprisingly, the soil concentration of AAs negatively affected the uptake of N derived of AAs, whereas the structure/complexity and degradability (presence or absence of aromatic rings) didn't have any significant effect (Sauheitl et al., 2009).

The study of Warren (2009) further develops this relationship between the substrate concentration effect on the proportional uptake of different N forms (nitrate, ammonium, glycine). He suggests that it is likely a reflection of kinetics of N uptake – at low substrate concentration the uptake responds the affinity, whereas at high concentrations it is determined by maximal enzyme velocity (Warren, 2009).

Plants are able to take up amino acids simultaneously with other N forms. And, as the concentrations of different N forms change along a gradient of succession and plant productivity, plants can have different preferences for the N source (Nordin et al., 2001). The organic nitrogen uptake by plants seems to be a common phenomenon and was described in several environments, such as boreal forests (Persson and Näsholm, 2001), and alpine (Lipson et al., 1999), arctic (Nordin et al., 2004) or tundra (Schimel and Chapin, 1996) ecosystems.

Even though it is still difficult to directly assess that plants rely to a greater degree on organic N, we can conclude its importance from several findings (Näsholm et al., 2009):

- The amount of inorganic N produced doesn't correspond the amount of total plant N uptake indicating that they must have other N sources
- In some ecosystems, the concentration of amino acids is similar or even higher than the concentration of inorganic N
- Many plants evolved mechanisms of organic N acquisition that in many features resembles the mechanism of inorganic N acquisition.

Organic N uptake by plants can depend on the growth stage of plants. Weigelt et al. (2005) conducted a pot experiment with five grass species and found a connection between plant growth rate and a preference for organic or inorganic N form. The fast growing plants with greater total biomass took up significantly more inorganic N compared to the slow growing species (Weigelt et al., 2005). Harrison et al. (2008) came to a similar conclusion - fast-growing plants take up more of added N, probably as a result of plant traits that regulate nutrient capture (Harrison et al., 2008).

2.1.6.2 Organic nitrogen uptake by microorganisms

There is a great variety of microorganisms in soil that possess a diverse number of enzymes enabling them to mediate the transformations of the N-cycle. The synthesis and expression of these enzymes require not only energy, but also carbon and nitrogen.

In their paper, Geisseler et al. (2010) review the pathways of nitrogen utilization by microorganisms. The production (synthesis and secretion) of extracellular depolymerases necessary for soil organic matter degradation (such as proteases, chitinases, peptidoglycan-hydrolases, *etc.*) is regulated through four major mechanisms: substrate induction, end-product repression, de-repression due to insufficient nutrient supply and constructive production. In a conceptual model they compare two pathways: mineralization-immobilization-turnover route (MIT) that includes mineralization of organic molecules followed by the uptake and assimilation of the released NH_4^+, and direct route that comprises all mechanisms for the direct uptake of organic N. The relative importance of each N uptake route is not static but undergoes dynamic changes over time. Three main factors determine which route will be more important: the form of N available, C sources and the N availability relative to C. These factors are, in turn, affected by environmental conditions of the site (temperature, soil aeration and moisture) (Geisseler et al., 2010). In connection with the new paradigm of the N-cycle (Schimel and Bennett, 2004), the soil heterogeneity is believed to play an important role in creating micro-sites with different conditions, which enables both routes to be dominant at the same time at the relatively same place (Geisseler et al., 2010). When N is limiting relative to C (= high C/N ratio), net immobilization occurs, which should gradually lead to the depletion of mineral soil N pool and cause shift to direct route in order to take up N from alternative sources while the N is a limiting factor. On the other hand, when C is limiting relative to N (= low C/N ratio), N containing organic compounds may be used as C sources (Geisseler et al., 2010; Tahovská et al., 2013). Microorganisms will then release the excess N in the form of NH_4^+, which will eventually

lead to a shift from direct route to MIT. The authors suggest that the strongest effect on the N-uptake route should be at the C/N ratio of 20-40 (Geisseler et al., 2010). Soils subjected to long-term loads of atmospheric N and S deposition will very likely have lower C/N ratio.

The effect of C/N ratio of ongoing microbial processes is described in a similar way by Hodge et al. (2000). They conclude that if C/N ratio is higher than 30:1, microorganisms will require other sources of N to meet their demands. Thus, they will immobilize N that could be taken by plants. When C/N ratio of the substrate declines to about 20, fungi start to release N (they have higher C/N ratio of their biomass than bacteria) whereas bacteria still assimilate. With C/N less than about 12.5, microorganisms will release the surplus N (often as NH_4^+) and the net mineralization will occur (Hodge et al., 2000). Similarly, C/N ratio lower than 15 is followed by net mineralization, whereas C/N ratio higher than 80 by net immobilization (Brady and Weil, 2002).

Bacteria have been reported to have a high ability to recycle N intracellularly (Bengton and Bengtsson, 2005). This mechanism is most probably helping them the most at low N concentrations and at low growth rates. On the other hand, when conditions allow for high growth rates of microorganisms and rapid turnover of microbial biomass, re-mineralization (mineralization of previously immobilized N by the microbial biomass) is very likely to occur. Moreover, high nitrate immobilization by microorganisms was observed, even at high NH_4^+ concentrations (Bengtson and Bengtsson, 2005). This contradicts the assumption that at high ammonium concentrations (when the excess ammonium is released due to C limitation of microbial biomass), nitrification prevails and nitrate immobilization is suppressed (Paul and Clark, 1996; Myrold, 2005; Taylor and Townsend, 2011), and makes the N cycling even more complex and intertwined.

One of the arguments against a significant organic N contribution to plant nutrition is the fact that plants cannot effectively compete for it against microbes (Näsholm et al., 2009). Studies on competition for available N between plants and microorganisms show that microbes are better competitors. However, the turnover of the microbial population is much faster and the N incorporated into biomass can be released back in the soil in a short time period. Plants retain N for longer periods of time. It means that, in long-term perspective, plants may become more competitive and the fraction of organic N absorbed may be of a significant importance (Kaye and Hart, 1997; Näsholm and Persson, 2001, Hodge et al., 2000). Similarly, in the short term, microorganisms proved to be better competitors for inorganic N sources (Näsholm and Persson, 2001).

Still, there are some constraints when interpreting the data. A complete separation of the microbial community (free-living and symbiotic microorganisms that actually help plants in nutrient uptake) and assessment of direct competition between plants and microorganisms for soil N is very difficult. There are many possible pathways and loops where nitrogen goes through at various rates and retention times and in various amounts (Hodge et al., 2000).

Chapman et al. (2006) suggest that plants are to some extent able to control N cycling. The effect of plant is species-specific. The composition of vegetation cover can influence microbial community composition and ongoing processes in soil (described more in detail in Chapter 2.2.2).

2.2 Forest ecosystems and their nitrogen cycle

Barnes et al. (1998) describe *forest* as a complex three-dimensional ecosystem where trees and other woody vegetation dominate. This system is a part of the landscape and interacts with other parts of the environment (Barnes et al., 1998). Forests influence the global ecosystem and its functioning in many ways. They store C, they are involved in global water cycle and other biogeochemical cycles through chemical elements and energy transformations. Forests, having a very low albedo in the range from 0.07 to 0.25% (which means that they absorb 75 to 93% of the solar radiation), contribute to the global bilance of temperature. Least but not last, forests offer a living space for numerous organisms (Perry, 1994).

The distribution of different forest types around the world is determined by climatic conditions. Generally, in the temperate zone deciduous, coniferous or mixed forests can be found. In the Central Europe, mixed forests dominate the lower elevations, whereas mountain areas are predominantly covered by coniferous forests with Norway spruce *(Picea abies)*. Semi-natural spruce forests in our conditions can be compared to boreal forests. This similarity can be explained by the Hopkins's bioclimatic law which states that the temperature change at 1000 metres of elevation can be compared to the temperature change at 5° of latitude (500 to 750 km) (Forest Ecology lecture, 2012; Hopkins, 1920). The similarities between central-European mountain spruce forests and boreal forests are not only in tree species composition but also in soil properties and litter composition. Typical soil type of these coniferous forests is podzol with characteristic eluvial and illuvial horizons. Slow decomposition of soil organic matter results from a generally low pH, frequent water-logging and from the chemical composition and structure of the organic material (lower content of easier degradable living tissues and a high content of resins and

waxes). Almost 2/3 of all organic matter represents the dead organic matter where nutrients are bound. Low nutrient demands and other adaptations help coniferous tree species to live and even "thrive" in these nutrient-poor ecosystems. Their needles are protected by cuticle and contain waxes and smaller and nested stomata. Further, chloroplasts reduce their size during winter and become inactive, which helps them to survive the harsh conditions (frost, desiccation). The rooting system of the conifers is usually in the upper soil layers because of the presence of mycorrhizal fungi that form partnerships with these trees and help them in gaining nutrients (Prach et al., 2009).

When considering the N cycle, these forest ecosystems represent a typical nitrogen-depleted site with slow-growing trees and other plant species in understory with low nutrient demands (Tamm, 1991). A large part of N source in these forests is represented by its organic forms being thus a large potential N pool not only for microorganisms, but also for plants (Näsholm et al., 1998; Jones and Kielland, 2002). Due to its complex composition, however, this pool may be difficult to decompose and utilize. This may explain the size of the pool, as well (Jones et al., 2005). As was already mentioned, larger pools tend to have a longer residence time and to turn over more slowly than the smaller pools, such as soil inorganic nitrogen (Myrold, 2005). Jones et al. (2005) suggest that not the actual size but the rate of flux through the particular N pools is more important (Jones et al., 2005). Due to human activities leading to increased atmospheric N deposition, many nutrient-limited ecosystems developed towards the nitrogen-saturation state (Tamm, 1991; Galloway, 1998).

2.2.1 Natural spruce forest versus "plantations"

The distribution of natural spruce forests in the Central Europe is determined by two main factors – wet and cold climate, and soil conditions unfavorable for many other tree species, such as waterlogged and shallow, undeveloped soils with a low nutrient content (Šantrůčková et al., 2010). The natural spruce forests in our conditions are basically found only in mountain areas above elevations of 950 m. In lower elevations, Norway spruce can be found azonally in waterlogged spruce forests or in cold depressions. All of these types can be found in the Šumava Mountains and in the Šumava National Park (Kučera, 2010).

The plantations of Norway spruce, on the other hand, are found in lower latitudes as well as in mountains and serve to commercial purposes. These extensive and very often even-aged monocultures are a result of the transition in forestry at the end of 18th century (Dobrovolný and Brázdil, 2003). They differ from (semi-)natural forests in many aspects. Commercial (managed) plantations are to a large extent dependent on human activities.

Management is thus the main driving force of the forest dynamics. These stands are mostly uniform in species composition and trees age distribution. Original species composition is replaced and usually one tree species (with favorable qualities) is preferred. Similarly, natural processes of the forest dynamics are replaced by wood logging (Šantrůčková et al., 2010).

The important aspect is also the absence of dead and decaying wood in plantations. One of the arguments for justification of dead wood removal (even in mountain spruce stands) is the effort to minimize the effect of bark beetle populations that would multiply in these logs and attack the adjacent stands (Wermelinger, 2004). However, many studies confirm that dead wood plays a very important role in mountain spruce forest regeneration as a micro-site with favorable conditions for many species of animals, plants and fungi (Freedman et al., 1996; Wu et al., 2005; Zielonka, 2006; Svoboda and Pouska, 2008; Svoboda and Zenáhlíková, 2009). In their study in the Bohemian Forest Mountains, Svoboda and Pouska (2008) observed that even though dead wood represented only about 5% of the forest floor cover, 50 to 80% of the young regenerating spruce seedlings and saplings grew on this "substrate" (Svoboda and Pouska, 2008; similarly also Svoboda and Zenáhlíková, 2009). Moreover, even forest stands managed in the past can after several decades of natural development turn into forest with valuable biological characteristics such as high dead wood amount, large seedling and sapling banks and heterogeneous structure (Svoboda and Zenáhlíková, 2009).

In mountain areas with acidified soils poor in nutrients, dead wood represents an important source of nutrients. Removal of dead wood may limit future natural regeneration of spruce stands. It can be substituted by artificial plantation, which is laborious, expensive and often ineffective (Svoboda et al., 2010).

Generally, different species composition develops in understory of natural and seminatural forests compared to that in plantation. The plants in understory can have effect on biochemical processes in soil which will be further discussed in Chapter 2.2.2, with respect to common understory species found in mountain spruce forests.

2.2.2   Spruce forest understory species

The biochemical processes in soil mediated by microorganisms are to a great extent influenced by the vegetation cover. Chapman et al. (2006) suggest that plants are more or less able to control N cycling. They distinguish between conservative (conifers, ericaceous plants, etc.) and extravagant plant species (grasses, most herbs, etc.) in connection to their

environment and ability to control N cycling. Conservative plants usually live in nutrient-poor habitats and evolved several strategies for obtaining nutrients (such as mycorrhizal symbiosis). They are considered to regulate the N cycling more strongly than extravagant species that usually use N mineralized by microorganisms (Chapman et al., 2006).

The composition of the litter determines its degradability which is further reflected in the microbial community involved in decomposition and in nutrient availability. The different decomposability and decomposition rate is usually ascribed to the content of lignin and soluble carbohydrates in litter. Litter mass loss (as a measure of decomposition rate) was found to be positively correlated to content of polyphenols and soluble carbohydrates and negatively to lignin content in litter (Osono and Takeda, 2005). Decomposition could be also hindered by low P and N availability (Šantrůčková et al., 2006). In case of N availability effect, it may support decomposition in the early stage when celluloses are being decomposed. Conversely, in the later stage when lignin is being decomposed, high N availability can slow down the decomposition rate through creating more recalcitrant aromatic compounds of N with lignin. N may also restrict the synthesis of lignin-degrading enzymes (Berg, 2000).

The tree cover at our experimental plots is dominated almost exclusively by Norway spruce with only sparse distribution of rowan on edges and in open sites. The herb layer is dominated by acidophilous grasses and herbs, such as *Calamagrostis villosa, Avenella flexuosa* and *Vaccinium myrtillus* (Jonášová and Prach, 2008). Therefore, we focus on these species and their litter quality more in detail.

There are reports on the effect of Norway spruce on soil properties and microbial community. Compared to deciduous tree species (birch), soil under spruce was characterized by higher C/N ratio of litter, lower pH, base saturation and by lower content of C and N bound to microbial biomass. On the other hand, spruce stands stored more carbon and nitrogen in the soil and thus seemed to sequester more soil carbon (Merilä et al., 2010; Hansson et al., 2011; Smolander and Kitunen, 2011; Kiikkilä et al., 2012). The decomposition rate of spruce material (wood, needles, bark) was reported to be lower compared to other dominants at our experimental plots (grasses, bilberry) (Šantrůčková et al., 2006). This is a result of high content of lignin and low content of polyphenols and soluble carbohydrates (Osono and Takeda, 2005; Shorohova et al., 2008). It might also be caused by low P and N availability (Šantrůčková et al., 2006). There is a difference in decomposition rate for particular parts of the tree, as well. The decomposition rate constant (based on percent mass remaining) of the spruce logs range from 0.026 (and 0.044 for snags)

(Yatskov et al., 2003) to 0.050 (Laiho and Prescott, 1999) and to 0.052 per year (Shorohova et al., 2008). Spruce bark decomposes even slower due to lower concentration of easier degradable carbohydrates (such as holocellulose) and higher content of tannins and lignin that can impede microbial colonization (Shorohova et al., 2008). Laiho and Prescott (1999) observed a relationship between initial N/P concentrations in logs and consequent release/gain of these nutrients during decomposition. Low initial concentrations of led to immobilization, while high concentrations were followed by release. Based on these results the authors suggest that the coarse woody debris of some tree species is not a significant source of available nutrients (N, P) but may actually compete for limiting nutrients with vegetation (Laiho and Prescott, 1999).

Grasses tend to produce easy degradable litter with low C/N ratio. From our dominants, *Calamagrostis villosa* was reported to have the fastest decomposition rate followed by *Vaccinium myrtillus* and *Avenella flexuosa* (Šantrůčková et al., 2006). These species decompose rapidly and their litter does not accumulate in the forest floor (Wardle et al., 2003). Fiala et al. (2005) suggest that *Calamagrostis* can effectively accumulate N in its biomass and thus has potential to reduce N losses from soil during the growth season (Fiala et al., 2005). On the other hand, Šantrůčková et al. (2006) argue that higher cover of *Calamagrostis* in the catchment of the Čertovo Lake supported higher microbial activity and might have contributed to higher N release from the litter (Šantrůčková et al., 2006) compared to the Plešné Lake where *Vaccinium myrtilus* is dominant (Svoboda et al., 2006). The decomposition rate of *Vaccinium* is fast (Wardle et al., 2003; Hilli et al., 2010) and the litter does not accumulate in the forest floor (Wardle et al., 2003). The cover of *Vaccinium* can indicate a thick layer of humus and low pH, which is a favorable micro-site for spruce seedling growth (Baier et al., 2005).

The last dominant is moss (*Polytrichum* spp.). Moss litter is both poor in N and recalcitrant and thus decomposes more slowly than the dead parts of some herbs and grasses and forms (Mikola, 1954. In: Smolander and Kitunen, 2002; Hobbie, 1996). Bryophytes are able to fix C and N from atmosphere and influence their environment through decreasing soil temperatures or increasing soil moisture. They are also able to change the density of soil organic matter and reduce the loss of organic N from ecosystem by decreasing decomposition (Turetsky, 2003).

2.2.3 *History of the forests in the Bohemian Forest National park*

The mountain Norway spruce forests located on the border of south-western part of Czech Republic with Germany and Austria represent one of the remaining areas of once largely distributed old-growth spruce forests throughout the Central Europe (Svoboda and Pouska, 2008). The history of the Bohemian Forest region was reviewed by Beneš (1995). In context of Central Europe, mature forest is considered a climax stage of vegetation. In the region of the Bohemian Forest Mountains, origins of forest cover can be dated back to the beginning of Holocene. The first tree "invaders" were willow, birch and pine, followed by hazel (around 7000 BC) and Norway spruce and later by beech (6000 BC) and fir. Other species such as oak, lime or elm are relatively scarce. The first significant impact of population on the appearance of this region occurred during the Middle Ages through expansion of arable land, through gold mining and pasture in forests. Later, in the 18th century, the largest areas of the Bohemian Forest were deforested due to development of glass production, trade and mining. An extensive grid of channels for tree logs transport was build. These activities affected not only the forest area but also the trees distribution. Both fir and beech declined (fir was used as a construction material, beech for heating in glassworks). Moreover, while spruce wigs are not tasty for cattle, both fir and beech were grazed, which eventually favored spread of spruce. After massive deforestation during the 18th century, regeneration of spruce forests was adopted in the following century mainly by Schwarzenberg family (Beneš, 1995). Forest stands in the Bohemian Forest were negatively affected by a series of windstorms in 1868 to 1870. The impacts were large due to previous overlogging and forest pasture. Even semi-natural stands that were able to resist the effects of windstorm succumbed to the bark beetle outbreak (Zatloukal, 1998). Further, during the second half of the 20th century, the Bohemian Forest experienced the impacts of the Industrial Revolution in similar amounts and rates as the whole Central Europe, which further impaired the forest stands. Until the 1950s the deposition of SO_4^{2-}, NO_3^- and NH_4^+ was relatively stable but increased rapidly in the following thirty years and culminated in the early 1980s. After measures had been taken, acid deposition gradually decreased (Kopáček et al., 2001), which was followed by regeneration and a decrease in nutrient loss from the glacier lake catchments (Vrba et al. 2003).

2.2.4 *Driving forces of the forest dynamics*

One of the most important driving forces in the natural development of forests is disturbance (Frelich, 2002). In Central Europe conditions, forests have to cope with, above

all, large-scale disturbances connected with spruce bark beetle (*Ips typographus*) outbreaks and windstorms (Fischer et al., 2002; Dobrovolný and Brázdil, 2003; Schelhaas et al., 2003; Wermelinger, 2004). Storm winds and bark beetle infestation influence the dynamics and structure in both near-natural and managed forest stands (Fischer et al., 2002). However, based on the historical reports, forests with original patterns of tree species composition and age-stages distribution were less susceptible to strong winds. Wind events were also not so frequent compared to the present state. The decreased resistence to windstorms is ascribed to the change in forestry at the turn of the 18th and 19th century leading to the establishment of large even-aged spruce monocultures. Moreover, these monocultures are very often found in unsuitable climatic conditions (Fanta, 1997; Dobrovolný and Brázdil, 2003) and were negatively affected by air pollution in the second half of the 20th century (Schelhaas et al., 2003). Large-scale slashes following severe windstorms generally occur in areas of mountain spruce forests affected by salvage logging in the past (Křenová and Vojtěch, 2007).

The spruce bark beetle is regarded one of the most significant pests in European forests causing large-scale tree diebacks, usually following severe windstorms. On the other hand, this species inherently belongs to all Norway spruce forest stands. As a pioneer species, bark beetle often starts the decomposition of dead wood. This is another aspect of its important role in forest dynamics (Wermelinger, 2004). The susceptibility of individual trees and forest stands is governed by many factors, such as exposition, tree age, and nutrient and water supplies of trees. The susceptibility of trees together with weather conditions and human measures, in turn, affect the performance of the insect outbreak (Wermelinger, 2004). It must be noted that non-autochthonous spruce stands are very likely to be more vulnerable to the effect of these two disturbance types (Dobrovolný and Brázdil, 2003; Wermelinger, 2004).

Svoboda et al. (2010) suggest that the interaction of bark beetle outbreaks and windstorms belongs to the forest stands in Šumava Mountains and has occurred historically (Svoboda et al., 2010). These two factors have been forming the forest stands for thousands of years and the forests are adapted to these dynamics (Šantrůčková et al., 2010). Thus, both bark beetle and windstorms should be seen as essential parts of the spruce forests, providing space, light and nutrients for new generations of the tree stands and thus encouraging restoration and regeneration of the forest (Jonášová and Prach, 2004; Müller et al., 2008; Jonášová and Matějková, 2007). However, in many cases this view is not held and the consequences of these natural disturbances are (even in national parks and their core zones)

still considered and treated as a threat to forest production and viability (Svoboda and Pouska, 2008).

2.2.5 Non-intervention vs. clear-cutting

Bark beetle outbreaks in managed forests are usually followed by clear-cutting (and by artificial reforestation) in order to prevent further spread of the beetle. Clear-cutting has long-term impact on soil organisms and ongoing processes (up to 10 years). The generally observed increase in microbial biomass is accompanied by increase in soil respiration, N mineralization and thus in decrease in C/N ratio. This, in turn, leads to significant losses of N and other nutrients after clear-cutting (Paul and Clark, 1996; Aber et al., 2002; Hazlett et al., 2007). Homyak et al. (2008) propose the application of wood chips as a tool for decreasing the negative effects of harvesting, such as nitrate leaching to waters. This is based on their observation that C/N ratio of wood chips decreased significantly (from 125:1 to 70:1) one year after their application at the clear-cut plots, suggesting that they have potential for N immobilization (Homyak et al., 2008).

Next to changes in soil biochemistry, there are also other negative effects, such as mechanical disruption of the forest floor or changes in microclimatic conditions. The absence of tree vegetation has several consequences. There is a decrease in nutrient uptake and respiration by plants, which leads to an increase in water passing through the system (Bohrmann et al., 1968). Moreover, the clear-cut plots tend to be more overheated due to the vegetation removal. Hais and Kučera (2008) observed an increase in soil surface temperature by 3.5 °C at non-intervention and by 5.2 °C in clear-cut plots (Hais and Kučera, 2008).

Forest management affects also the vegetation cover and composition of fungal community. Clear-cutting can lead to loss of species richness of ectomycorrhizal fungi which negatively alters the fungal community and their functioning in soil (Byrd et al., 2000). One reason for this decline and fungal community composition shift is the disruption of the network of mycorrhizal hyphae in soil resulting in reduced colonization (Smith and Read, 2008). In case of changes in the vegetation cover, there is evidence that clear-cutting supports expansion of pioneer species, such as competitive grasses. Bryophytes, on the other hand, seem to be susceptible to the changes in microclimate at the clear-cut plots and decline not only in % cover but primarily in diversity (Fenton et al., 2003; Palviainen et al., 2005; Jonášová and Prach, 2008). Dwarf shrubs (such as *Vaccinium* spp.) decreased after clear-cutting but still remained a significant nutrient sink and were able to recover after few years (Palviainen et al., 2005). Compared to clear-cut plots, at plots left without intervention, both

mosses and herbs survived relatively well (Jonášová and Prach, 2008) and the regeneration of tree cover was faster (Jonášová and Prach, 2004).

However, in the core zones of the Bavarian Forest and Bohemian Forest National Parks the aftermath measures were and are a matter of discussion. On the German side, a large-scale subalpine forest dieback followed the bark beetle population boom in 1995. The pattern of natural regeneration changed but after ten years it was observed in nearly all (99.1%) inventory plots (Heurich, 2009). On the Czech side, regeneration at non-intervention and even at clear-cut and reforested plots was observed (Zatloukal et al., 2001; Jonášová and Prach, 2004). The tree species composition of the regenerated forest stands was, however, much closer to the natural forest conditions at non-intervention plots (Jonášová and Matějková, 2007). The regeneration of Norway spruce was positively affected when plots were left without any management (Hrežíková, 2008). Zatloukal et al. (2001) concludes that the regeneration of spruce under dead trees and at clear-cut plots is sufficient for re-establishment of a new forest generation and reforestation is therefore inappropriate. The contribution of other tree species (such as mountain-ash *Sorbus aucuparia* or sycamore maple *Acer pseudoplatanus*) to regeneration is, however, quite small and should be fostered (Zatloukal et al., 2001).

While there are data on development of vegetation cover, data on soil chemistry and biochemistry are still scarce.

3   AIMS

Estimation of mineral and microbial N concentrations in soils of semi-natural mountain Norway spruce forests under four dominant plant species and under dead wood with respect to different human intervention (spontaneous succession x clear-cutting) after windstorm and bark beetle events

4   HYPOTHESES

Central hypothesis: The forest dieback together with consequent management practices lead to changes in vegetation cover and affect the processes of nitrogen transformation in soil

Specific hypotheses:

1) The concentration of mineral and microbial N will be the highest in the litter horizon where the majority of transformation processes takes place
2) Concentrations of N in microbial biomass will be higher than concentrations of mineral N forms (nitrates and ammonium)
3) Concentration of N bound to microbial biomass will be higher at plots left without intervention
4) Concentrations of mineral and microbial N will differ under the four dominant plant species and under dead wood. The distribution in the soil profile will be similar (as described in hypothesis 1)

5 MATERIALS AND METHODS

5.1 Site description

The study area Březník is located in the central part of the Bohemian Forest Mountains, in the first and second zones of the Bohemian Forest National Park (N 48° 58' – 48°59'; E 13° 25' – 13° 27'). The elevation ranges from 1175 to 1280 m. The experimental plots follow the former research made by Jonášová and Prach (2004, 2008) in areas affected by storm event and bark beetle outbreak in 1997 and 1998. There are two types of stands differing in management – S stands (climax mountain spruce forests without human intervention after the bark beetle attack), P stands (climax mountain spruce forests where clear-cutting was applied in spring 1997 and only wood chips were left) (Jonášová and Prach, 2008). Originally, 12 plots were established. However, for the purposes of the current research, only 6 plots - always three from each type of management (S3, S5, S7, P2, P3, P5) - are being monitored with installed dataloggers and sampled for soil chemical and biological analyses (Figure 3).

The bedrock is formed predominantly of gneiss, partly combined with. The dominant soil type developing under the mountain spruce forests are podzols that are low in pH and nutrient-poor. Soils and waters have been exposed to acid deposition in the 2nd half of the 20th century and exhibit acidification till present (Kopáček et al., 2001). At the beginning of 20th century the pH of the soils was around 5.3 and until today pH declined to around 4.5 and less (Hruška, 2005).

The tree cover is dominated almost exclusively by Norway spruce with only sparse distribution of rowan on edges and in open sites. The herb layer is dominated by acidophilous grasses and herbs, such as *Calamagrostis villosa, Avenella flexuosa* and *Vaccinium myrtillus* (Jonášová and Prach, 2008).
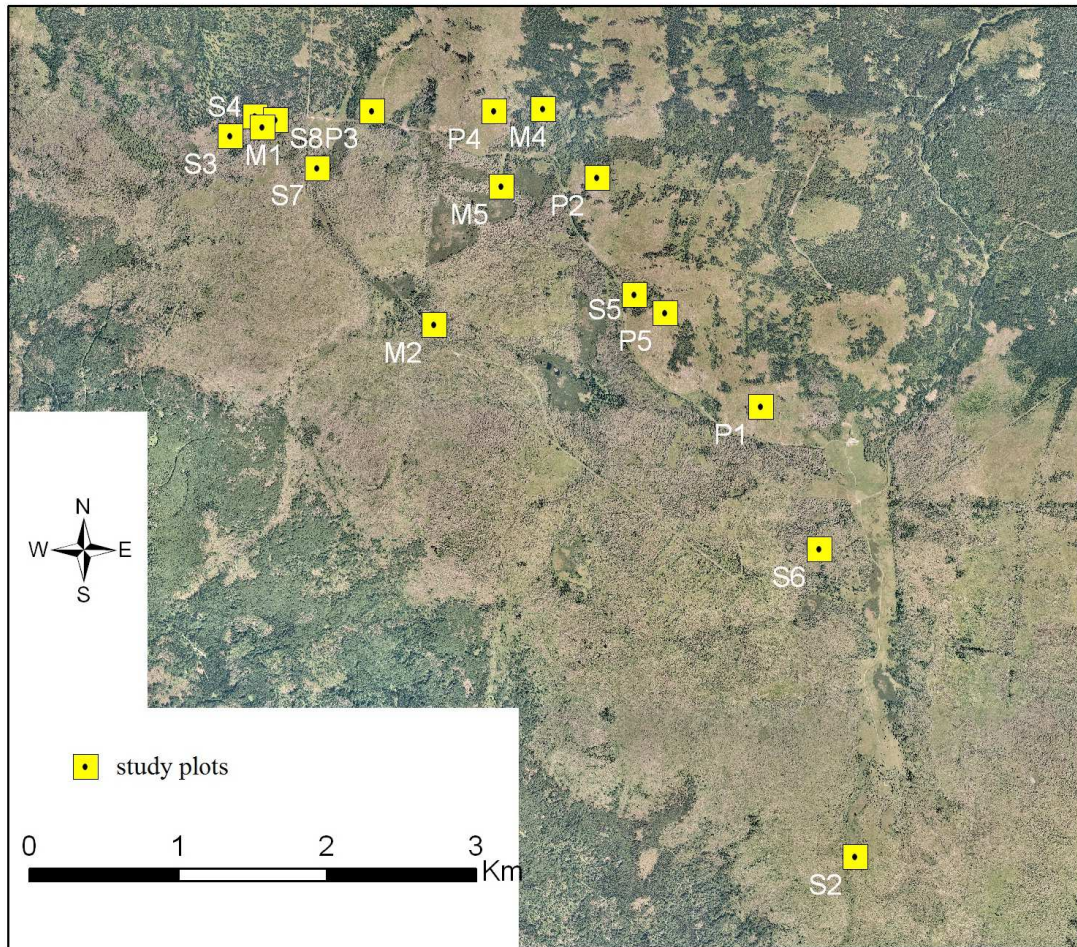
Figure 3. Aerial view on the whole Březník site with the experimental plots.

5.2 Soil sampling and preparation

Concentrations of extractable C and N, of microbial C and N, together with C/N ratio of biomass and rates of ammonification and nitrification were measured at the experimental plots. The effect of management and plant dominant on these soil properties was studied. To study the effect of plant dominant and management, soil was sampled at each plot under five selected dominants (dominants were selected according to Hrežíková, 2008) – moss (Mch) (*Polytrichum* spp.), two grass species *Avenella flexuosa* (M) and *Calamagrostis villosa* (T), bilberry *Vaccinium myrtillus* (Bo), and dead wood (D) in 3 replicates from 3 horizons: litter, organic (0-10 cm) and mineral layer (10-30 cm). Sampling took place in October and November 2011 and 2012 and the samples were put into cold room immediately after arriving to the laboratory.

Soil samples were then sieved (5 mm diameter), weighed, and a composite sample for each plot was made from 3 replicates in few days after sampling. A small part of soil

was used to determine the dry weight, total CN and pH for each combination (plot – dominant – horizon) and rest of the soil samples were then stored wet in plastic bag at 4°C until being used for further analyses.

Basic soil characteristics are given in Table 1. Soil pH and total soil C and N concentrations (C_{tot}, N_{tot}) were measured in 2011. Soil pH_{KCl} was measured in 1M KCl (weigh of dry soil to volume of extraction solution was 1:20) according to Petrenko and Berezhnyak (2008). The pH values ranged from 3.0 to 4.1 and from 2.9 to 4.0 in S and P plots, respectively, indicating a highly acidic soils. The average values for both S and P plots in the 0-10-cm horizon were 3.3 ± 0.2 and, in the 10-30-cm horizon 3.8 ± 0.2. C_{tot} and N_{tot} concentrations were measured on elementar analyzer (Micro-cube elementar analyser, Germany).

Table 1. Soil pH under the five dominants in the two lower horizons. Dry weight of soil (DW) is given for all horizons. The abbreviations stand for: Bo-*Vaccinium myrtillus*, D-dead wood, M-*Avenella flexuosa*, Mch-moss, T-*Calamagrostis villosa*, S-non-intervention plots, P-clear-cut plots.

dominant	site	litter DW	0-10 cm DW	0-10 cm pH	10-30 cm DW	10-30 cm pH
Bo	S	0.34 ± 0.07	0.47 ± 0.06	3.2 ± 0.1	0.62 ± 0.07	3.8 ± 0.0
	P	0.25 ± 0.05	0.38 ± 0.06	3.1 ± 0.2	0.58 ± 0.07	3.8 ± 0.1
D	S	0.26 ± 0.03	0.41 ± 0.10	3.1 ± 0.1	0.61 ± 0.12	3.7 ± 0.2
	P	0.24 ± 0.01	0.35 ± 0.06	3.3 ± 0.0	0.55 ± 0.04	3.7 ± 0.1
M	S	0.23 ± 0.02	0.35 ± 0.13	3.3 ± 0.2	0.63 ± 0.14	3.9 ± 0.1
	P	0.28 ± 0.03	0.32 ± 0.02	3.7 ± 0.0	0.53 ± 0.08	3.9 ± 0.1
Mch	S	0.19 ± 0.02	0.38 ± 0.15	3.5 ± 0.2	0.59 ± 0.13	3.9 ± 0.1
	P	0.31 ± 0.08	0.47 ± 0.04	3.3 ± 0.1	0.58 ± 0.00	3.8 ± 0.1
T	S	0.23 ± 0.00	0.43 ± 0.09	3.2 ± 0.2	0.64 ± 0.07	3.7 ± 0.1
	P	0.23 ± 0.02	0.37 ± 0.08	3.3 ± 0.1	0.53 ± 0.05	3.7 ± 0.1

5.2.1 *Net ammonification and nitrification rate*

A long-term aerobic incubation method modified according to Ste-Marie and Paré (1999), Zhu and Carreiro (1999) and Schmidt and Belser (1982) was used.

Moist soil was incubated for 3 weeks at 10°C in two laboratory replicates for each extraction time. Concentration of NO_3^- and NH_4^+ were measured in sulphate extract after one week (week 1) and at the end of incubation (week 3). Net rate of ammonification and nitrification was calculated as a difference between NO_3^- and/or NH_4^+ concentration after 3 and 1 week of incubation.

The incubation was conducted during January and February 2012 (the 2011-samples) and in June 2013 (the 2012-samples). For the 2011-samples, 5 g of each soil sample was put into 100ml NTS-flasks. The flasks were covered with parafilm, perforated, and incubated at 10°C until extraction. The samples were extracted with 40 ml 0.5M K_2SO_4. The 2012-samples were incubated in 40 ml glass vials (2.5 g of litter, 5 g of 0-10 cm and 10-30 cm horizons) and were extracted with 20 ml of 0.5M K_2SO_4. All the extracts were shaken in a horizontal vortex (1 hour, 150 strokes per minute), centrifuged (10 min, 4000g), filtered through 0.45 µm glass fibre filter and frozen in scintillation counter vials for further analysis. NO_3^- and NH_4^+ concentrations were measured spectrophotometrically on FIA (Flow Injection Analyzer, Foss Tecator).

Calculations:

The amount of nitrates and ammonium in soil

$$\mathbf{N = (c\ N\text{-}NO_3^- - B) * V / (m * DW)} \quad \mathbf{[\mu g\ N\text{-}NO_3^- * g^{-1}\ DW]}$$ (similarly for ammonium)

$c\ N\text{-}NO_3^-$............concentration of nitrates in extract [mg N-NO_3^- * l^{-1}]

B.......................concentration of nitrates in blanc (0.5M K_2SO_4) [mg N-NO_3^- * l^{-1}]

V......................volume of the extractant [ml]

m......................wet soil weight in the extract [g]

DW……………dry weight of the soil

The nitrification and ammonification rate

The nitrification rate was expressed as amount of nitrates produced per g DW and day [µg N-NO_3^- * g^{-1} * d^{-1}]. Similarly, the ammonification rate was expressed as amount of ammonium produced per g DW and day [µg N-NH_4^+ * g^{-1} DW* d^{-1}].

$$\mathbf{v = (N_t - N_0)/ t} \quad \mathbf{[\mu g\ N\text{-}NO_3^- * g^{-1}\ DW * d^{-1}]\ and\ [\mu g\ N\text{-}NH_4^+ * g^{-1}\ DW * d^{-1}]}$$

N_0......................amount of the specific N form in soil at the beginning of incubation (week 1)
[µg N-NO_3^- * g^{-1} DW] and [µg N-NH_4^+ * g^{-1} DW]

N_t......................amount of the specific N form in soil at the end of incubation (week 3)
[µg N-NO_3^- * g^{-1} DW] and [µg N-NH_4^+ * g^{-1} DW]

t………………incubation time [days]

5.2.2 Microbial biomass assessment – Chloroform Fumigation Extraction method (CFE)

Along with ammonification and nitrification, I measured microbial biomass using the CFE method, modified by Vance et al. (1987). The core of this method is that the soil samples (with their microbial community) are subjected to chloroform vapors. It disrupts the cell walls of the microorganisms and causes the cell protoplasm to be released into the soil sample. These organic compounds can be extracted and measured for extractable N and C (N_{ext} and C_{ext}). To calculate microbial N and C in soil extract (N_{mic} and C_{mic}), non-fumigated samples (N_{ext} NF, C_{ext} NF) are subtracted from the fumigated ones (N_{ext} F, C_{ext} F) and divided by conversion factor, which determines proportion of microbial C released after fumigation, which is extractable from soil.

Soil (4 replicates) was weighed (5 g) into 100ml NTS-flasks. Two flasks were extracted (40 ml 0.5M K_2SO_4, shaken in vortex, centrifuged, filtered and frozen until the analyses) immediately (non-fumigated control) and other two were closed into a dissicator and evacuated with chloroform for 24 hours. After that, chloroform was removed and the rest of its vapor was cleared away with a vacuum pump. The samples were then processed in the same way as the non-fumigated ones. Carbon and nitrogen contents were analyzed on LiquiTOC II (Elementar, Germany).

The 2012-samples were incubated in 40 ml glass vials (2.5 g of litter, 5 g of 0-10 cm and 10-30 cm horizons) in 4 replicates and were extracted with 20 ml of 0.5M K_2SO_4. Compared to the 2011-samples that were extracted for microbial biomass measurements directly after being weighed, the 2012-samples were extracted after one week of incubation at 10°C.

Calculations:

The amount of extractable C and N (calculated for both fumigated and non-fumigated samples)

$$\text{C(N)}_{ext}\ [\mu g\ C\ (N)*\ g^{-1}\ DW] = C\ (N)_{ext}\ [mg*\ l^{-1}] * m/(V * DW)$$

C $(N)_{ext}$ [mg * l^{-1}].......C(N) in the soil extract, data from the analyzer

(* dilution – 10× or 20×)

V............................... volume of the extractant [ml]

m...............................wet soil weight [g]

DW…………….……dry weight of the soil

The amount of microbial C and N

$$\mathbf{C_{mic}\ [\mu g\ C^*\ g^{-1}\ DW] = [C_{ext}\ (F) - C_{ext}\ (NF)]\ /0.38}\ \text{(similarly for N}_{mic})$$

C_{mic}........................microbial C concentration $[\mu g\ C^*\ g^{-1}\ DW]$

C_{ext} (F) ……………extractable C concentration in fumigated sample $[\mu g\ C^*\ g^{-1}\ DW]$

C_{ext} (NF) …………extractable C concentration in control sample $[\mu g\ C^*\ g^{-1}\ DW]$

0.38.......................conversion factor for C flush (Vance et al., 1987)

0.54………………..conversion factor for N flush (Vance et al., 1987)

5.3 Statistical analysis of the data set

Raw data were processed and all studied soil characteristics were calculated in MS Office Excell 2007 (Microsoft). For the statistical analysis only mean values for laboratory replicates were used. All data (except of values of ammonification and nitrification rate) were log-transformed to ensure the normal distribution. The values of ammonification and nitrification rates were negative in several cases, which made impossible to use this correction. The statistical analysis was processed in the programme Statistica for Windows 9.1 (Statsoft Inc.) with the use of ANOVA test, namely General Linear Model (GLM) analysis which allows us to include hierarchic design and interactions of parameters as well. The hierarchy between site and treatment *site(management)* takes into account the fact that the sites within the same management (non-intervention *S,* as well as clear-cut *P*) can differ much more than sites with different management. The effect of different parameters and their interactions were also analyzed (*management*year, dominant*management, dominant*management*year, dominant*year*). The parameter *site* was random, while the other variables (*dominant, management, year*) were fixed. The analyses were supplemented and checked with the multiple comparisons of means (Tukey HSD test).

The data for each horizon were analyzed separately, for the effect of horizon across the whole dataset was too strong and suppressed significant effects of all other factors. All data in graphs are presented without the log-transformation.

Several values differed markedly from others and were excluded from the statistical analysis:

i) litter: N_{ext} and N_{mic} concentration under *Calamagrostis* at P2 (2011), C_{mic} concentration under moss at P2 (2011), microbial C/N ratios under moss and *Vaccinium* at S7 (2012), under *Avenella* at S5 (2012) and under *Vaccinium* at S3 (2012)

ii) 0-10-cm horizon: microbial C/N ratio under dead wood at S5 and under moss at S7 (both 2012)

iii) 10-30-cm horizon: N_{mic} concentrations under *Avenella* S3 and under *Vaccinium* at S7 (both 2011)

Calamagrostis at P2 was excluded because of markedly low concentration of extractable N (N_{ext}) and high microbial N concentration (N_{mic}) in litter. N_{ext} concentration was even lower than the N_{ext} concentration in the 10-30-cm horizon under the very same dominant and it is very likely a result of errors during samples processing. The low value of N_{ext} affected the

high value of N_{mic} that was one order of magnitude higher than in other samples. Microbial carbon concentration (C_{mic}) in litter under moss at P2 2011 was again one order of magnitude higher than other samples. N_{mic} concentration in soil under *Avenella* S3 and *Vaccinium* S7 (both 2011) were <0, which suggests improper fumigation. Values of the microbial C/N ratio excluded from the statistical analysis were considerably higher than the values of other samples.

6 RESULTS

6.1 C/N ratio of soil

The C/N ratio of the Březník soils ranged from 20.5 to 41.5 (28.4 ± 4.8 (for mean values see Table 2). Soil C/N ratio was significantly affected by horizon (F=18.33, p=0.000001, DF=2). It decreased in order from 10-30 cm > litter > 0-10 cm. Despite high variability within the non-intervention sites, C/N ratio was significantly affected by site and management (F=3.56, p=0.012096, DF=4) and was lower at the clear-cut plots and S5 (see Figure 4).

The effect of dominant was not significant. However, the lowest C/N ratio was found in soil under moss and *Calamagrostis* at clear-cut plots (**23.2** ± 1.88 and **23.3** ± 0.96, respectively), whereas *Vaccinium* and *Avenella* at non-intervention plots had the highest C/N values (**34.5** ± 4.73 and **35.0** ± 7.72, respectively). The concentrations of C_{tot} and N_{tot} in soil were positively correlated (Figure 5).

Table 2. Soil C/N ratio under five dominants. Mean values (± s.d., n=3) are given for the two different managements. The abbreviations stand for: Bo-*Vaccinium myrtillus*, D-dead wood, M-*Avenella flexuosa*, Mch-moss, T-*Calamagrostis villosa*, S-non-intervention plots, P-clear-cut plots.

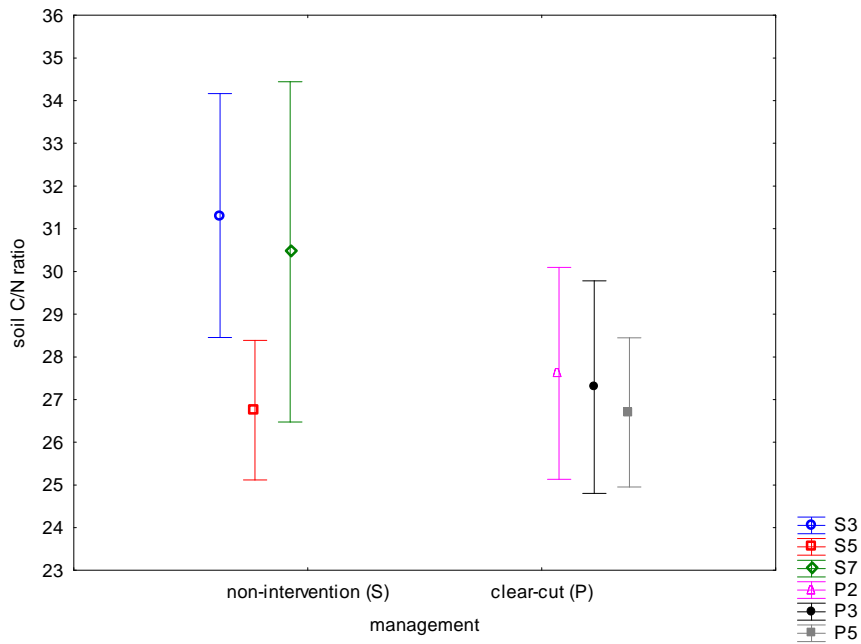| dominant	site	litter	0-10 cm	10-30 cm
Bo	S	**30.6** ± 1.7	**27.5** ± 0.9	**34.5** ± 4.7
	P	**29.3** ± 1.7	**25.0** ± 3.7	**28.8** ± 2.3
D	S	**30.6** ± 2.3	**26.8** ± 0.8	**32.3** ± 6.1
	P	**31.4** ± 3.7	**24.8** ± 2.0	**26.6** ± 2.7
M	S	**25.0** ± 2.5	**26.0** ± 2.0	**35.0** ± 7.7
	P	**24.9** ± 1.9	**25.1** ± 1.5	**31.8** ± 3.8
Mch	S	**33.6** ± 3.6	**27.8** ± 2.4	**33.0** ± 6.2
	P	**30.1** ± 4.3	**23.2** ± 1.9	**29.8** ± 3.5
T	S	**25.3** ± 0.8	**24.8** ± 0.4	**32.8** ± 5.4
	P	**24.8** ± 0.4	**23.3** ± 1.0	**28.1** ± 1.8

Figure 4. The effect of site and management on soil C/N ratio.
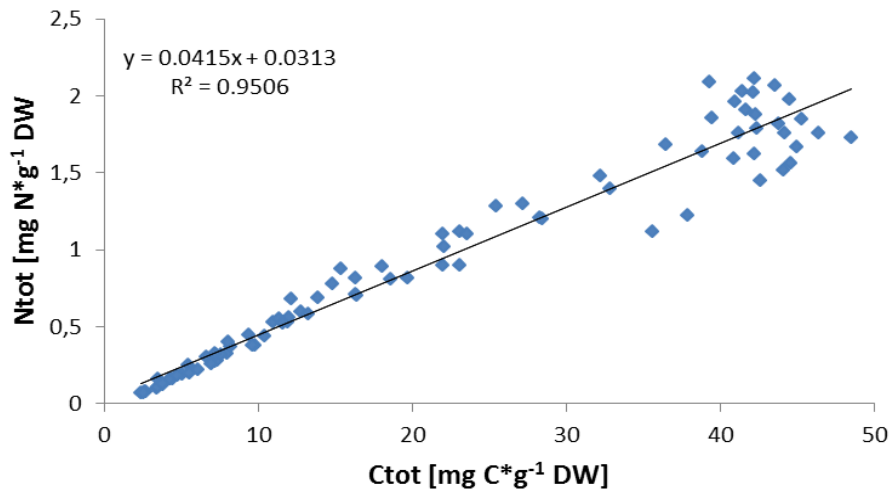

Figure 5. The correlation between concentrations of C_{tot} and N_{tot} in soil.

6.2 Extractable carbon concentration (C_{ext})

The distribution of C_{ext} was quite uniform being the highest in the litter layer and decreasing along the soil profile. Mean values of C_{ext} concentration in litter for 2011 (and 2012) were 824.1 ± 150.1 (1108.7 ± 221.1) and 930.2 ± 225.4 (1247.3 ± 208.0) µg C*g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 0-10-cm horizon mean values for 2011 (and 2012) were 348.61 ± 92.3 (362.8 ± 187.5) and 409.5 ± 64.9 (500.5 ± 161.5) µg C*g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 10-30-cm

horizon mean values for 2011 (and 2012) were 219.1 ± 55.0 (235.6 ± 123.1) and 237.6 ± 52.9 (250.4 ± 67.8) µg $C*g^{-1}$ DW at non-intervention and clear-cut plots, respectively.

Extractable carbon in the litter layer was significantly affected by management (F=33.8, p=0.004363, DF=1) in favor of clear-cut plots (Figure 6). The high numbers of clear-cut plots are consistent in both years.

The effect of management alone in the two lower layers was not significant due to high variability among the non-intervention sites. Despite this variability, concentration of extractable carbon in the two lower horizons was significantly affected by site and management (0-10-cm layer: F= F=10.277, p=0.000012, DF=4; 10-30-cm layer: F=9.164, p=0.000033, DF=4) (Figure 7 and 8). Clear-cut plots together with S5 had higher C_{ext} concentrations than S7 and S3. Similar pattern was found in other characteristics as well (e.g. C_{mic} or N_{ext} in the 0-10-cm horizon).
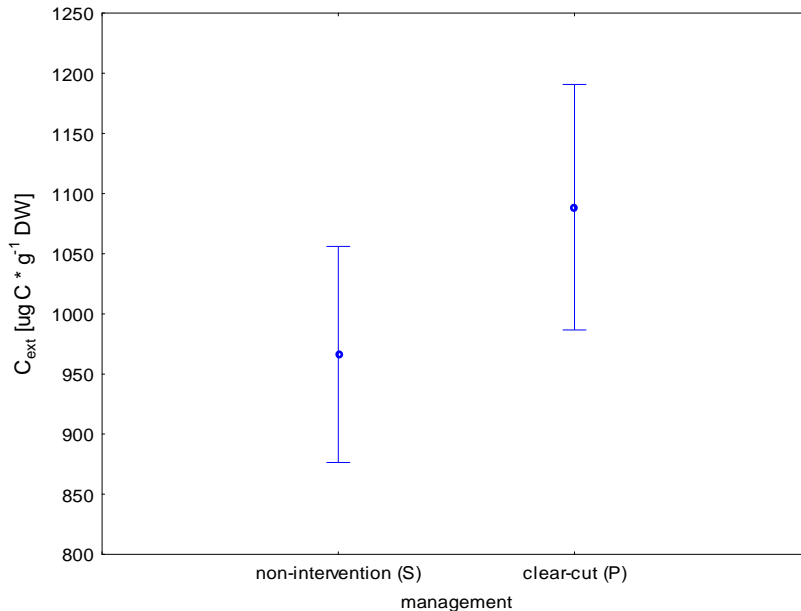

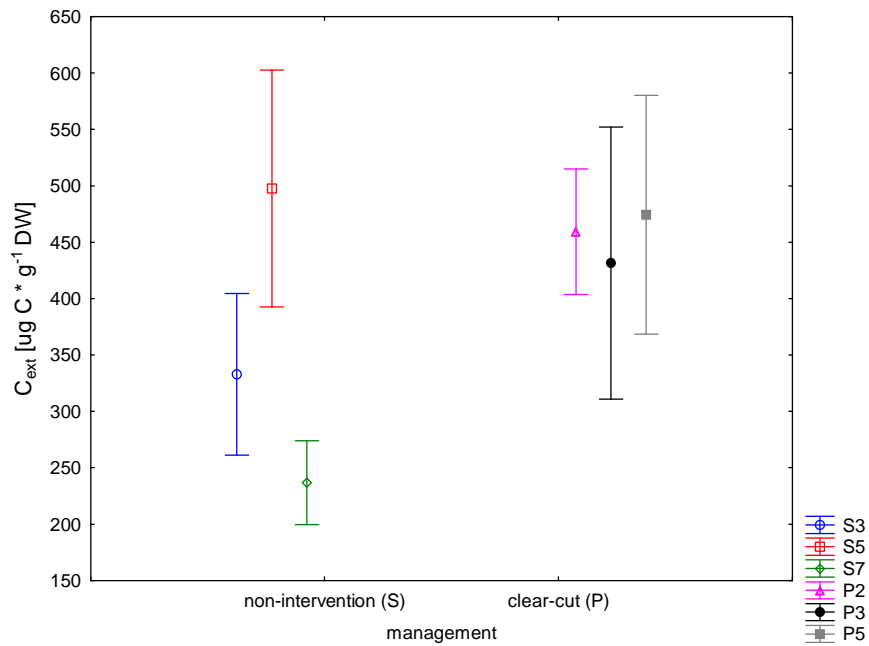**Figure 6**. The effect of management on C_{ext} concentration in litter.

Figure 7. The effect of site and management on C_{ext} concentration in the 0-10-cm layer.
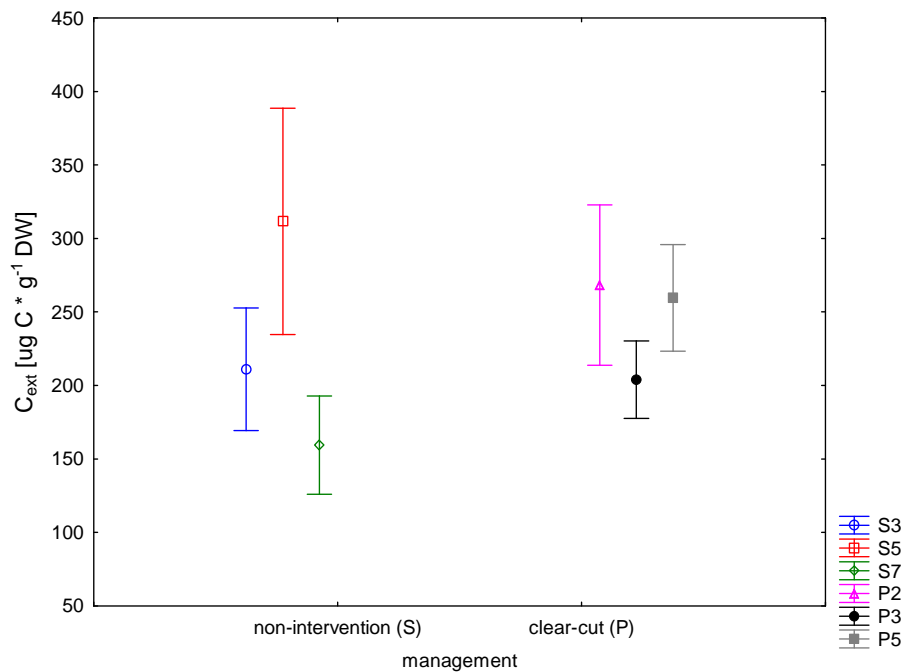

Figure 8. The effect of site and management on C_{ext} concentration in the 10-30-cm layer.

In the litter layer, the effect of dominant was only significant in interaction with year (F=3.25, p=0.022552, DF=4). Dead wood didn't differ from other dominants in 2012 (except of moss) but was significantly higher in concentration of C_{ext} than all dominants in 2011. For mean concentrations of C_{ext} and other characteristics under all dominants in the three soil horizons see Tables 3-5.

There was no significant effect of dominant on concentrations of extractable carbon in the two lower layers.

Concentrations of microbial C and N were positively correlated with concentration of extractable (see Figure 9 and Figure 10 for C_{mic} and N_{mic}, respectively).
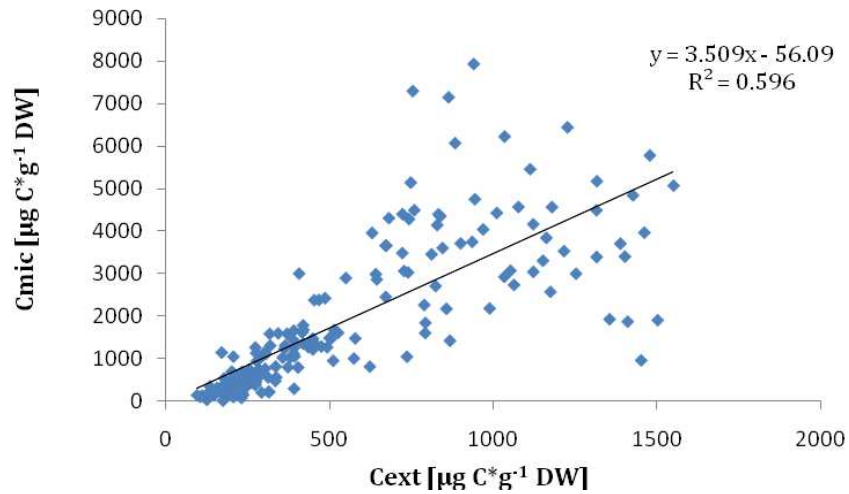

Figure 9. Positive correlation between concentrations of extractable C in soil (C_{ext}) and C bound to microbial biomass (C_{mic}). The graph comprises the data from 2011 and 2012 and from all three horizons.
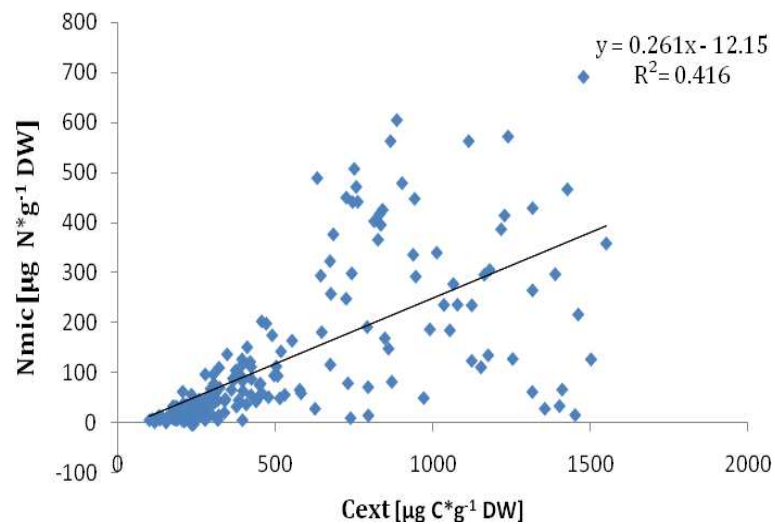

Figure 10. Positive correlation between concentrations of extractable C in soil and N bound to microbial biomass. The graph comprises data from both sampling years and from all three horizons. Note that, compared to microbial C (Figure 9), the scale is different reflecting lower concentrations of N bound to microbial biomass compared to microbial C.

Table 3. Concentrations of extractable C and N (C_{ext}, N_{ext}) and of microbial C and N (C_{mic}, N_{mic}) in **litter**. Mean values (± s.d., n=3) for the five dominants are given. For abbreviation see Table 2.

dominant	site	C_{ext} [µg C*g^{-1} DW]		N_{ext} [µg N*g^{-1} DW]		C_{mic} [µg C*g^{-1} DW]		N_{mic} [µg N*g^{-1} DW]	
		2011	2012	2011	2012	2011	2012	2011	2012
Bo	S	**743** ± 67	**1298** ± 91	**90** ± 32	**413** ± 36	**4153** ± 348	**4155** ± 531	**390** ± 52	**133** ± 122
	P	**851** ± 187	**1045** ± 79	**196** ± 59	**218** ±98	**4753** ± 504	**3788** ± 623	**461** ± 77	**269** ± 47
D	S	**793** ± 191	**1318** ± 125	**256** ± 63	**278** ± 83	**3130** ± 389	**2068** ± 961	**277** ± 15	**51** ± 42
	P	**845** ± 110	**1456** ± 37	**231** ± 63	**440** ± 152	**2795** ± 535	**2584** ± 978	**267** ± 75	**136** ± 62
M	S	**856** ± 32	**964** ± 205	**336** ± 54	**263** ± 92	**4069** ± 266	**2818** ± 730	**441** ± 27	**104** ± 65
	P	**935** ± 350	**1167** ± 124	**325** ± 98	**443** ± 89	**4647** ± 502	**4060** ± 1092	**488** ± 17	**301** ± 123
Mch	S	**813** ± 90	**852** ± 98	**144** ± 80	**180** ± 58	**6502** ± 1589	**3095** ± 762	**454** ± 13	**92** ± 41
	P	**1109** ± 173	**1097** ± 158	**150** ± 104	**118** ± 60	**9495** ± 3831	**3691** ± 774	**517** ± 72	**264** ± 23
T	S	**915** ± 213	**1112** ± 45	**382** ± 70	**501** ± 238	**2944** ± 520	**3694** ± 458	**292** ± 80	**202** ± 71
	P	**910** ± 93	**1471** ± 66	**287** ± 180	**449** ±223	**5250** ± 1269	**4853** ± 860	**847** ± 491	**449** ± 174

Table 4. Concentrations of extractable C and N (C_{ext}, N_{ext}) and of microbial C and N (C_{mic}, N_{mic}) in **the 0-10-cm horizon.** Mean values (± s.d., n=3) for the five dominants are given. For abbreviation see Table 2.

dominant	site	C_{ext} [µg C*g^{-1} DW]		N_{ext} [µg N*g^{-1} DW]		C_{mic} [µg C*g^{-1} DW]		N_{mic} [µg N*g^{-1} DW]	
		2011	2012	2011	2012	2011	2012	2011	2012
Bo	S	**296** ± 20	**272** ± 100	**32** ± 11	**55** ± 27	**984** ± 296	**1575** ± 1037	**56** ± 19	**66** ± 61
	P	**392** ± 72	**440** ± 48	**49** ± 13	**81** ± 49	**1231** ± 319	**1470** ± 170	**92** ± 38	**88** ± 4
D	S	**378** ± 82	**437** ± 245	**76** ± 25	**79** ± 47	**1198** ± 116	**894** ± 393	**64** ± 23	**21** ± 15
	P	**402** ± 25	**703** ± 183	**66** ± 8	**154** ± 17	**1574** ± 196	**1496** ± 80	**106** ± 15	**76** ± 4
M	S	**454** ± 123	**341** ± 169	**75** ± 16	**52** ± 19	**1085** ± 308	**943** ± 381	**72** ± 31	**31** ± 20
	P	**474** ± 30	**554** ± 115	**98** ± 20	**98** ± 21	**1844** ± 388	**1710** ± 857	**133** ± 61	**100** ± 63
Mch	S	**312** ± 47	**378** ± 212	**55** ± 31	**86** ± 59	**1156** ± 341	**1330** ± 852	**70** ± 25	**51** ± 47
	P	**444** ± 39	**345** ± 48	**62** ± 20	**48** ± 24	**1771** ± 498	**1011** ± 48	**123** ± 40	**59** ± 7
T	S	**303** ± 2	**386** ± 133	**60** ± 3	**131** ± 89	**1001** ± 167	**1342** ± 230	**71** ± 24	**49** ± 10
	P	**336** ± 41	**462** ± 77	**75** ± 18	**98** ± 35	**1129** ± 372	**2175** ± 694	**99** ± 28	**150** ± 46

Table 5. Concentrations of extractable C and N (C_{ext}, N_{ext}) and of microbial C and N (C_{mic}, N_{mic}) in **the 10-30-cm horizon.** Mean values ($\pm$ s.d., n=3) for the five dominants are given. For abbreviation see Table 2.

dominant	site	C_{ext} [µg C*g⁻¹ DW]		N_{ext} [µg N*g⁻¹ DW]		C_{mic} [µg C*g⁻¹ DW]		N_{mic} [µg N*g⁻¹ DW]	
		2011	2012	2011	2012	2011	2012	2011	2012
Bo	S	263 ± 37	274 ± 130	18 ± 6	15 ± 5	308 ± 183	598 ± 554	16 ± 21	23 ± 26
	P	285 ± 82	266 ± 56	22 ± 5	29 ± 15	386 ± 62	646 ± 57	20 ± 11	46 ± 14
D	S	238 ± 37	214 ± 85	21 ± 9	18 ± 10	382 ± 182	228 ± 97	21 ± 11	8 ± 3
	P	221 ± 34	254 ± 52	30 ± 6	32 ± 6	506 ± 119	443 ± 164	24 ± 15	27 ± 7
M	S	198 ± 49	277 ± 171	17 ± 10	17 ± 6	190 ± 187	441 ± 367	22 ± 25	18 ± 22
	P	235 ± 25	306 ± 96	20 ± 4	27 ± 8	350 ± 61	532 ± 202	14 ± 6	26 ± 8
Mch	S	163 ± 16	245 ± 92	16 ± 9	25 ± 18	247 ± 119	364 ± 328	13 ± 13	16 ± 11
	P	221 ± 34	210 ± 21	22 ± 9	16 ± 8	310 ± 93	320 ± 14	17 ± 5	19 ± 4
T	S	234 ± 60	168 ± 77	18 ± 5	22 ± 16	350 ± 117	240 ± 108	25 ± 9	5 ± 0
	P	226 ± 37	217 ± 37	33 ± 11	30 ± 12	477 ± 101	510 ± 190	31 ± 10	30 ± 7

6.3 Microbial carbon (C_{mic})

Similarly to C_{ext} concentration, the distribution of microbial C (C_{mic}) was quite uniform being the highest in the litter layer. As mentioned above, concentration of C_{mic} was positively correlated with concentration of extractable C (Figure 9). The mean values of C_{mic} concentrations in the litter for 2011 (and 2012) were 4159.5 ± 1494.7 (3165.8 ± 1011.5) and 5387.9 ± 2885.7 (3795.2 ± 1144.0) µg C*g⁻¹ DW at non-intervention and clear-cut plots, respectively.

For the 0-10-cm horizon the mean values for 2011 (and 2012) were 1084.8 ± 273.2 (1216.7 ± 705.6) and 1509.8 ± 465.6 (1572.3 ± 627.2) µg C*g⁻¹ DW at non-intervention and clear-cut plots, respectively. For the 10-30-cm horizon the mean values for 2011 (and 2012) were 295.3 ± 175.4 (374.3 ± 364.4) and 405.6 ± 116.8 (490.2 ± 181.4) µg C*g⁻¹ DW at non-intervention and clear-cut plots, respectively.

The effect of management alone was not significant because of the high variability among plots of the same management. The combined effect of site and management was significant in all three horizons. In litter (F=5.88, p=0.000997, DF=4), S5 plot was significantly lower than P2 and P5 plots (Figure 11). In both the 0-10-cm layer (F=7.22, p=0.000225, DF=4) and the 10-30-cm layer (F=15.57, p=0.000000, DF=4), S7 plot had the lowest C_{mic} concentrations (see Figure 12 and 13).

The effect of dominant (for mean values of C_{mic} under dead wood and four plant dominants see Tables 3-5) was significant only in litter (F=13.50, p=0.000001, DF=4) (Figure 14) as

well as the effect of dominant*year (F=8.20, p=0.00089, DF=4). Dead wood was significantly lower than all other dominants which were not significantly different from each other.

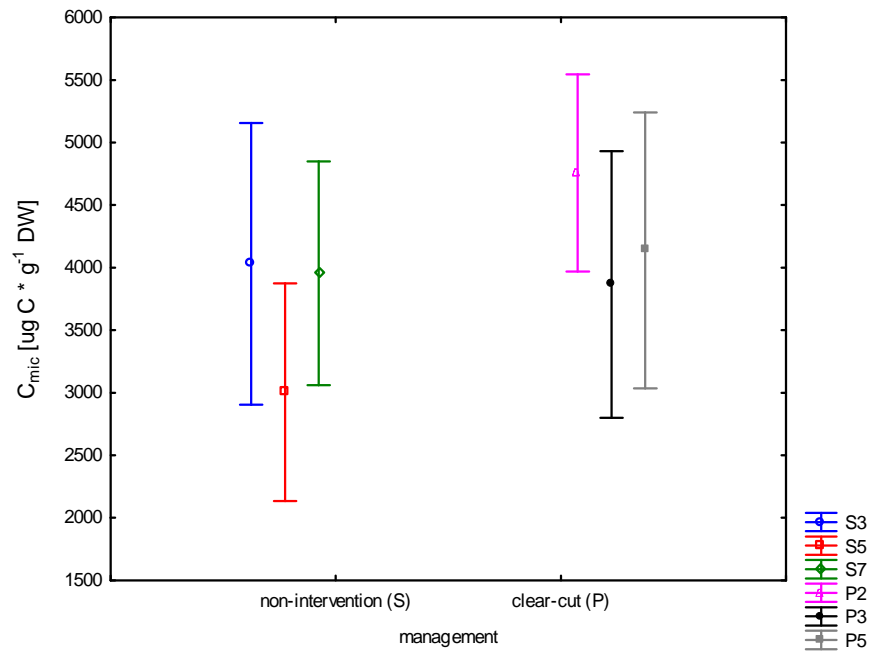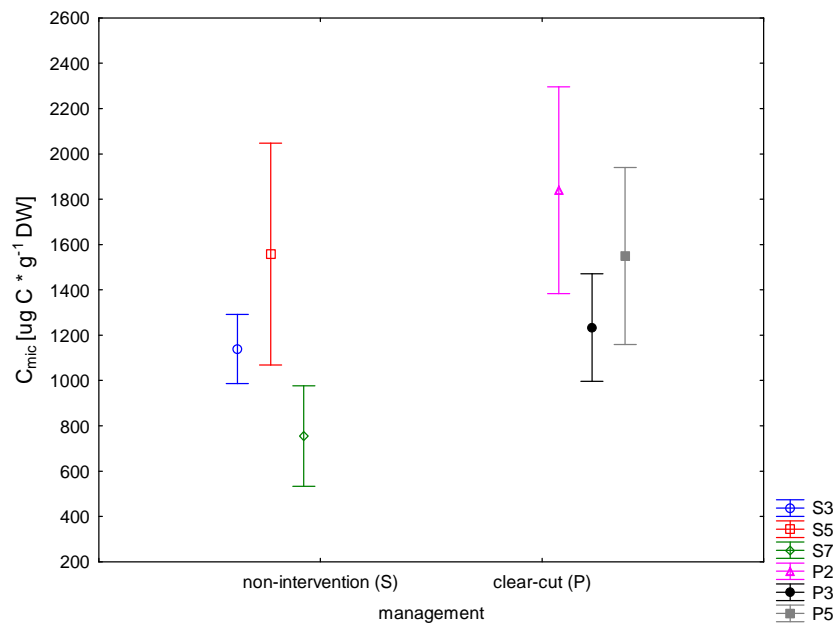**Figure 11**. The effect of site and management on C_{mic} concentration in the litter layer.

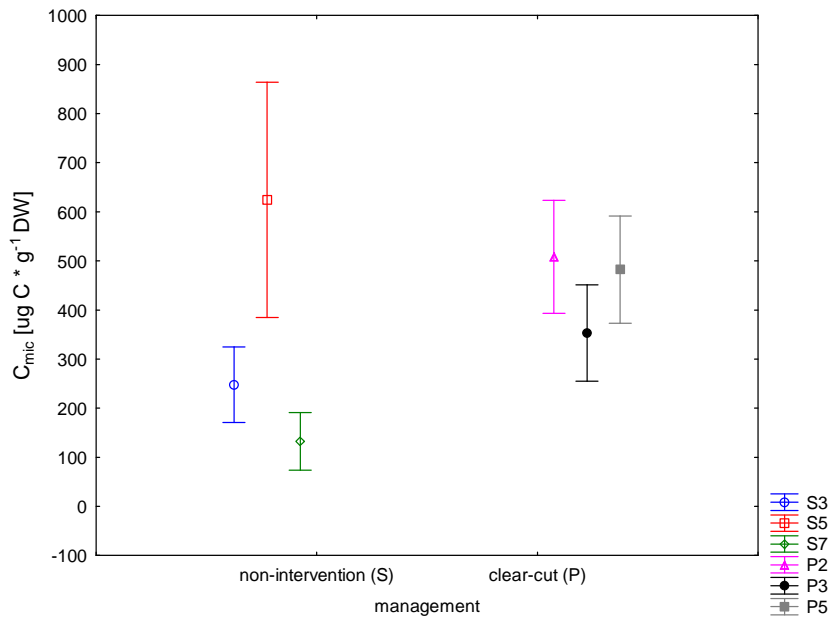**Figure 12.** The effect of site and management on C_{mic} concentration in the 0-10-cm layer.

Figure 13. The effect of site and management on C_{mic} concentration in the 10-30-cm layer.
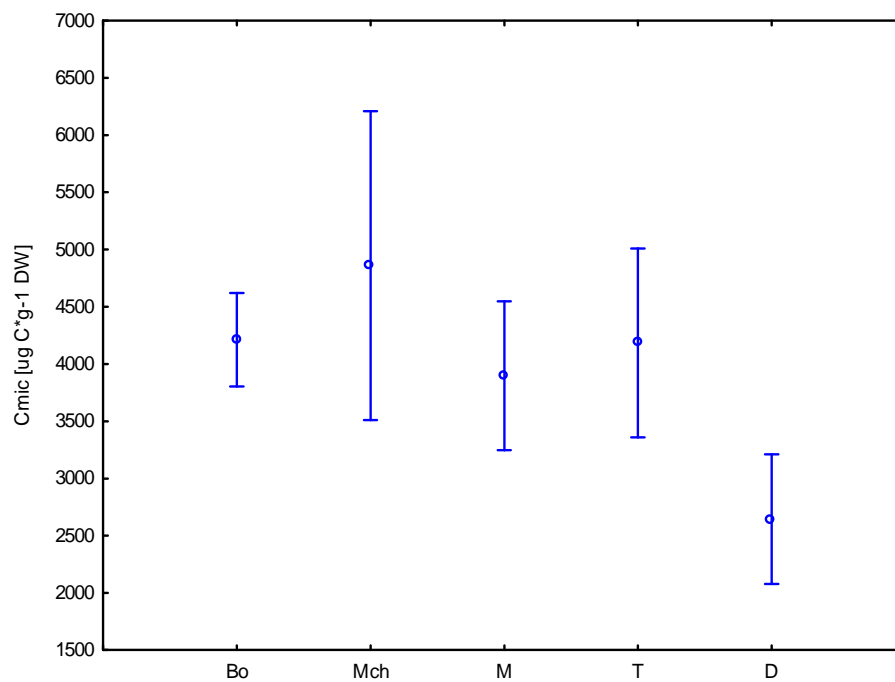

Figure 14. Microbial carbon concentration (C_{mic}) in litter as affected by dominant. For abbreviations see Table 2.

6.4 Extractable nitrogen concentration (N_{ext})

The distribution of extractable N (N_{ext}) was relatively uniform being the highest in the litter layer and decreasing along the soil profile. The mean values of N_{ext} concentrations

in the litter layer for 2011 (and 2012) were 241.5 ± 127.0 (326.9 ± 168.8) and 237.8 ± 126.4 (333.4 ± 195.1) µg N*g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 0-10-cm horizon the mean values for 2011 (and 2012) were 59.3 ± 25.4 (80.4 ± 61.3) and 70.1 ± 23.1 (95.9 ± 46.3) µg N*g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 10-30-cm horizon the mean values for 2011 (and 2012) were 17.9 ± 8.1 (19.2 ± 12.6) and 25.5 ± 8.9 (26.7 ± 11.7) µg N*g^{-1} DW at non-intervention and clear-cut plots, respectively. Management alone did not significantly affect the extractable nitrogen concentration in any of the three horizons due to high variability within the non-intervention plots. Management and site, on the other hand, affected significantly N_{ext} concentration in the two lower horizons (0-10-cm: F=11.86, p=0.000003, DF=4; 10-30-cm: F=23.31, p=0.0000000, DF=4) (Figure 15 and 16). In both layers the pattern is similar as for C_{ext} concentration – S7 has the lowest concentration compared to all other plots.
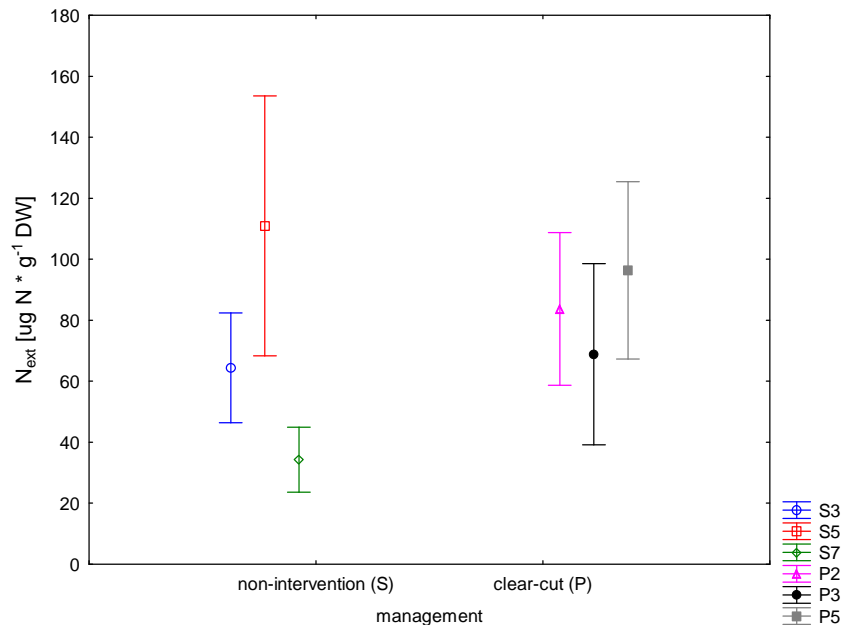

Figure 15. The effect of site and management on N_{ext} concentration in the 0-10-cm layer.
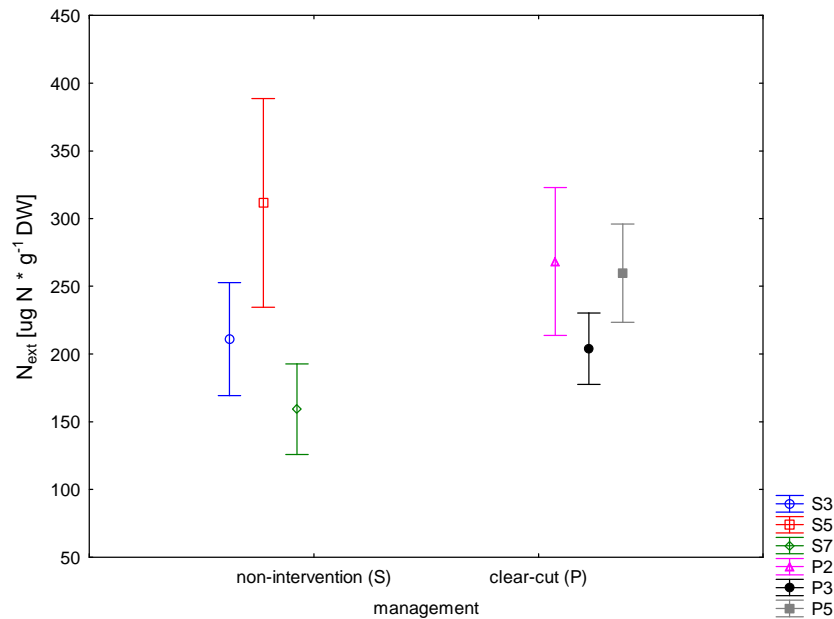
Figure 16. The effect of site and management on N_{ext} concentration in the 10-30-cm layer.

Dominant had a significant effect on N_{ext} concentration in the litter layer (for concentrations of N_{ext} under all dominants see Tables 3-5) (F=9.19, p=0.000035, DF=4), as well as in the 0-10-cm layer (F=5.26, p=0.001925, DF=4). In litter, moss together with *Vaccinium* had significantly lower N_{ext} concentration compared to dead wood and both grass species (Figure 17). In the 0-10-cm layer, *Vaccinium* had significantly lower N_{ext} concentrations compared to both grass species and dead wood (Figure 18). There was no significant effect of dominant on N_{ext} concentration in the 10-30-cm horizon.
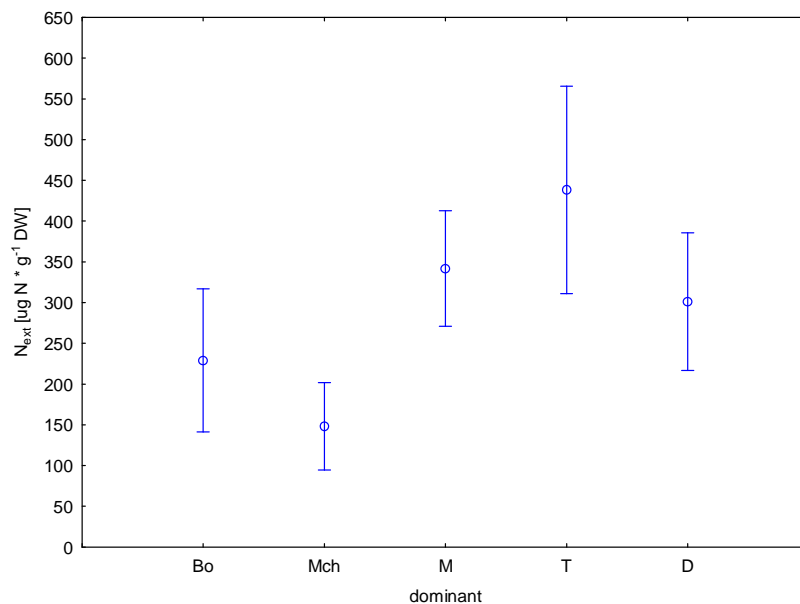

Figure 17. The effect of dominant on N_{ext} concentration in the litter layer. For abbreviation see Table 2.
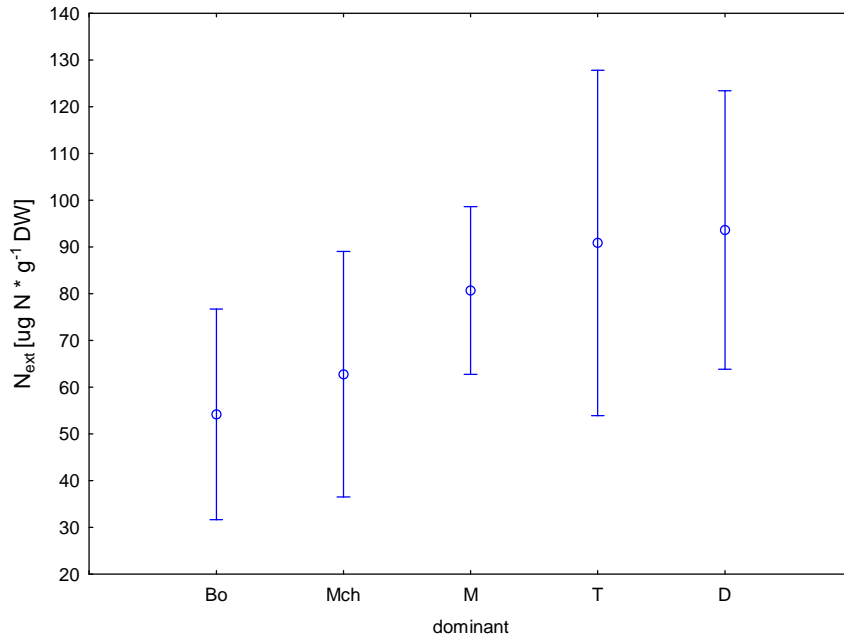
Figure 18. The effect of dominant on N_{ext} concentration in the 0-10-cm layer. For abbreviation see Table 2.

6.5   Microbial nitrogen concentration (N_{mic})

Similar to C_{ext} and C_{mic}, the distribution of N bound to microbial biomass was relatively uniform being the highest in the litter layer and decreasing along the soil profile. Mean concentration of N_{mic} in litter for 2011 (and 2012) was 370.7 $\pm$ 86.6 (116.3 $\pm$ 89.9) and 443.4 $\pm$ 118.2 (284.0 $\pm$ 142.8) µg N*g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 0-10-cm horizon the mean values for 2011 (and 2012) were 66.7 $\pm$ 25.2 (43.5 $\pm$ 39.7) and 110.6 $\pm$ 42.3 (94.8 $\pm$ 46.6) µg N*g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 10-30-cm horizon mean values for 2011 (and 2012) were 19.5 $\pm$ 17.5 (14.2 $\pm$ 17.2) and 21.2 $\pm$ 11.4 (29.3 $\pm$ 12.3) µg N*g^{-1} DW at non-intervention and clear-cut plots, respectively. For mean concentrations of N_{mic} under all dominants see Tables 3-5. Microbial N concentration in the litter layer was significantly affected by management (F=32.12, p=0.004685, DF=1) (Figure 19). N_{mic} was significantly higher at clear-cut than at managed plots. In the 0-10-cm layer, the effect of management was nearly significant (F=7.59, p=0.05115, DF=1). Similarly to the litter layer, N_{mic} concentrations were higher at the clear-cut plots. The combination of site and management had significant effect on N_{mic} concentrations in the two lower horizons. In the 0-10-cm layer (F=3.49, p=0.016523, DF=4), there was again the similar pattern as for N_{ext} and C_{ext} (Figure 20). S7 plot was significantly lower than all other plots except of S3. In the 10-30-cm layer (F=4.76, p=0.003715, DF=4), both S7 and S3 plots have significantly lower concentrations of N_{mic} (Figure 21).
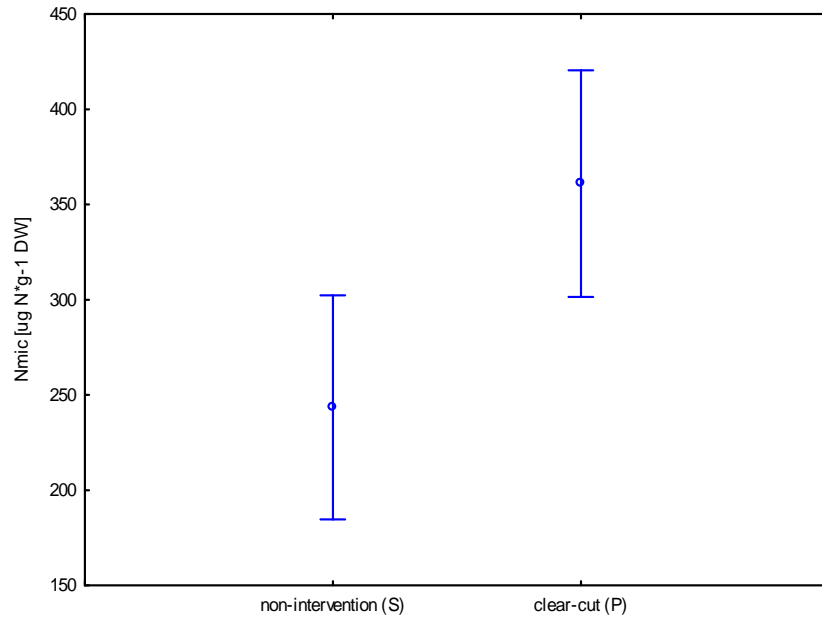
Figure 19. Effect of management of concentration of microbial N in litter.
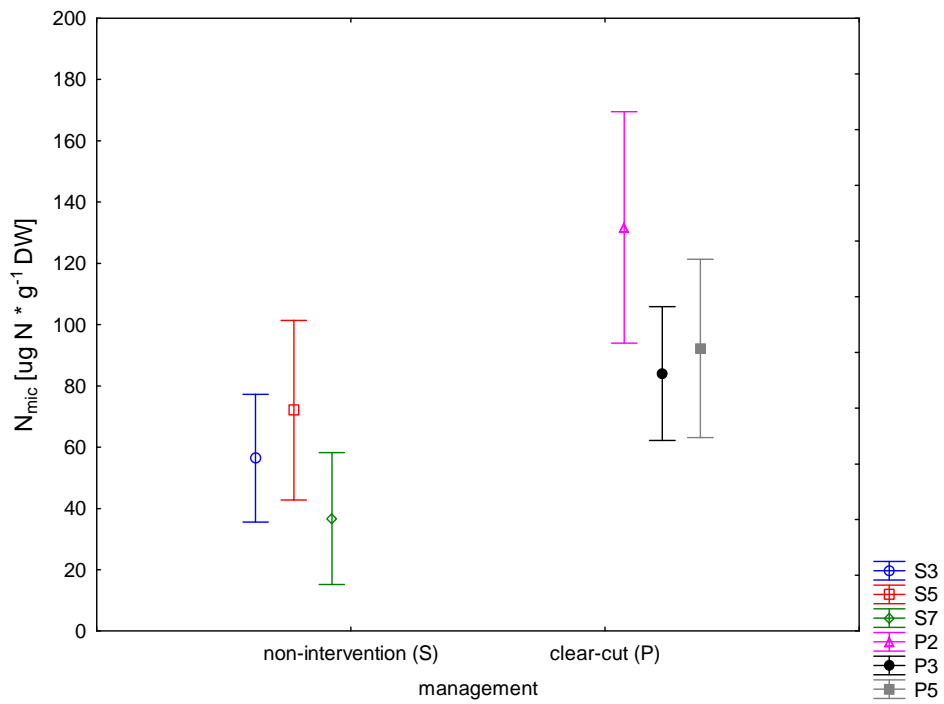

Figure 20. Effect of site and management of concentration of microbial N in the 0-10-cm layer.
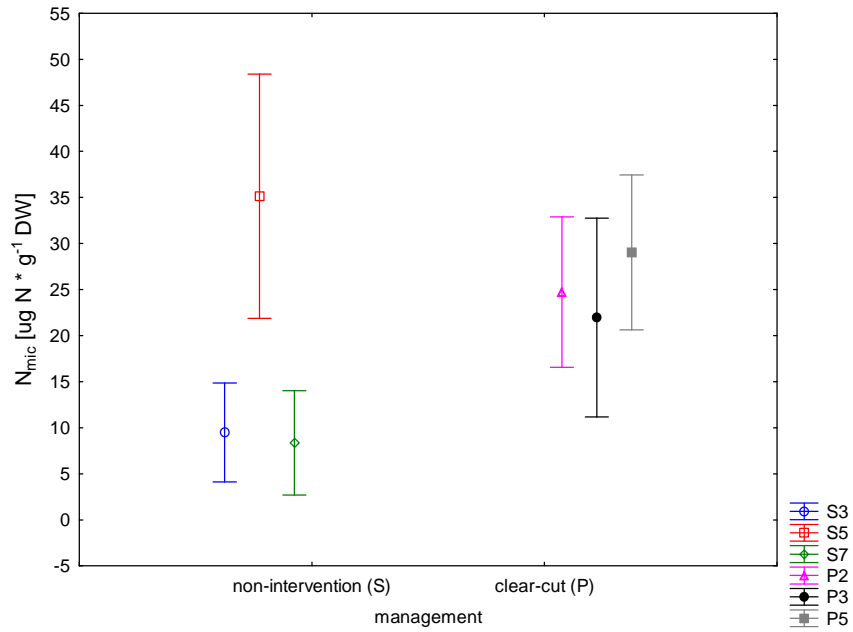
Figure 21. Effect of site and management of concentration of microbial N in the 0-10-cm layer.

The effect of dominant was significant only in the litter layer (F=4.24, p=0.006634, DF=4) where under dead wood the concentration of N_{mic} was significantly lower (Figure 22). The differences among other dominants were insignificant. For mean concentrations of N_{mic} under four plant dominants and dead wood see Table 3-5.

The N_{mic} concentration was positively correlated with concentration of organic carbon in soil in both years (C_{ext}) (see Figure 10).

Figure 22. The effect of dominant on Nmic concentration in the litter layer. For abbreviations see Table 2.

6.6 C/N ratio of the microbial biomass

Mean values of microbial C/N ratio in litter for 2011 (and 2012) were 11.2 ± 2.5 (44.6 ± 33.1*) and 11.3 ± 4.7 (15.1 ± 4.6) at non-intervention and clear-cut plots, respectively. For the 0-10-cm horizon the mean values for 2011 (and 2012) were 17.3 ± 3.7 (34.5 ± 13.9*) and 14.5 ± 4.0 (17.7 ± 4.0) at non-intervention and clear-cut plots, respectively. For the 10-30-cm horizon mean values for 2011 (and 2012) were 15.8 ± 7.3* (33.3 ± 17.3*) and 24.6 ± 13.2 (17.3 ± 4.2) at non-intervention and clear-cut plots, respectively. The asterisk (*) indicates that outlying values (extremely high or <0) were among the replicates. They were removed from the mean calculation and also excluded from the statistical analysis. For mean values of C/N_{mic} under all dominants see Table 6.

Table 6. Mean C/N ratio under the five dominants in all three horizons. The asterisk (*) indicates cases with C/N ratio<0. These values were excluded from the mean calculation and from the statistical analysis. For abbreviation see Table 2.

		litter		0-10-cm		10-30-cm	
		2011	2012	2011	2012	2011	2012
B	S	10.7±0.7	63.5±36.3	18.2±2.0	32.8±9.8	22.9±10,1*	37.9±12.4
	P	10.4±0.7	14.5±3.5	14.7±3.0	16.7±1.8	30.3±21.1	15.5±5.4
Mch	S	14.3±3.4	45.3±27.9	16.8±1.4	44.1±23.4	18.1±4.8	19.7±12.5
	P	18.1±5.7	13.8±1.7	14.6±0.8	17.2±1.4	20.3±8.5	17.84±3.8
M	S	9.3±1.1	58.1±50.4	16.1±2.7	36.5±10.1	5.4±2.7*	80.8±65.3*
	P	9.5±1.0	14.7±3.1	16.8±7.1	20.4±6.4	28.5±9.0	20.4±3.8
T	S	10.4±1.1	21.1±9.0	15.1±3.2	27.9±2.3	14.0±1.2	45.3±20.5
	P	7.6±2.5	11.7±2.4	11.5±2.3	14.6±2.3	16.8±5.1	16.6±3.0
D	S	11.4±2.0	54.3±17.4	20.5±5.3	58.7±40.9	18.4±1.1	28.7±11.2
	P	11.1±2.8	20.6±5.7	14.9±1.2	19.8±2.2	27.3±10.4	16.1±2.6

Microbial C/N ratio was significantly affected by management in litter (F=10.501, p=0.028019, DF=1) and in the 0-10-cm horizon (F=22.285, p=0.009167, DF=1) and was significantly higher at the non-intervention plots (Figure 23 and 24).

Dominant had a significant effect on microbial C/N ratio only in the litter layer (F=5.329, p=0.002098, DF=4) (Figure 25). Microbial C/N ratio was significantly higher in soil under dead wood but was also markedly variable compared to plant dominants.

In both the litter layer (F=41.844, p<10^{-6}, DF=1) and the 0-10-cm layer (F=36.026, p=0.000001, DF=1) the effect of year was significant. C/N ratio of the microbial biomass was higher in 2012 than in 2011.

Figure 23. The effect of management on microbial C/N ratio in litter.

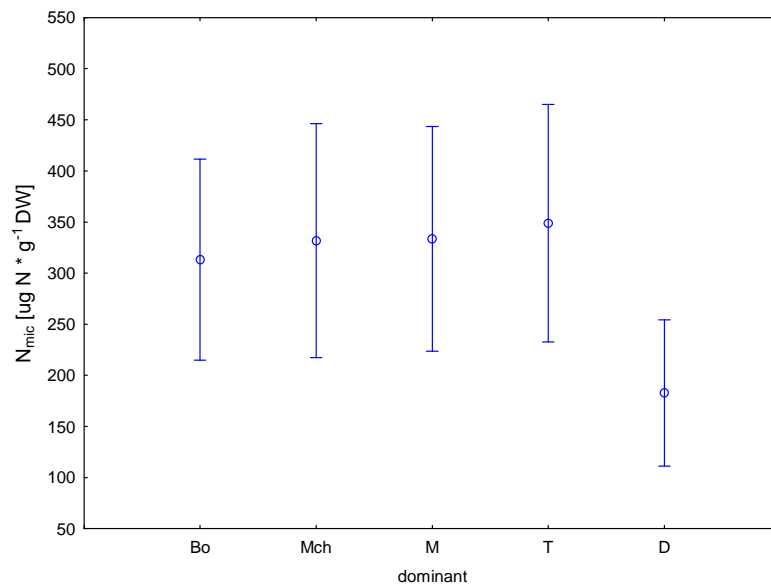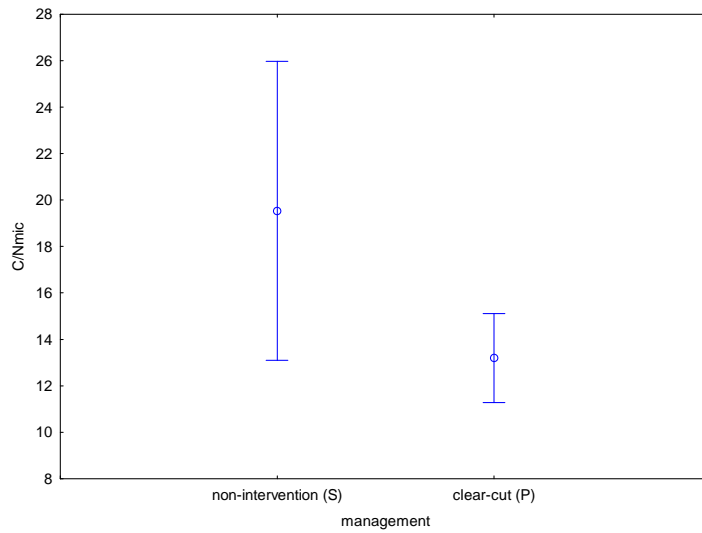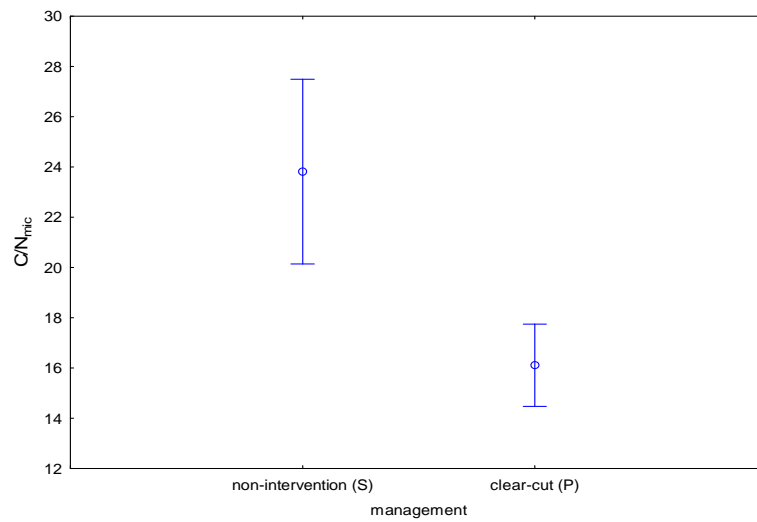**Figure 24**. The effect of management on C/N ratio of microbial biomass in the 0-10-cm layer.
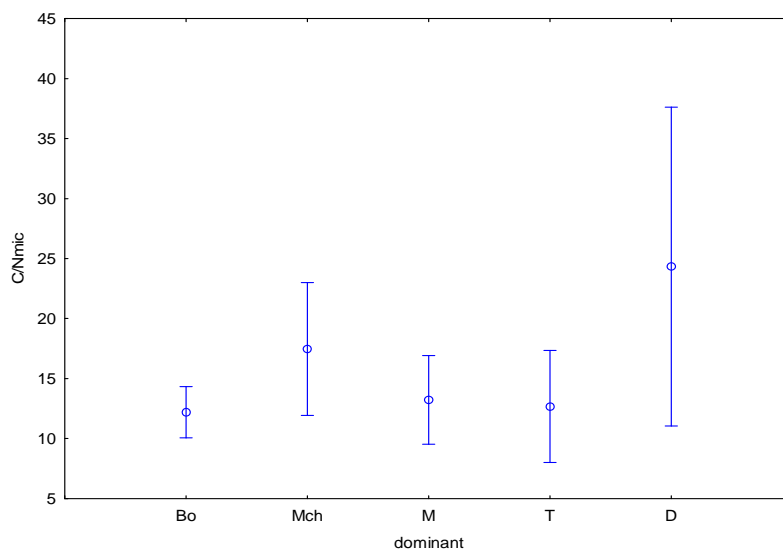

Figure 25. The effect of dominant on microbial C/N ratio in litter. For abbreviations see Table 2.

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6.7 Mineral forms of nitrogen – concentrations of ammonium and nitrates

In case of mineral forms of nitrogen (concentrations of NH_4^+ and NO_3^-) the pattern of distribution was quite different than that of C_{ext}, C_{mic}, N_{ext} and N_{mic}. In most cases, NH_4^+ concentrations were the highest in the litter and were higher than NO_3^- concentrations in this layer. NH_4^+ concentration decreased significantly along the soil profile. There were large differences between litter and the two other horizons. The concentration of nitrates in the two lower layers, on the other hand, was quite high and there were not that large differences between the three horizons. Opposite to the litter layer, in the 0-10-cm and 10-30-cm horizons the concentrations of NO_3^- were much higher than those of NH_4^+. For concentrations of NH_4^+ and NO_3^- under the five dominants see Tables 7-9.

Mean values of NH_4^+ concentration in litter for 2011 (and 2012) were 131.4 ± 93.8 (401.4 ± 249.1) and 225.6 ± 160.4 (389.3 ± 291.2) µg $N-NH_4^+$ *g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 0-10-cm horizon the mean values for 2011 (and 2012) were 22.3 ± 43.4 (28.3 ± 43.4) and 5.9 ± 6.4 (30.0 ± 35.1) µg $N-NH_4^+$ *g^{-1} DW at non-intervention and clear-cut plots, respectively. For the 10-30-cm horizon the mean values for 2011 (and 2012) were 0.5 ± 0.9 (1.4 ± 1.1) and 1.2 ± 2.8 (0.7 ± 0.3) µg $N-NH_4^+$ *g^{-1} DW at non-intervention and clear-cut plots, respectively.

Table 7. Concentrations of ammonium and nitrate and the rates of ammonification and nitrification in the litter layer. Mean values (± s.d.) are given for each dominant under the two management practices. The asterisk (*) indicates negative rates of ammonification and/or nitrification in at least one out of three replicates. For abbreviations see Table 2.

		N-NH₄⁺ [µg N-NH₄⁺*g⁻¹ DW]		NO₃⁻ [µg N-NO₃⁻*g⁻¹ DW]		ammonification rate [µg N-NH₄⁺*g⁻¹ DW *d⁻¹]		nitrification rate [µg N-NO₃⁻*g⁻¹ DW *d⁻¹]	
		2011	2012	2011	2012	2011	2012	2011	2012
Bo	S	36 ± 12	555 ± 120	18 ± 6	177 ± 83	1.6 ± 0.4	4.6 ± 1.9	0.6 ± 0.3	0.3 ± 0.2
	P	221 ± 78	305 ± 177	38 ± 30	15 ± 6	2.9 ± 1.9	1.1 ± 0.5	1.6 ± 0.9	0.2 ± 0.2*
D	S	141 ± 53	285 ± 59	122 ± 67	195 ± 118	1.9 ± 0.8	2.3 ± 0.3	1.1 ± 0.2	0.8 ± 0.3
	P	193 ± 21	411 ± 259	119 ± 69	267 ± 56	2.5 ± 1.9	2.6 ± 0.8	1.6 ± 0.2	2.2 ± 0.9
M	S	228 ± 4	332 ± 142	146 ± 35	133 ± 71	2.6 ± 0.7	2.9 ± 0.6	2.6 ± 0.6	0.7 ± 0.4
	P	242 ± 103	618 ± 116	72 ± 44	100 ± 45	-1.8 ± 2.7*	4.4 ± 1.1	4.2 ± 4.4	0.8 ± 0.2
Mch	S	59 ± 68	177 ± 115	42 ± 48	119 ± 8	2.4 ± 1.4	0.1 ± 0.1	0.8 ± 0.7	0.0 ± 0.0
	P	34 ± 42	88 ± 77	23 ± 28	41 ± 27	1.0 ± 0.8	1.3 ± 0.8	1.7 ± 1.3	0.2 ± 0.3
T	S	192 ± 95	658 ± 317	216 ± 42	250 ± 211	2.6 ± 0.8	2.9 ± 1.5	1.4 ± 0.8	0.4 ± 0.4*
	P	438 ± 181	524 ± 370	105 ± 75	239 ± 133	2.2 ± 1.1	4.0 ± 1.5	3.5 ± 1.3	4.4 ± 1.7

Table 8. Concentrations of ammonium and nitrate and the rates of ammonification and nitrification in the 0-10-cm layer. Mean values (± s.d.) are given for each dominant under the two management practices. The asterisk (*) indicates negative rates of ammonification and/or nitrification in at least one out of three replicates. For abbreviations see Table 2.

		N-NH$_4^+$ [µg N-NH$_4^+$*g^{-1} DW]		NO$_3^-$ [µg N-NO$_3^-$*g^{-1} DW]		ammonification rate [µg N-NH$_4^+$*g^{-1} DW *d^{-1}]		nitrification rate [µg N-NO$_3^-$*g^{-1} DW *d^{-1}]	
		2011	2012	2011	2012	2011	2012	2011	2012
Bo	S	**0.5** ± 0.2	**47.3** ± 40.3	**16.4** ± 7.0	**41.6** ± 4.6	0.3 ± 0.1	0.4 ± 0.4	0.2 ± 0.1	0.6 ± 0.3
	P	**2.3** ± 2.0	**62.1** ± 67.9	**35.4** ± 16.3	**50.0** ± 8.2	0.2 ± 0.3	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.1
D	S	**3.3** ± 0.7	**8.5** ± 3.5	**66.3** ± 57.5	**131.1** ± 73.0	0.1 ± 0.2*	0.03 ± 0.04	1.5 ± 0.2	0.3 ± 0.1
	P	**0.7** ± 0.1	**75.4** ± 6.7	**61.8** ± 20.8	**148.3** ± 11.0	0.1 ± 0.1*	0.5 ± 0.4	0.7 ± 0.4	0.7 ± 0.4
M	S	**8.0** ± 10.3	**10.0** ± 3.5	**62.2** ± 51.8	**67.6** ± 27.0	0.1 ± 0.0	0.2 ± 0.2	0.3 ± 0.1	1.4 ± 0.2
	P	**9.2** ± 6.4	**40.4** ± 25.1	**82.4** ± 25.2	**117.0** ± 44.1	0.1 ± 0.0	0.3 ± 0.3	0.5 ± 0.2	0.6 ± 0.7
Mch	S	**3.0** ± 0.8	**12.3** ± 5.0	**54.3** ± 68.4	**146.2** ± 119.8	0.3 ± 0.1	0.6 ± 0.3	0.5 ± 0.2	0.1 ± 0.3*
	P	**0.5** ± 0.2	**15.1** ± 13.1	**28.9** ± 16.6	**53.8** ± 37.1	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.3	0.8 ± 1.0*
T	S	**3.6** ± 4.4	**87.1** ± 70.0	**54.7** ± 24.0	**153.5** ± 95.9	0.03 ± 0.03	0.0 ± 0.0*	0.5 ± 0.1	0.2 ± 0.1
	P	**28.3** ± 35.7	**14.6** ± 8.1	**67.9** ± 9.4	**147.5** ± 47.9	0.2 ± 0.2	0.5 ± 0.5	0.7 ± 0.1	0.6 ± 0.1

Table 9. Concentrations of ammonium and nitrate and the rates of ammonification and nitrification in the 10-30-cm layer. Mean values (± s.d.) are given for each dominant under the two management practices. The asterisk (*) indicates negative rates of ammonification and/or nitrification in at least one out of three replicates. For abbreviations see Table 2.

		N-NH$_4^+$ [µg N-NH$_4^+$*g^{-1} DW]		NO$_3^-$ [µg N-NO$_3^-$*g^{-1} DW]		ammonification rate [µg N-NH$_4^+$*g^{-1} DW *d^{-1}]		nitrification rate [µg N-NO$_3^-$*g^{-1} DW *d^{-1}]	
		2011	2012	2011	2012	2011	2012	2011	2012
Bo	S	**0.2** ± 0.1	**1.7** ± 0.8	**9.9** ± 7.0	**13.1** ± 7.7	0.02±0.0	0.00±0.02*	0.05±0.02	0.1±0.1
	P	**0.5** ± 0.2	**0.7** ± 0.4	**12.9** ± 1.1	**35.9** ± 19.2	0.03±0.04	0.06±0.01	0.05±0.02	0.4±0.2
D	S	**0.2** ± 0.1	**0.3** ± 0.1	**13.7** ± 9.6	**27.3** ± 12.2	0.02±0.01	0.04±0.01	0.1±0.1	0.1±0.1
	P	**0.5** ± 0.2	**0.8** ± 0.1	**19.9** ± 3.5	**37.4** ± 6.8	0.01±0.02*	0.04±0.02	0.1±0.1	0.2±0.1
M	S	**1.5** ± 1.8	**2.2** ± 1.3	**13.1** ± 15.1	**14.8** ± 10.0	0.04±0.02	0.1 ±0.1*	0.1±0.1	0.2±0.2
	P	**0.3** ± 0.1	**0.5** ± 0.2	**18.0** ± 6.0	**37.0** ± 13.6	0.04 ±0.03	0.1 ± 0.0	0.0±0.2*	0.3±0.1
Mch	S	**0.5** ± 0.1	**0.9** ± 0.8	**11.5** ± 13.8	**34.4** ± 31.8	0.03±0.0	0.04±0.05*	0.1±0.1	0.3±0.3
	P	**0.5** ± 0.1	**0.6** ± 0.3	**10.6** ± 4.0	**17.2** ± 10.9	0.02±0.03	0.04±0.02	0.1±0.03	0.1±0.1
T	S	**0.4** ± 0.2	**2.1** ± 0.7	**11.4** ± 6.2	**32.7** ± 25.6	0.03±0.01	0.01±0.01	0.1±0.02	0.1±0.1
	P	**4.1** ± 5.2	**0.8** ± 0.4	**24.3** ± 13.1	**34.1** ± 15.4	0.04±0.03	0.03±0.03*	0.1±0.1	0.4±0.3

Due to high variability within the same management, the effect of management alone on concentrations of ammonium and nitrates was not significant. The combined effect of site and management was significant only for nitrates in all three horizons – in litter (F=3.08, p=0.027875, DF=4), in the 0-10-cm layer (=8.35, p=0.000072, DF=4), and in the 10-30-cm horizon (F=11.02, p= 0.000006, DF=4) (Figures 26-28).
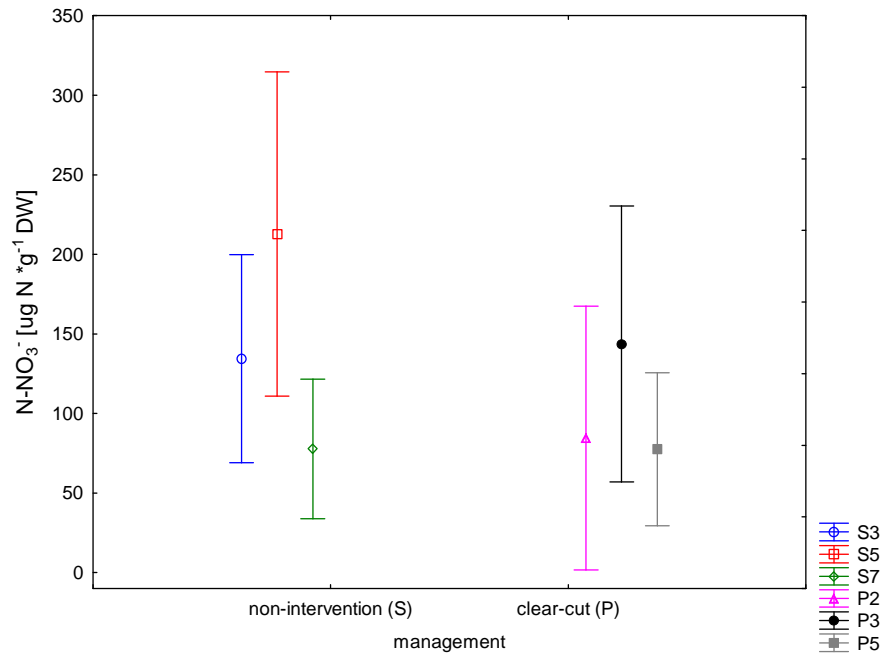

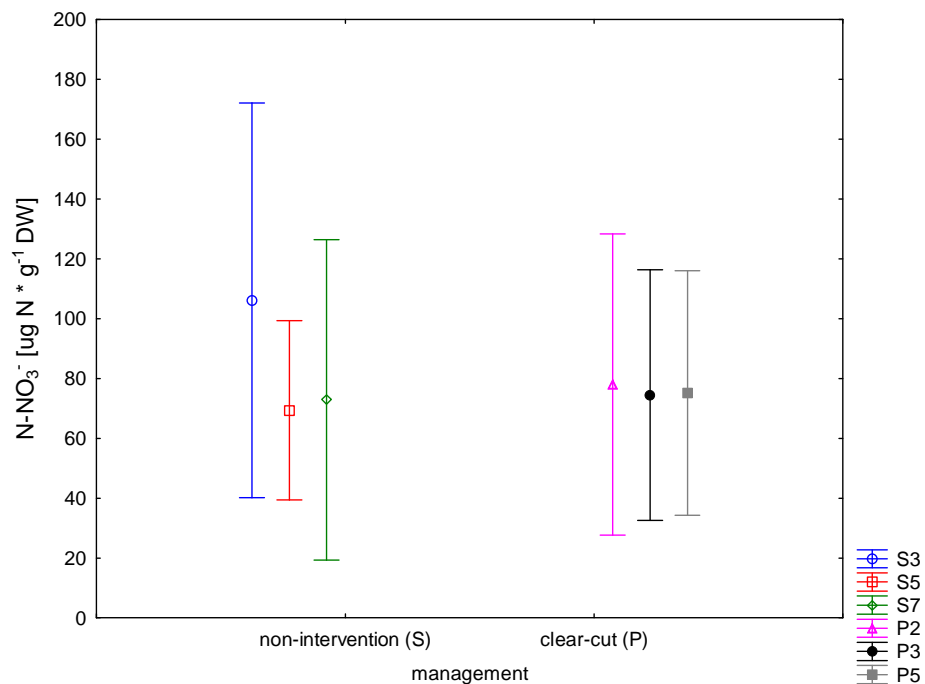**Figure 26**. The effect of site and management on NO_3^- concentration in the litter layer.

Figure 27. The effect of site and management on NO_3^- concentration in the 0-10-cm layer.
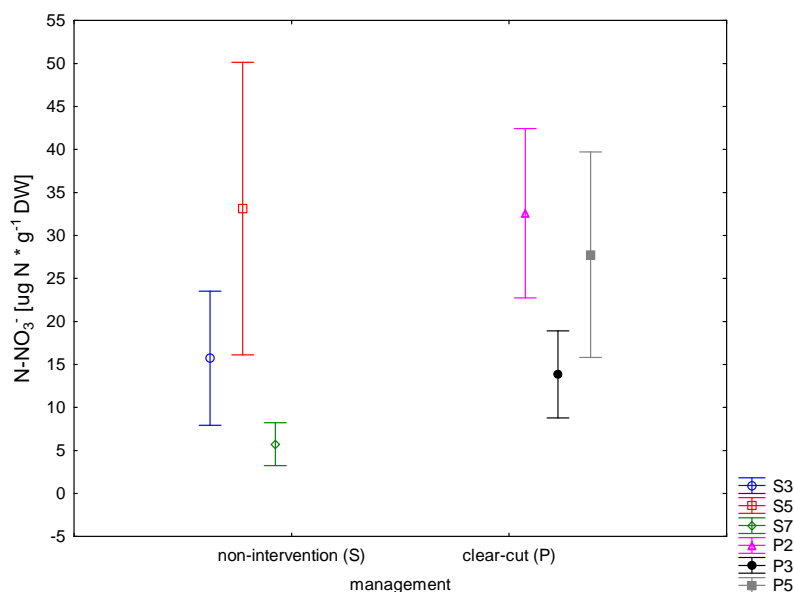
Figure 28. The effect of site and management on NO_3^- concentration in the 10-30-cm layer.

In the litter layer, dominant affected significantly NH_4^+ concentration (F=8.98, p=0.000039, DF=4) (Figure 29) and NO_3^- concentration (F=10.61, p=0.000009, DF=4) (Figure 30). In both cases, NO_3^- and NH_4^+ concentrations were the lowest under moss and the highest under *Calamagrostis* and *Avenella*. Nitrate concentration in soil under moss and *Vaccinium* was highly variable and was significantly lower than all other dominants. In the 0-10-cm horizon, nitrates were affected by dominant (F=5.95, p=0.000884, DF=4) and again moss (together with *Vaccinium*) had lower concentrations than other dominants (Figure 31).

Figure 29. the effect of dominant on ammonium concentration in litter. For abbreviations see Table 2.

Figure 30. The effect of dominant on nitrate concentration in the litter layer. For abbreviations see Table 2.

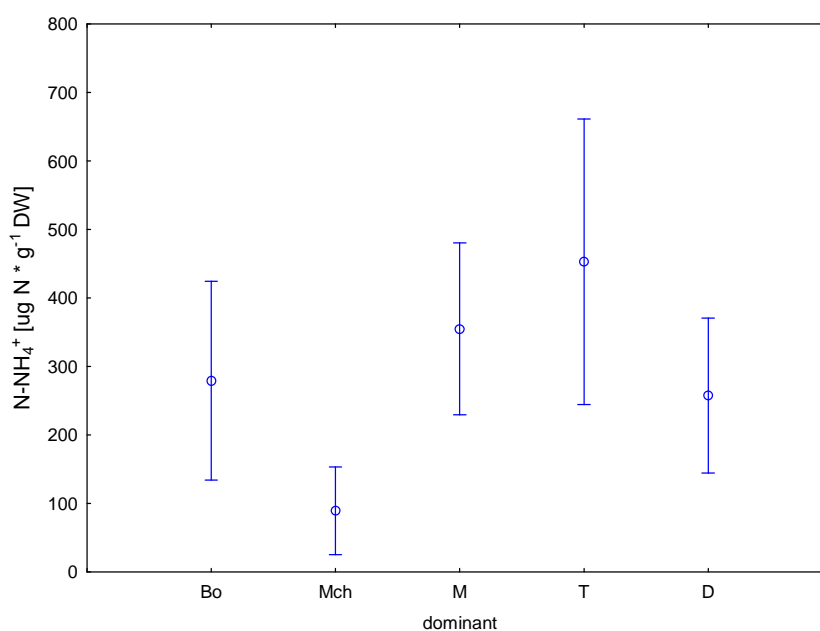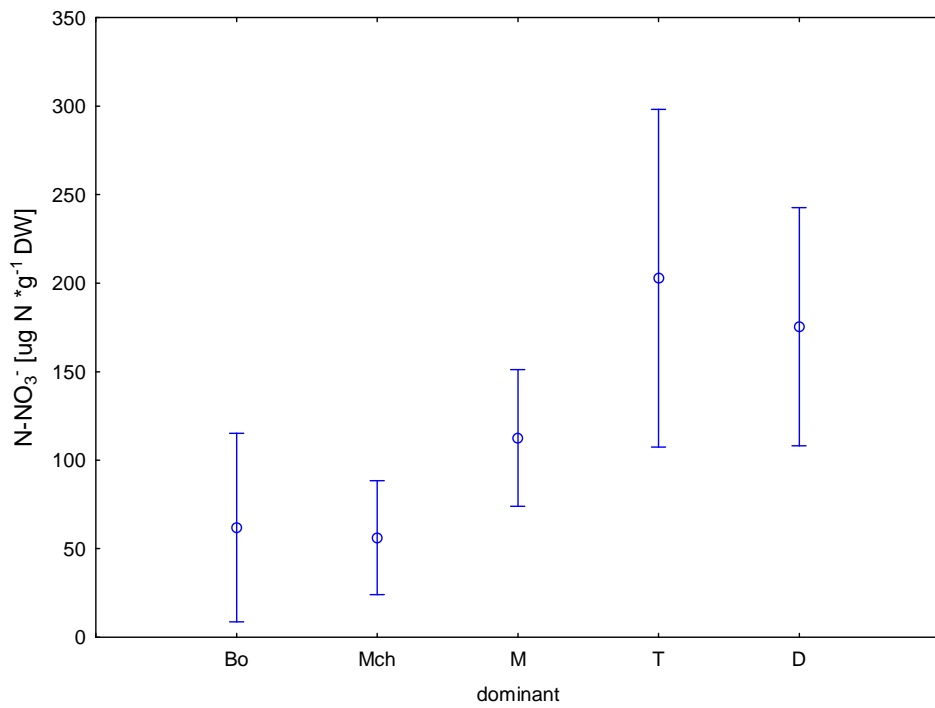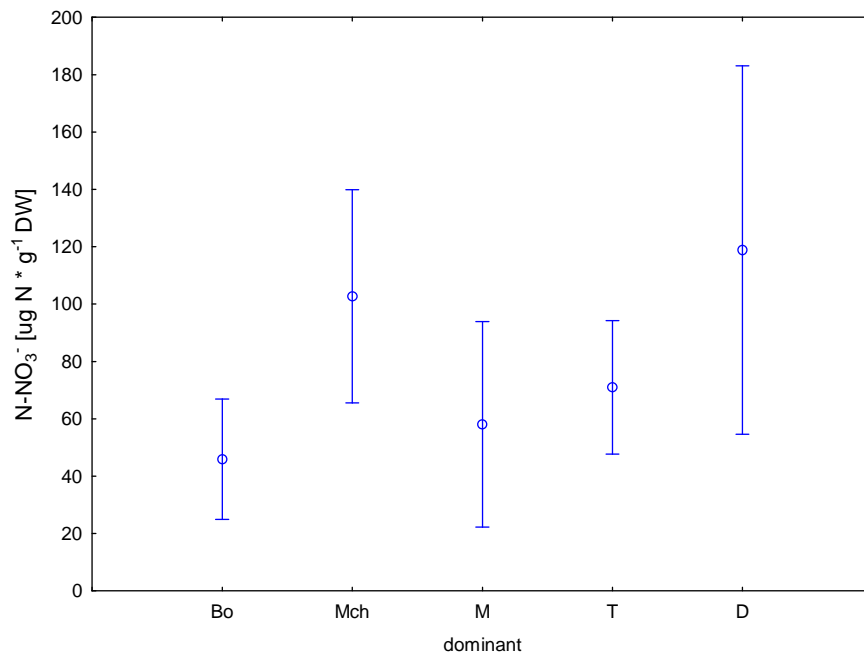**Figure 31**. The effect of dominant on nitrate concentration in the 0-10-cm layer. For abbreviations see Table 2.

6.8 Ammonification and nitrification rate

Ammonification rates in litter were positive in all cases except of S5 *Avenella* 2012. The mean ammonification rates at non-intervention and clear-cut plots in 2011 (and 2012) were 2.22 ± 0.98 (1.36 ± 2.5) and 2.56 ± 1.82 (2.67 ± 1.7) µg $N\text{-}NH_4$ $*g^{-1}$ DW $*d^{-1}$, respectively. The mean nitrification rates at non-intervention and clear-cut plots in 2011 (and 2012) were 1.32 ± 0.9 (2.53 ± 2.5) and 0.44 ± 0.4 (1.55 ± 1.8) µg $N\text{-}NO_3$ $*g^{-1}$ DW $*d^{-1}$, respectively. In the 0-10-cm horizon nitrification rate was higher than that of ammonification in most cases but tended to be lower under *Vaccinium*. The mean values for non-intervention and clear-cut plots in 2011 (and 2012) were 0.17 ± 0.2 (0.25 ± 0.3) and 0.14 ± 0.2 (0.37 ± 0.4) µg $N\text{-}NH_4^+$ $*g^{-1}$ DW $*d^{-1}$, for ammonification, and 0.61 ± 0.5 (0.52 ± 0.5) and 0.50 ± 0.3 (0.58 ± 0.6) µg $N\text{-}NO_3^-$ $*g^{-1}$ DW $*d^{-1}$, for nitrification. In the 10-30-cm layer the rates were very low, about 10 times lower than in the 0-10-cm layer. And again, in most cases, nitrification rate was higher than the ammonification rate. For mean values of ammonification and nitrification rates under the four plant dominants and dead wood see Table 7-9.

Ammonification rate was neither affected by management nor by dominant. Nitrification rate was significantly affected by site and management in the two 10-30-cm layer (F=4.13, p= 0.007, DF=4) and S3, S7 and P3 plots had the lowest rates of nitrification. The other three plots (S5, P5 and P2) have similar nitrification rates (Figure 32). Management alone did not have any significant effect.

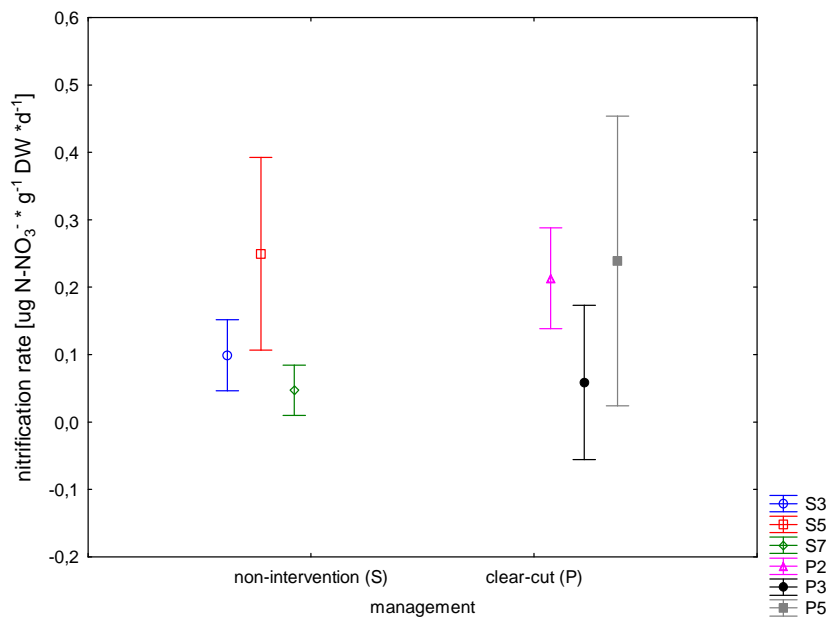**Figure 32**. The effect of site and management on the nitrification rate in the 10-30-cm layer.

Nitrification was affected by dominant only in the 0-10-cm layer (F=5.06, p=0.002444, DF=4). The lowest nitrification rate was found under *Vaccinium*. It differed significantly only from *Calamagrostis* (Figure 33).
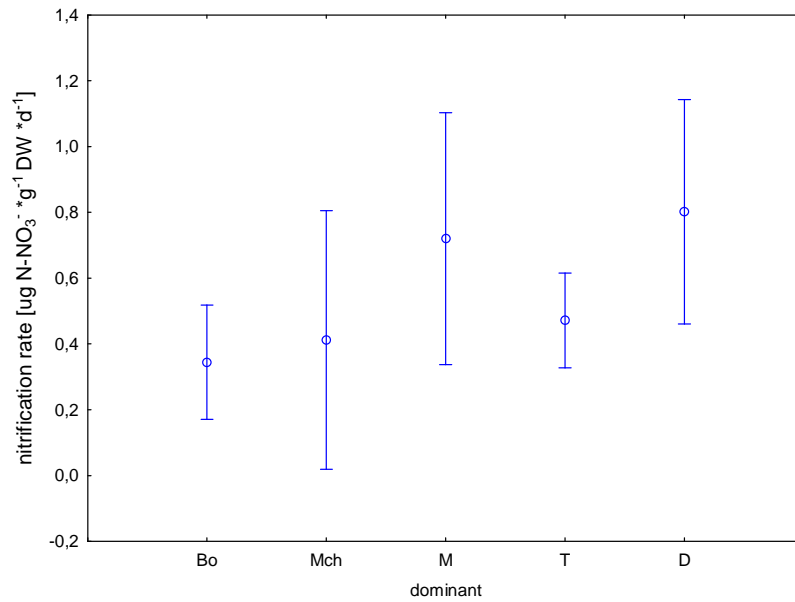

Figure 33. The effect of dominant on the nitrification rate in the 0-10-cm layer. For abbreviations see Table 2.

7 DISCUSSION

The dynamics of nitrogen transformations is dependent above all on the C/N ratio of the organic material (Brady and Weil, 2002). The critical C/N ratio of organic material indicating a shift from N limitation ($\rightarrow$ nitrogen assimilation) to C limitation ($\rightarrow$ nitrification) is estimated on around 20 to 25 (Paul and Clark, 1996; Myrold, 2005). The C/N ratio of decomposed organic material influences the soil C/N. According to studies undertaken in European forest ecosystems, soils with C/N ratio <25 (Gundersen et al. 1998; Dise et al., 1998; Kopáček et al., 2002a, 2002b) or even <32 (Šantrůčková et al., 2006) are at higher risk of nitrate leaching due to a decrease in N immobilization. Further, N saturation is often linked to the shift in the microbial community towards bacteria (lower fungi/bacteria ratio) and by a transition towards C limitation (Nilsson et al., 2012; Kopáček et al., 2013). The C/N ratio of Březník soils ranged from 20.5 to 41.5 (28.4 ± 4.8). This would suggest that the soils are near the break point between N and C limitation and are at risk of excess N release but still might favor fungal communities. Soil under the two grass species (both N-rich plants with a low C/N ratio) with the C/N ratio around 25, are at higher risk of nitrate leaching, whereas under *Vaccinium* and moss with average C/N values in litter around 30 the situation is better. The ongoing processes can change as we go deeper in the soil. In nitrate-leaching prevention (as a function of C/N ratio) the 10-30 cm horizon seems to be the most favorable.

7.1 The concentrations of all N forms are the highest in the litter layer (Hy 1)

The upper soil layers (litter and humus horizons) of the spruce forests in the Bohemian Forest Mountains can contain up to 40% of the total available N (Šantrůčková et al., 2009). Our results confirm the hypothesis that the concentrations of both extractable and microbial carbon and nitrogen are found in the litter layer. The concentration of these N forms decreased along the soil profile. Concentrations of microbial carbon and nitrogen were positively correlated with the extractable C content in soil indicating that the C availability determines the microbial abundance and thus also microbial activity. The respiration data from our soils confirm the highest microbial activity in the litter layer (Otáhalová, unpublished data). Similarly, the mineralization was more pronounced in the litter layer.

The similar distribution in soil was for dissolved mineral nitrogen forms. NH_4^+ concentration decreased significantly along the soil profile. In case of NO_3^- concentration, however, the differences between litter and the two lower horizons were relatively small with a substantial number of cases when concentration of nitrates in the 0-10-cm layer was even higher than

that in litter. This may be a result of higher NO_3^- mobility. Nitrates have negative charge and their sorption to soil colloids is thus much weaker compared to ammonium with positive charge (Brady and Weil, 2002). Higher concentration of nitrates in the lower soil layers may be also caused by lower immobilization of nitrates by microorganisms due to a decrease in microbial abundance compared to the litter layer.

7.2   Concentration of microbial N compared to mineral forms of N in soil (Hy 2)

The comparison of microbial and mineral N concentrations appeared to be quite difficult to explain and find any pattern as some results of N_{ext} were apparently ruled by error. It seems that the discrepancy was connected with (i) analytical problems of measuring N concentration and with (ii) fumigation.

(i)   In many cases, especially when ammonium N concentration was high, we measured lower N_{ext} content compared to that of mineral N forms (NH_4^+ + NO_3^-). This is unrealistic, as N_{ext} is comprised of both organic and mineral N forms. The difference between N_{ext} and N_{min} should be always > 0. The discrepancy can be connected with repeated freezing and with different accuracy of machines for N_{ext} and N_{min} analyses (LiquiTOC, FIA). It is very likely that due to repeated freezing the organic forms condensate, which in turn negatively affects their measurement in LiquiTOC (Šantrůčková, Říhová and Vaněk, personal communication). Moreover, there can be an additional effect of different sensitivity of both N_{ext} and N_{min} measurements. The detection limit of FIA (for N_{min} content analysis) is around 10 µg*l^{-1}, whereas the detection limit of LiquiTOC (for N_{ext} content analysis) is around 50 µg*l^{-1} (Čapek, personal communication). The analytical difficulties brought underestimation of N_{ext} mainly, but they could also affect N_{mic}, but to a less extent. N_{mic} is calculated as a difference of N_{ext} in fumigated and non-fumigated sample from the same soil.

(ii)   In some cases (S3 *Avenella* and S7 *Vaccinium* 2011 in the 10-30-cm layer), N_{ext} content in fumigated samples was lower than in non-fumigated, which led to negative values of N_{mic} concentration.

Despite these discrepancies there was a trend towards higher microbial N concentrations compared to the content of mineral forms of N in soil. This is in agreement with other studies in spruce forest floor biochemistry (Šantrůčková et al., 2009; Tahovská et al., 2013). Microbial biomass is an important N pool. Changes in the microbial N pool can cause changes in N transformations in soil.

7.3 Concentration of N bound to microbial biomass is higher at plots left without intervention (Hy 3)

Microbial nitrogen concentration in the soils of both managed and non-intervention plots were lower compared to other study done in the unmanaged area of the Bohemian Forest National Park after forest defoliation caused by bark beetle attack (Tahovská et al., 2010). The differences may be also caused by different sampling time and incubation condition.

In the litter layer, opposite to our assumption, microbial biomass N was significantly higher at clear-cut plots. In the two lower layers the effect of management alone was not significant due to high variability among the non-intervention plots. The clear-cut plots, however, tend to have higher concentrations of microbial N that the non-intervention plots. The C/N ratio of the microbial biomass was significantly lower in the two upper layers of these plots. It was accompanied by lower soil C/N ratio. An explanation can be the general dominance of grass species in vegetation cover since they are good competitors and thrive in light conditions after the tree harvesting. Both grass species *(Calamagrostis villosa, Avenella flexuosa)* have been reported to have low C/N ratio of their biomass that is thanks to that easily decomposable (Wardle et al., 2003). This may also explain the higher content of N in microbial biomass at the clear-cut plots. Grasses, in general, do not form symbiotic association with ectomycorrhizal fungi (EMC fungi) but with arbuscular mycorrhizal fungi (Wang and Qiu, 2006) that are more connected with the transport of P to their host plants than with the transport of N (Smith and Read, 2008). The extensive rhizosphere rich in bacteria and the lack of EMC fungi may further explain the lower microbial C/N ratio. As a result of high microbial activity and decomposition of organic material of low C/N ratio, the N cycling is accelerated and enhances the risk of N leaching as observed in Šantrůčková et al. (2006). From this point of view, clear-cutting poses higher risk to the nutrient cycling in forest floor.

There was high variability among the plots with the same management, especially at the non-intervention plot. The S7 plot had significantly lower values than other sites in several soil properties in the two lower horizons (e.g. N_{ext}, N_{mic}, C_{ext}). The lower variability in the clear-cut plots might be related to the more uniform vegetation cover by grasses, as mentioned above.

7.4 Concentration of N differs under the five dominant plant species, the distribution along the soil profile remains similar (Hy 4)

The four sampled plant dominants and decaying wood had a significant effect on many soil properties. It must be noted, however, that not all of them can be called "dominants" at all sampling sites. For our research, dominants were chosen according to field study in the same area of Březník in 1998 (Hrežíková, 2008). From that time vegetation cover has been changed and in 2009 grasses had the highest % coverage even at the non-intervention. Nevertheless the studied species are generally the most important plants in spruce forest understory vegetation.

Differences among individual dominants in nutrient concentrations depend on the quality of their litter that influences the decomposition rate and affects the soil C/N ratio as well. From our dominants, the decomposition rate of *Calamagrostis* was reported to be the highest, followed by *Vaccinium*, *Avenella* and spruce needles (Šantrůčková et al., 2006). High lignin and low polyphenol and soluble carbohydrates content slow decomposition (Osono and Takeda, 2005), which is the case of spruce wood and other parts of the tree (bark, needles). Low decomposition rate of spruce needles may also be caused by low P and N availability that could hinder the decomposition (Šantrůčková et al., 2006).

DEAD WOOD: According to our results, soil under dead wood was characterized by the highest concentration of extractable carbon, the lowest C_{mic} content and by lower N_{mic} concentration compared to other dominants. More over, in soil under dead wood there was the highest base cation content and the lowest concentration of aluminium ions (Krausová, 2011). The highest base cation content may be surprising since soils under spruce were reported to have lower base cation content compared to soils under deciduous trees such as birch (Merilä et al., 2010; Hansson et al., 2011; Smolander and Kitunen, 2011; Kiikkilä et al., 2012).The cation-exchange capacity (CEC) was higher in soils under dead wood and moss compared to both grass species. The microbial C/N ratio was significantly higher in dead wood litter but was also more variable than in litter of plant dominants. This might, together with the relatively high soil C/N ratio under dead wood (around 30), support higher abundance of fungi that appear to play a crucial role in N retention in forest soil (Nilsson et al., 2012; Kopáček et al., 2013). On the other hand, we observed high concentration of both mineral forms of nitrogen in soil under dead wood as well as higher nitrification rates. This might limit the fungal community. Their role could be taken over by actinomycetes which have finer mycelia and smaller biomass than fungi and are able to compete with fungi in

lignin degradation (Waldrop et al., 2004). Under a relatively high soil C/N ratio, they would immobilize N to ensure their demands. The increased content of mineral N forms can be also explained by the fact that the trees were growing in condition with elevated N availability.

GRASSES AND BILBERRY: The soil under both grasses was overall the richest in all forms of N (microbial and mineral). Further, compared to other dominants, under *Calamagrostis* the nitrification rate tended to be higher than ammonification rate even in litter, which indicates high mineral N availability in the late autumn. As already mentioned, *Calamagrostis villosa* has a potential to effectively accumulate N in its biomass and thus reduce N losses from soil during the growth season (Fiala et al., 2005). On the other hand, Šantrůčková et al. (2006) suggestthat *Calamagrostis* supported higher microbial activity and might have contributed to higher N release from the litter during fall and winter (Šantrůčková et al., 2006). In the soils under the both grass species, the lowest concentrations of base cations and the highest concentrations of aluminium were found. The cation-exchange capacity was also significantly lower compared to dead wood or moss (Krausová, 2011). Grass species produce high content of organic acids that bound the base cations that are then leached from soil. The above findings indicate that higher grass cover can be accompanied by risk of N leaching, low base cations saturation and high concentration of aluminium, which might further deepen the negative effects of acidification. The decomposition of *Vaccinium* litter is fast (Wardle et al., 2003; Hilli et al., 2010) and the cover of *Vaccinium* can indicate a thick layer of humus and low pH, which is a favorable micro-site for spruce seedling growth (Baier et al., 2005). When considering the effect of dominating vegetation cover on a larger scale, lower cover of grasses and higher cover of bilberry might also partially explain the lowest concentrations of nitrogen forms at S7 in the 0-10-cm layer. Even though vegetation cover has changed since 1998, S7 still has the highest abundance of bilberry (25.5% vs. <1% at all other sites) from all other sites and the lowest cover of grasses (30% vs. 60-70%, except of P5 where grasses cover about 30% accompanied by *Luzula* with almost 30%) (Hrežíková, unpublished data).

MOSS: Bryophytes are able to fix C and N from atmosphere and influence their environment through decreasing soil temperatures or increasing soil moisture. They are also able to change the density of soil organic matter and reduce the loss of organic N from ecosystem by decreasing decomposition (Turetsky, 2003). This could explain the overall lowest concentrations of all N forms in moss. In soil under moss the CEC was found to be higher compared to soil under grass species. Moss, similarly to bilberry, shows positive effect on soil properties, such as higher base cation content, CEC and microbial C/N ratio.

7.5　The effect of the sampling year

Next to the effects of dominant and management, a significant effect of year was observed that might be connected with different temperatures and precipitation in the studied years. The mean month temperature in summer was higher in 2012, while in September and October was higher in 2011 (Pavlas, unpublished data). The mean annual precipitation in this area is 1100 mm. Compared to 2011, the year 2012 was drier during the vegetation season with significant increase in precipitation in August (in case of the South Bohemia region, the precipitations doubled in 2012 compared to 2011, www.chmi.cz).

We found significant differences in several soil properties. In litter, both extractable and microbial carbon concentrations were higher in 2012. The microbial C/N ratio was higher in 2012 as well, whereas the concentration of N bound to microbial biomass was significantly higher in 2011. Both mineral forms of N (NH_4^+ and NO_3^-) and the ammonification rate were again higher in 2012. Concentrations of mineral N forms were higher in 2012 also in the two lower layers. This increase in mineral N concentrations might be the effect of the wet period following the drought period that stimulates microbial activity and mineralization (Denef et al., 2001).

The effect of the year has to be interpreted with caution, as it can also reflect spatial variability and, in the case of extractable C and N and microbial C and N, the slight differences in soil incubation before analyses. Soil sampling was not performed at exactly the same places in the year 2011 and 2012. In 2011, soil was extracted without preincubation while one week preincubation was used in 2012. The preincubation could thus bring increase in extractable C and N and in microbial biomass. Higher NH_4^+ and NO_3^- concentrations in 2012 in all three soil horizons can reflect increased mineralization due warm and dry early autumn.

8 CONCLUSION

We assessed carbon and nitrogen concentrations together with potential ammonification and nitrification rates in soil at 6 plots affected by bark beetle in the late 1990s. Different management practices (non-intervention vs. clear-cutting) were applied. Although having different starting conditions, all the sites went since then through a succession. After 16 years we can see that, in case of soil properties, the differences are small and the sites are getting closer. The higher variability among the non-intervention sites compared to theclear-cut sites can be a result of the uniform management at the clear-cut plots. Our results suggest that the effect of management on soil conditions might be linked to the development of vegetation cover. The expansion of grass species may be connected with adverse changes in soil chemistry, such as increase in availability of nitrogen and aluminium or decrease in base cations content and overall cation-exchange capacity. Grass species obviously expand more at disturbed and opened clear-cut plots.

Since we have no data on the soil properties before the bark beetle infestation and forest dieback we cannot directly say how this disturbance event affected the nutrient transformation processes in soils of both S and P plots. However, clear-cutting very likely caused a shift in microbial community towards bacteria due to an increase in N availability and also due to mechanical disturbance of soil that negatively affects fungal mycelia. It also allowed succession of grass species on a larger scale than at the non-intervention sites that again contribute to a decrease in soil C/N ratio. Soil is a very heterogeneous environment, where nutrient-rich and nutrient-poor micro-sites might be found very close to each other. On a larger scale, however, the vegetation cover seems to be of a high importance not only due to preventing erosion but also due affecting the soil microbial community composition and nutrient cycling. This suggests that the clear-cut plots have a higher potential to become N saturated and release more nitrogen than the sites left to natural succession.

Our results show that clear-cut management used in the Bohemian Forest Mountains in the Březník area did not bring any substantial deteriorating effect on soil biochemistry, but distinct trend of increased microbial N concentration and decreased C/N ratio of microbial biomass and soil and thus a higher potential of N leaching are still obvious 16 years after clear-cutting of the forest. It clearly indicates that clear-cut is less appropriate way of forest management, especially in the mountain spruce forests that are exposed to acidification and other disturbances, such as windstorms followed by bark beetle infestation. It appears that the negative effect of clear-cutting on soil biochemistry is closely connected to the expansion

of grasses. At those sites, where vegetation more typical for spruce forest stands (such as bilberry, mosses) develops, no apparent negative effect on soil biochemistry was found. The application of wood chips after trees were cut and removed might have had a positive effect on the soil biochemistry and could have potentially alleviated the negative effects of clear-cutting. Regarding the management from a long-term perspective, input of nutrients from decaying wood which is higher at non-intervention plots must not be omitted.

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