

School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice
Faculty of Science



**Nitrogen availability and transformation in soils
of acidified and nitrogen saturated mountain forest
ecosystems**

Ph.D. Thesis

Mgr. Karolina Tahovská

Supervisor: prof. Ing. Hana Šantrůčková, CSc.

Department of ecosystem biology, Faculty of Science, University of South
Bohemia in České Budějovice

Supervisor specialist: prof. Ing. Jiří Kopáček, Ph.D.

Institute of Hydrobiology, Biology Centre of the ASCR, České Budějovice

České Budějovice 2012

This thesis should be cited as:

Tahovská K., 2012: Nitrogen availability and transformation in soils of acidified and nitrogen saturated mountain forest ecosystems, Ph.D. Thesis Series, No. 5. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 137 pp.

Annotation

Nitrogen availability and transformation in acidified and N saturated soils of Czech (The Bohemian Forest, Ore Mountains) and Ukraine (Pop Ivan massif) mountain forest ecosystems were investigated. The study was primarily focused on the role of microbial immobilization in soil N retention. The study was based on field measurements (ion exchange resins), analyses of selected soil biochemical and microbial characteristics, and on laboratory experiments (¹⁵N labelling).

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice 10.9. 2012

.....
Karolina Tahovská

This thesis originated from a partnership of Faculty of Science, University of South Bohemia in České Budějovice and Institute of Hydrobiology, Biology Centre of the ASCR, supporting doctoral studies in the Ecosystem Biology study programme.



Financial support

This study was financially supported by Grant Agency of the Academy of Science of the Czech Republic (KJB600960907), by Ministry of Education, Youth and Sports (MSM6007665801), The Czech Science Foundation Grant Agency (GA206/07/1200; P504/12/1218), by Norway financial mechanism (EEA NFM CZ0051) and by Grant Agency of the University of South Bohemia (GAJU 142/2010/P).

"If I have seen a little further, it is by standing on the shoulders of Giants"

Isaac Newton

Acknowledgements

Finally! Many years and almost two children later I've actually finished. Firstly, I would like to thank my supervisor Hana Šantrůčková for her broad support during all the years, her time and useful comments during work planning, writing manuscripts, and their revisions. I further thank my supervisor specialist Jiří Kopáček for his advice and time during discussions of the results. I am also very grateful to all colleagues and friends, which I met on my way and who always made the work atmosphere pleasant, inspiring and funny and did not hesitate to help when I needed it: Bára Pivničková, Dan Vaněk, Eva Kaštovská, Tomáš Pícek, Peťa Čapek, Jiří Kaňa, Jiří Bárta, Michal Choma, Michal Šorf, Tomáš Hájek, Jarda Vrba, Jana Vrbová, Martina Vašková, Dáša Sirová, Zuzka Urbanová, and Keith Edwards. I am grateful also to Filip Oulehle from the Czech Geologic Survey for giving me the opportunity to study Ukrainian soils and sharing his ideas with me. Special thanks belong to my husband and parents for their support and patience. Last but not least I thank my little son absolute love for his sometimes tired, but always loving mother.

List of papers and author's contribution

The thesis is based on following papers, which are referred in the text by their Roman numerals.

- I. Šantrůčková, H., **Tahovská, K.**, Kopáček, J., 2009. Nitrogen transformations and pools in N-saturated mountain spruce forest soils. *Biology and Fertility of Soils* 45 (4), 395 – 404. (IF = 2.319) *K. Tahovská participated in soil sampling, carried out all laboratory measurement, participated in data evaluation, manuscript writing, and revision.*
- II. **Tahovská, K.**, Kopáček, J., Šantrůčková, H., 2010. Nitrogen availability in Norway spruce forest floor - the effect of forest defoliation induced by bark beetle infestation. *Boreal Environment Research* 15 (6), 553 – 554. (IF = 1.803) *K. Tahovská optimized the resin method, carried out field measurements and analyses of resins in laboratory, participated in biochemical analyses, wrote the manuscript and completed revisions.*
- III. Kaňa, J., **Tahovská, K.**, Kopáček, J., Changes in soil chemistry after bark beetle infestation and forest dieback. *Biogeochemistry (in press)* DOI: 10.1007/s10533-012-9765-5. (IF = 3.069) *K. Tahovská participated in sample preparation and in part of the biochemical analyses, data assembly and writing, and revision of the manuscript.*
- IV. **Tahovská, K.**, Kaňa, J., Bárta, J., Oulehle, F., Richter, A., Šantrůčková, H., Microbial N immobilization is of great importance in acidified mountain spruce forest soils (*submitted to Soil Biology & Biochemistry*) *K. Tahovská participated in soil sampling, was responsible for preparation and maintenance of the experiments, carried out ¹⁵N labelling experiments and subsequent analyses, was responsible for data assembly, their evaluation, wrote the manuscript, and completed revisions.*
- V. Oulehle, F., Evans, C., Hofmeister, J., Krejčí, R., **Tahovská, K.**, Persson, T., Cudlín, P., Hruška, J., 2011. Major changes in forest carbon and nitrogen cycling caused by declining sulphur deposition. *Global Change Biology* 17 (10), 3115 – 3129. (IF = 6.862) *K. Tahovská participated in soil sampling, carried out net N mineralization and nitrification measurements and participated in writing and revisions of the manuscript.*

Content

1. GENERAL INTRODUCTION	1
1.1 Background	1
1.2 Acidification of forest soils	3
1.3 Introduction to microbial N transformation in soil	5
1.4 N saturation – definitions	7
1.5 N transformations in N saturated soil	8
2. AIM AND HYPOTHESES	13
3. EXPERIMENTAL SITES	14
4. PRESENT INVESTIGATION	17
5. CONCLUSIONS AND FUTURE PROSPECT	20
REFERENCES	24
RESEARCH ARTICLES	35
paper I	37
paper II	49
paper III	63
paper IV	81
paper V	121
Curriculum vitae	

ABBREVIATIONS

Al – aluminium

ATP – adenosine triphosphate

BC – base cations

C – carbon

Ca – calcium

CaCO₃ – calcium carbonate

(Ca, Mg, Ca)SiO₃ – silicate minerals

CO₂ – carbon dioxide

DNRA – dissimilatory nitrate reduction to ammonium

DOC – dissolved organic carbon

DON – dissolved organic nitrogen

EMEP – European Monitoring and Evaluation Programme

H – hydrogen

HNO₃ – nitric acid

H₂SO₄ – sulphuric acid

K – potassium

Mg – magnesium

Na – sodium

N – nitrogen

NH₃ – ammonia

NH₄ – ammonium

N₂O – nitrous oxide

NO₃ – nitrate

NO_x – mono-nitrogen oxides

P – phosphorus

S – sulphur

SO₂ – sulphur dioxide

SOM – soil organic matter

1. General Introduction

1.1 Background

Atmospheric pollution related to anthropogenic activity has become one of the main factors affecting nutrient cycling of ecosystems over the last two centuries (e.g. Norton and Veselý, 2003; Kopáček and Posch, 2011). Like the whole of Central Europe, the mountain area of the Czech Republic has been strongly acidified through emissions coming predominantly from the most industrialized European region located at the German-Polish-Czech border, - the so called area of “Black Triangle” (Berge, 1997). Emissions in the Czech Republic reached their maxima in 1980s (Fig 1., Kopáček and Veselý, 2005; Kopáček and Hruška, 2010) and since then have significantly decreased (86% in SO_2 , 53% in NO_x and 35% in NH_3 emissions, Kopáček et al., 2011; Kopáček and Hruška, 2010) due to the restructuring of industry and agriculture after political changes in 1990s (desulphurization of power stations, decrease in energy and cattle production and the application of synthetic fertilizers).

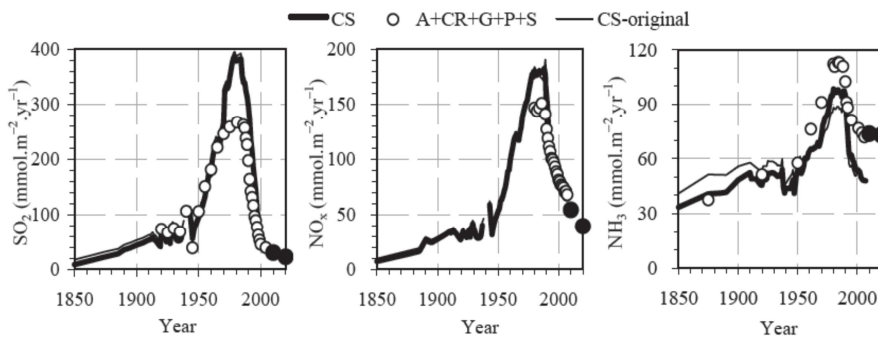


Figure 1: Trends in SO_2 , NO_x and NH_3 emissions in the Czech Republic and Slovakia (CS). Empty points are for middle Europe (Austria, Czech Republic, Germany, Poland, and Slovakia). Black points are presumed central European emissions for 2010 and 2020 (EMEP (2009) models) (taken from Kopáček and Hruška, 2010).

In the Bohemian Forest, the area where most of the research presented in this thesis was conducted, the acid depositions of sulphur (S) and nitrogen (N) compounds corresponded closely to the emissions trends in the Czech Republic (e.g. Kopáček et al., 1997; Oulehle et al., 2006). Sulphur and N depositions peaked in 1980s (Fig 2.), then both decreased rapidly till 2000s when total N

depositions stabilized while the S deposition continued to slow down. The atmospheric N deposition was dominated by nitrate (NO_3^-) from 1960 to 2000, while ammonium (NH_4^+) prevailed from 1850 to 1960 and again after 2010 (Kopáček and Hruška, 2010).

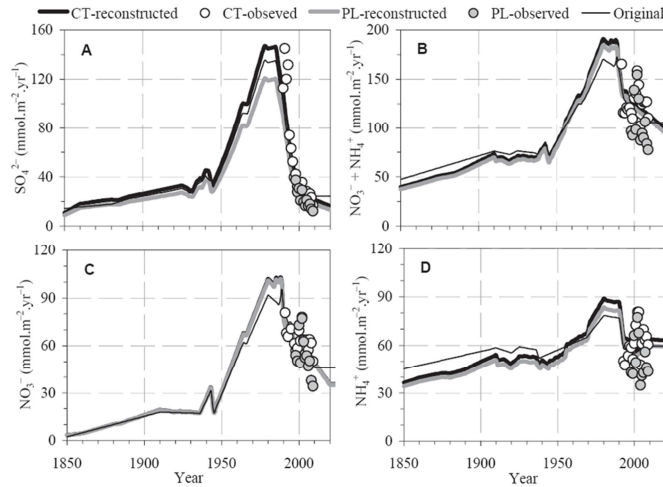


Figure 2: Trends in SO_4^{2-} (A), NO_3^- (C) and NH_4^+ (D) deposition in the Bohemian Forest (taken from Kopáček and Hruška, 2010)

Despite the reductions of S and N emissions in Europe, global rates of NO_x and NH_3 emissions have been increasing or stable, mostly as a consequence of the industrial development in Asia (Kopáček and Posch, 2011; Smith et al., 2011). The recovery of waters and soils in a response to decreased S depositions which is currently observed (e.g. Evans et al., 2001; Nedbalová et al., 2006; Šantrůčková et al., 2007; Oulehle et al., 2008) may eventually slow down, as the role of N depositions is expected to increase in the future and the effects of climate change are also suspected to become more pronounced (e.g. Murdoch et al., 1998; Keetle et al., 2003; Norton and Veselý, 2003; Veselý et al., 2003). In addition, many of the original central European forest stands have been almost completely altered into even-aged coniferous monoculture, which has contributed to widespread expansion of pest infections and forest dieback. From these points of view the ongoing recovery of soils and consequently waters from acidification may be delayed and the extent of future acidification is rather difficult to predict. Hence, acidification still remains an important topic of current ecological research worldwide.

1.2 Acidification of forest soils

Soil acidification is defined as a process of pH reduction and/or lowering of soil acid-neutralizing capacity. In mountain areas with higher rainfall (~1000 mm) and coniferous or mixed forests, it can occur naturally in the long-term through decomposition of organic matter. During decomposition, CO₂ and organic acids are released and base cations (Ca²⁺, Mg³⁺, K⁺, Na⁺) are leached from the soil by forming soluble salts of the bicarbonate ions. Acidification is also driven by the oxidation/reduction processes of N and S compounds in the soil. For example, H⁺ and NO₃⁻ are released by the oxidation of NH₄⁺ (nitrification) and a strong acid (HNO₃) is formed. In the overall reaction of nitrification 2H⁺ are generated for every N (Sprent, 1987).

Acidity can partly be neutralized by weathering processes and related cation exchange reactions in soil (soil buffering capacity). Soils on bedrocks containing carbonate (CaCO₃) or silicate minerals ((Ca,Mg,Ca)SiO₃) are less susceptible to acidification, because their bedrocks release base cations (and remove H⁺) at relatively high rates (Sverdrup, 1990). In the Czech mountain region, with shallow soils laying mostly on acid bedrocks (e.g. schist, gneiss), the increased atmospheric acidity overwhelmed the weathering reactions and caused an exhaustion of soil neutralizing capacity (anthropogenic acidification). For example, the current exchangeable pH_{CaCl2} values in the Bohemian Forest soils are 2.5 – 3.3 in the organic horizons (Kopáček et al., 2002a,b) and base saturation is only 12 – 15 % on average, while the modelled pre-industrial values of base saturation were 12 – 27 % (in 1860, Majer et al., 2003).

Another important factor that can support soil acidification is the transformation of original forests to fast-growing spruce monocultures (*Picea abies* in Czech Republic). Acid precipitation under coniferous species (throughfall deposition) is generally higher than under deciduous trees due to their higher leaf area index and longer leaf retention period (e.g. Augusto and Ranger, 2001; Oulehle et al., 2007). The leaf litter quality and litter degradability also determine the rate of soil acidification (e.g. Reich et al., 2005). Spruce needle litter is rich in lignin and other phenolic compounds, and poor in nutrients (high lignin-to-N ratio, high phenolics-to-available phosphorus ratio). Consequently it is more resistant to biological degradation and decomposes slowly, producing more organic acids during decomposition (Binkley, 1995; Hobbie et al., 2006; Šantrůčková et al., 2006; Bárta et al., 2010). In addition, the uptake of base cations by spruces biomass is high and, reaches the maximum

after 40 - 50 years (Hruška and Cienciala, 2005). When spruce is harvested in managed forests, organic matter is removed and nutrients cannot return via decomposition back to the soil.

It is generally accepted, based mostly on *in vitro* experiments that most microorganisms prefer near neutral or slightly alkaline soil conditions for optimal growth (e.g. Killham, 1994). However, soil is rich in heterogeneous micro-environments and can host plentiful different groups of microorganisms with large metabolic diversity (Schimel and Bennett, 2004; Alewell et al., 2006; Manzoni and Porporato, 2007). It is highly probable that microbial processes that are restricted in acidic conditions *in vitro* would exhibit functional redundancy and would run under acidic conditions in field. For example, nitrification was for a long time considered to be negligible in acidic soils, but results from the last decades clearly demonstrate that this is not the case (reviewed in de Boer and Kowalchuk, 2001). Recently, it was discovered that Archaea rather than bacteria are capable of ammonia oxidation under acidic soil conditions. A possible explanation is that the activity of bacterial ammonia oxidizers is restricted by the reduced availability of NH_3 under low pH, because of its ionization to NH_4^+ (Frijlink et al., 1992). Under these conditions the NH_3 diffusion decreases, energy requirements for NH_4^+ transport increase, and Archaea are probably better adapted for such environments (Nicol et al., 2008; Zhang et al., 2012).

Soil pH was found to influence the composition of microbial community and the activity of some functional groups e.g. fungi/bacteria (e.g. Pennanen et al., 1998; Rousk et al., 2009). Generally, fungi have been showed to be more tolerant to acidic soil conditions than bacteria, having a wide pH-growth optimum (reviewed in Strickland and Rousk, 2010; Rousk and Bååth, 2011). However, Sinsabaugh (2010) showed that the activity of certain enzymes produced mainly by fungi (phenol oxidases and peroxidases) is inhibited by low soil pH across different ecosystems, which may be the reason for reduced degradability of soil organic matter in acidified soils.

Anthropogenic acidification is connected with increased mobilization of ionic form of aluminium in soils (Al^{3+} , e.g. Dise et al., 2001; Drábek et al., 2005) that is known to have toxic effect on plants and microorganisms (Illmer et al., 1995; Joner et al., 2005; Kochian 2005). Aluminium is present naturally in alumina-silicates (micas, feldspars and their weathering products) and also in non-silicate minerals (e.g. gibbsite, variscite, alunite) (Pitter, 2009). Aluminium is mobilised in soil through chemical weathering of Al minerals by the effect of

CO₂, weak organic acids (export of organic Al complexes) and strong acids such as HNO₃ and H₂SO₄ (export of ionic Al) (Vance et al., 1996). In soils with pH higher than 6, Al mobility is low, but when the soil pH decreases below 4.7, mobile Al³⁺ dominates the soil solution pool (e.g. Mulder and Cresser, 1994; Sposito, 1996; Pierzynski et al., 2000). In the acidified Bohemian Forest soils, exchangeable Al³⁺ represents on average 60% of the total soil exchangeable capacity (Kopáček et al., 2002a,b). Low ratio of base cations (BC) to Al in acidified soils (molar ratio BC/Al < 1) affects negatively the physiology of spruce trees leading to declined root growth and branching, water deprivation, elevated defoliation and higher sensitivity to pest infections (e.g. Foy, 1983; Heim et al., 2000; Puhe and Ulrich, 2001). Considerable amount of Al³⁺ is also leached from acidified soils affecting the nutrient status of waters. Kopáček et al. (2000) showed that Al, incoming through terrestrial losses in acidified forest soils, immobilized phosphorus (P) in glacial lakes. Consequently, Vrba et al. (2006) showed that under Al³⁺ excess in lake, P metabolism and food web structures are altered. Elevated concentrations of Al³⁺ can also affect the metabolism of microorganisms in the soil. Aluminium can induce rigidity of their membranes and causes the inhibition of certain enzymes (Rosswall et al., 1986; Illmer et al., 1995; 2003). Besides, Al addition was shown to suppress the decomposition of soil organic matter (SOM) by the precipitation of dissolved organic carbon (DOC), thus reducing its bioavailability (Scheel et al., 2007; 2008) and this can have an effect on the composition of soil microbial community (Joner et al., 2005). Although, the toxic effect of Al³⁺ is indisputable, it is often difficult to separate the direct effect of Al³⁺ on microbial metabolism from the effect of low pH, because both are closely related.

1.3 Introduction to microbial N transformation in soil

Nitrogen is essential nutrient for living organisms. It is present in the environment in various chemical forms, from diverse forms of organic N (amino acids, nucleotides, amines etc.) to mineral N (ammonium, nitrate) and gases (dinitrogen gas, nitrous oxide, nitric oxide and nitrogen oxide). The most important part of its cycle occurs in the soil.

Generally, the key process of the N cycle in soil is the turnover of organic matter through decomposition. Decomposition includes both the physical breakdown and biochemical transformation of complex organic compounds into simpler organic and inorganic molecules. Organic N-containing

polymers are not directly bioavailable, because of their complexity. They must be cleaved by extracellular enzymes secreted by microorganisms (depolymerization) to release monomers (amino acids, amino sugars, and nucleic acids, Fig 3., Schimel and Bennet, 2004). These monomers constitute the soil available DON pool and are immobilized by both microorganisms and plants that compete with each other in the N limited ecosystems. Organic monomers are then converted by microorganisms into mineral N forms in the processes of N mineralization.

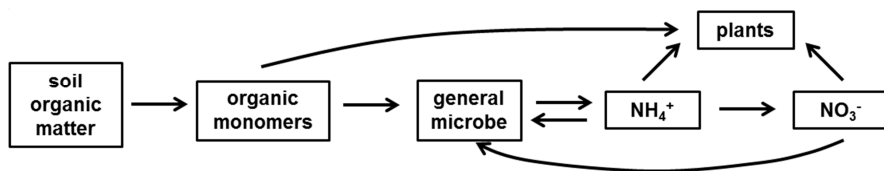


Figure 3: Depolymerisation as a regulatory mechanism of N cycle (simplified from Schimel and Bennett, 2004).

The first step of N mineralization is the microbial degradation of simple organic compounds to ammonium (ammonification). This process is driven by the need of heterotrophic microorganisms for energy and C. Thus, ammonium as a result of ammonification can be considered a by-product of microbial catabolism. Released ammonium can then be converted to nitrate through nitrification. Nitrification is the production of NO_3^- from the oxidation of reduced N compounds. Most often we speak about autotrophic nitrification, the two-step process of oxidizing NH_4^+ to NO_3^- (conversion of ammonia to nitrite and nitrite to nitrate) serving as the energy source for nitrifying microorganisms. However, several heterotrophic microorganisms from bacteria (e.g. *Arthrobacter*) to fungi (e.g. *Aspergillus*) can also oxidize either ammonium or organic N to NO_2^- and NO_3^- , but gain no energy from this process (e.g. Sylvia et al., 2005). It is still not clear how they benefit from this pathway.

Both inorganic forms of N derived from N mineralization may once again be incorporated into soil organic matter by microbial and plant immobilization. Microorganisms immobilize NH_4^+ by two primary pathways depending on the NH_4^+ concentration (dehydrogenase and glutamine synthetase-glutamate synthase pathway) and incorporate it into glutamate. Once glutamate is formed, it can be transferred to other carbon (C) skeletons by transaminase

reactions to form other amino acids (Sylvia et al., 2005). Nitrate can be reduced by microorganisms in three processes: nitrate immobilization, denitrification and dissimilatory nitrate reduction to ammonium (DNRA). All the processes require energy. Denitrification and DNRA differ from NO_3^- immobilization by the fact that reduced N is not used by the cell. Nitrates serve in these processes as electron acceptors for the cell's energy metabolism in conditions of oxygen deficiency. Both processes are coupled to electron transport phosphorylation (generating ATP). Final products of denitrification are N_2O and N_2 , while DNRA releases NH_4^+ to the soil. Denitrification gains slightly less energy per mol of NO_3^- than DNRA (560 and 600 $\text{kJ mol}^{-1} \text{NO}_3^-$, for denitrification and DNRA, respectively, Tiedje et al., 1989). Via denitrification N is ultimately returned to the atmosphere as a gas. Small part of the gas may also come from nitrification.

1.4 N saturation - definitions

Nitrogen is usually a limiting nutrient in temperate forest ecosystems with low anthropogenic pollution and its main input occurs through atmospheric deposition, with the exception of forests with N fixing plant species (Keeney, 1980, Moldan and Černý, 1994). Most of the N is accumulated (immobilized) in soil and plant biomass and N losses are minimal, dominated mostly by dissolved organic N (DON) (Kortelainen et al., 1997) originating from the transformation of plant biomass and SOM.

When the anthropogenic loading of N is elevated for a long period of time, however, the ecosystem is no longer able to immobilize all deposited N and the state of N saturation is achieved (Agren and Bosatta, 1988). The definition of N saturation is not uniform in literature, but ecosystems are generally considered to be N saturated when NO_3^- appears in soil leachate (Stoddard 1994, Venterea et al., 2004). In its widest meaning, N saturation can be viewed as a permanent alteration of the soil N cycle from closed internal cycling to an open cycle where the excess N flows out of the ecosystem (Kämäri et al., 1992).

In the traditional N saturation model, the response of an ecosystem to elevated N load is divided into several biogeochemical stages (Fig 4., Aber et al., 1989, 1998). Basically, enhanced N deposition increases N availability to plants and decreases C-to-N ratio of their litter. During decomposition the organic soil horizons are enriched by N, which accelerates N mineralization (stage 1) and

nitrification, while gross N immobilization continually decreases (stage 2). This results in elevated NO_3^- leaching from the ecosystem (stage 3). The ecosystem N retention capacity in this traditional view relies primarily on plant uptake as microbial immobilization can be limited by C. This concept has led to partial marginalization of the importance of microbial N immobilization processes in the N saturated soils.

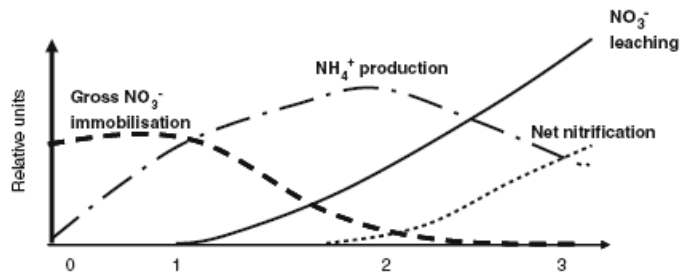


Figure 4: Stages of N saturation according to Emmett (2007) and based on Aber et al. (1989, 1998).

Novel conceptual model of N saturation (Lovett and Goodale, 2011) is based on an assumption that added N can flow simultaneously to all N sinks, which do not reach their N saturation capacity, but accumulate N at rates lower than the rate of N input. In both models, however, N mineralization and nitrification are assumed as driving force processes of N transformation, while the direct incorporation of mineral N into microbial biomass is expected to be less important. Lovett and Goodale (2011) further pointed out that rather than a series of stages, N saturation is the result of the interaction of several dynamic factors that control N sinks. This was confirmed recently by modelling soil N retention of the ecosystem in relation to carbon C turnover (Oulehle et al., 2012).

1.5 N transformation in N saturated soil

Nitrate concentrations in unpolluted forest soils tend to be low and net nitrification rates (i.e. change of NO_3^- concentrations per unit of time) are usually negligible. These observations led to a paradigm, that as ecosystem mature, losses of N are reduced and N cycling becomes tightly closed with nitrification turned off (Sylvia et al., 2005). But it may be possible that nitrification is running and NO_3^- produced is immobilized by microbial biomass. The importance of microbial NO_3^- immobilization in N retention ability of these ecosystems has been already shown (Davidson et al., 1992; Stark and Hart,

1997; Perakis and Hedin, 2001).

Data describing how N saturation changes soil N transformation in forest ecosystems are relatively heterogeneous despite more than 20 years of intensive research. It is still not entirely clear where the leached NO_3^- originates. Some studies showed that atmospherically deposited N can be directly leached out without encountering biological sink (physical bypass, e.g. Curtis et al., 2005; Schleppi et al., 1998). Others, however, proved that leached N undergoes microbial transformation (e.g. Nadelhoffer et al., 1999; Hagedorn et al., 1999; Pardo et al., 2004) (Fig 5.). Studies based on dual isotopic measurements (^{15}N together with ^{17}O or ^{18}O in NO_3^-) revealed that leaching of deposited NO_3^- through physical bypassing accounts for only 10 - 30% of leached N in forested watersheds and occurs usually during sudden rain events, during snow-melting, or in soil-poor rocky watersheds (reviewed in Curtis et al., 2011). Curtis et al. (2011) further emphasized that direct leaching of deposited N might be significant also in experimental studies with high N additions where the excess of NH_4^+ can reduce biological NO_3^- uptake.

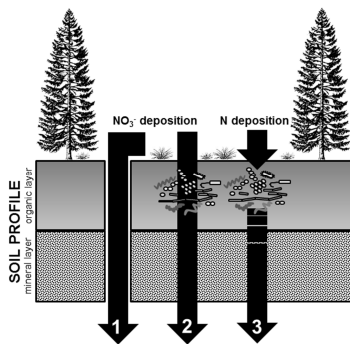


Figure 5: Pathways of NO_3^- leaching (modified from Curtis et al., 2011).

Nitrogen from deposition may be leached out without encountering biological sink (1 – physical bypassing), as a result of reduced N demand of plants and microorganisms (2 – N saturation), or may originate from both N sources – nitrate and ammonium involving enhancement of nitrification (3).

Long term acid input was reported to reduce microbial C pool and sometimes also microbial N immobilization (e.g. McNulty et al., 1996; Aber et al. 1998; Corre et al. 2003; Compton et al., 2004; Wallenstein et al., 2006; Treseder, 2008). However, considerable uncertainty remains concerning the mechanism. Microorganisms may suffer by lack of base cations or by Al toxicity and may be limited by available organic C substrate (Fog, 1988; Mulder et al. 2001; Waldrop et al., 2004; Scheel et al. 2007). Regardless the possible limitation of microbial growth and N uptake, the use of stable isotopes in N addition experiments showed that much of deposited N became rapidly immobilized in soil (e.g. Melin et al., 1983; Aber et al., 1998; Nadelhoffer et al.,

2004; Zak et al., 2004). Although it has been partly attributed to an abiotic process (e.g. Davidson et al., 1991; Potthast et al., 1996; Johnson et al., 2000; Perakis and Hedin, 2001; Compton and Boone, 2002; Corre et al., 2007; Huygens et al., 2008; Morier et al., 2008), there is also strong evidence that leaching of NO_3^- from forest soils is largely dependent on the N immobilization activity of the microbial community (e.g. Hart et al., 1994; Aber et al., 1998; Bengtsson and Bergwall, 2000; Zogg et al., 2000; Bengtsson et al., 2003).

Microorganisms can immobilize both, organic and inorganic N sources into their biomass (e.g. Merrick and Edwards, 1995; Nordin et al. 2001). Many studies support the idea that organic N is the main source for heterotrophic microorganisms (e.g. Dunn et al., 2006; Gonod et al., 2006). Regarding mineral N, it is still generally accepted that, as a consequence of energy constraints, NH_4^+ is favoured over NO_3^- as a source for microorganisms (Rice and Tiedje, 1989; Schimel and Firestone, 1989; Gundersen et al., 1998; Booth et al., 2005; Perelo et al., 2006). The assimilatory NO_3^- reduction (NO_3^- immobilization) requires additional energy for the conversion of NO_3^- to NH_4^+ and for the subsequent incorporation into amino acids. The reduced microbial NO_3^- immobilization by high levels of available NH_4^+ was recognized as a cause of N leaching rather than the enhancement of nitrification itself in some N saturated forest ecosystems (Emmett, 2007; Geisseler et al., 2010). Nevertheless, Curtis et al., (2011) emphasized that the production of NO_3^- must be considered as one of the sources of N leaching, because in some studies where only ammonium sulphate was used as a fertilizer NO_3^- leaching occurred.

Assimilatory NO_3^- reduction is regulated by the N availability and is expected when energy is in excess relative to availability of NH_4^+ or organic-N compounds. Stark and Hart (1997) suggested that there are at least three reasons why immobilization of NO_3^- can occur: (1) high rates of C addition may result in N limitation to microbial biomass and NO_3^- uptake increases, (2) spatial variability of C inputs may result in N mineralization and nitrification micro-sites poor in C (organic matter is decomposed to get C and excess of N is released as NH_4^+) and adjacent NO_3^- immobilization C rich micro-sites and (3) fungal populations can transport NO_3^- from micro-sites with high N mineralization and nitrification to C rich micro-sites. This idea was expanded by Schimel and Bennett (2004) in the assessment of the N source spatial variability. Basically, when N availability in the soil increases, microorganisms become less N limited and begin to mineralize N (i.e. release N in excess as NH_4^+). Produced NH_4^+ is nitrified in N rich micro-sites. Mineral N diffused from N rich micro-

sites to N poor micro-sites is immobilized by microorganisms and plants. Depolymerisation in such conditions is still regulating the mechanisms of the N cycle, however, micro-sites variability of different N substrates and redox conditions increases as well as the heterogeneity of microbial processes. As the mineral N availability further increases, the competition between plants and microorganisms is less substantial, a lot of mineral N remains in the soil solution, nitrifiers prosper, NO_3^- becomes the dominant N form and can be leached out.

An increased load of NO_3^- through atmospheric deposition should probably shift the microbial N immobilization towards NO_3^- , because microorganisms are also capable of synthesizing protein transporters for the uptake of NO_3^- (González et al., 2006), the synthesis of which is induced by the availability of NO_3^- itself (Stark and Hart, 1997; Moir and Wood, 2001). Bengtson and Bengtsson (2005) showed that at least some bacteria in liquid soil cultures (excluding micro-sites variability) immobilized NO_3^- even when NH_4^+ was available. Booth et al. (2005) reported in a meta-analysis that microbial immobilization of NO_3^- is common in forest ecosystems.

Nitrate leaching as an ecosystem response to elevated mineral N input deposition is characteristic by its large variability. Many manipulative N addition experiments were done in various types of ecosystems, with inconsistent results. Ecosystem response differs particularly in the early rates of NO_3^- leaching and in the seasonal course. Relatively high N load can sometimes lead to ambiguous reactions when neither N mineralization nor nitrification are enhanced (reviewed in Lovett and Goodale, 2011; Curtis et al., 2011). It could be partly caused by different designs of the experiments (e.g. duration and dose of N addition, type of acid addition - N with or without S, type of mineral N form added etc.) and also in the initial conditions under which the experiments proceeded. Among all factors that can influence N retention capacity of the ecosystem (e.g. history of land-use; bedrock, soil and vegetation composition; landscape characteristics; hydrology) the highest attention was paid to the soil C pool size (Evans et al., 2006a; Rothwell et al., 2008), soil C-to-N ratio (Dise et al., 1998; 2009; Gundersen et al., 1998) and the quality of organic C (Paavolainen et al., 1998; Strauss and Lamberti, 2000, 2002). Carbon-to-nitrogen ratio of upper soil organic horizons was found to be negatively related to N leaching mainly in ecosystems with lower N input, up to $30 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (Dise et al., 1998). Above this level the variability of the data increased, suggesting other important factors causing the heterogeneity. Other evidence

that ecosystem N retention capacity is related to organic C is the negative correlation between concentrations of DOC and NO_3^- in streams and soils (Kopáček and Hejzlar, 1998; Goodale et al., 2005). Taylor and Townsend (2011) showed that this nonlinear relation based probably on resource stoichiometry and C limitation of heterotrophic metabolism exists across soils, freshwater ecosystems, and oceans worldwide. This is not surprising, as soil microorganisms are predominantly heterotrophs (e.g. Barton and Northup, 2011). Hence, soil N immobilization capacity should rely primarily on the availability of soil organic C. Therefore, soil C cycle should not be neglected when considering factors affecting N retention capacity of the ecosystems, especially in N saturated conditions.

Over the last two decades, increases of DOC concentrations in streams and lakes have been observed across Europe and North America (Driscoll et al., 2003; Monteheit et al., 2007). The rising trends of DOC concentrations were ascribed, among others, to decreased S deposition (Clark et al., 2006; Evans et al., 2006b; Monteheit et al., 2007), but can also be caused by increasing decomposition of complex organic compounds after N loading went down. In fact, there are several indications that decomposition of older, humified, lignin rich organic matter is suppressed and C is accumulated in soil under chronic S and N deposition (Fog, 1988; Green et al., 1995; Berg and Matzner, 1997; Magill and Aber, 1998; Hagedorn et al., 2003). Acid deposition is accompanied by a decrease of the fungi-to-bacteria ratio and N mineralization was found to be negatively related to it (Schmidt et al., 2004; Högberg et al., 2007). Moreover, Högberg et al. (2010) showed that conifers reduce their C allocation to the roots (up to 60 %), which affects primarily the mycorrhizal fungi. Fungi are generally responsible for the decomposition of complex litter (including lignin and humic substances) and can translocate nutrients through their hyphae network, which gives them the opportunity to overcome nutrient limitations during decomposition (Boberg et al., 2010). Therefore their lowered activity could contribute to suppressed organic matter decomposition. During the recovery phase, decomposition could be restored and accumulated C could be released. Goodale et al. (2005) came with the hypothesis that the increases of DOC concentrations is linked to decreasing trends of NO_3^- concentration observed at the same time. They explain low NO_3^- concentrations by increased microbial N immobilization or denitrification in soils. However, greater understanding of all the mechanisms and experimental evidences are still missing.

2. Aim and hypotheses

The overall aim of the thesis was to describe N availability and transformation in acidified and N saturated mountain forest soils. Since there are few data concerning microbial N immobilization, the principal hypothesis was, that **microbial N immobilization plays a crucial role in soil N transformation and retention.**

Specific hypotheses

- (h1) most of the N transformation processes occurs in the upper soil horizons
- (h2) microbial biomass N pool is substantially higher than both mineral N pools
- (h3) enhanced terrestrial N export is accompanied by increased *in situ* N availability
- (h4) high *in situ* N availability is caused by increased net N mineralization processes
- (h5) microbial N immobilization occurs immediately without delay
- (h6) N sources (organic and mineral) are immobilized into microbial biomass simultaneously
- (h7) organic N is immobilized preferentially over mineral N
- (h8) NO_3^- is immobilized even in N saturated soils
- (h9) N immobilization is related to C availability
- (h10) the composition of microbial community (fungi/bacteria) affects N immobilization pattern

Objectives

In order to address the principal hypothesis it was necessary to evaluate the size of microbial biomass N pool and determine its relative importance as related to other soil N pools. We further used the method for *in situ* measurement of N availability and evaluated long-term data obtained from field in a relation to results acquired in laboratory incubations of soils to find out to what degree we are able to extrapolate the data on the ecosystem level. In the follow up work we determined N fluxes through main soil N pools and described microbial immobilization of different N forms. The last, but not the least important part of the work was focused on the possible links among the N transformations, soil chemistry, C content, and the composition of microbial community in N saturated forest soils.

3. Experimental sites

The studies were carried out in four forested mountain areas. Two of them were Czech sites in The Bohemian Forest with spruce plantations, but unmanaged for more than a century (Veselý, 1994) and undergoing recovery from anthropogenic acidification (1). Another mature spruce experimental stand (planted in 1930s) was also located in the Czech Republic, but in the Ore Mountains currently undergoing recovery from extremely high S depositions (2). The third site is represented by pristine spruce forests of the Carpathian biosphere reserve. This site hasn't been impacted by direct human activity (3).

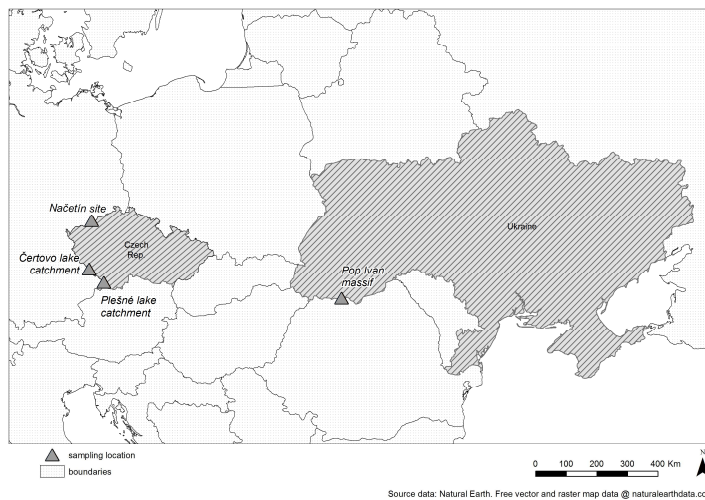


Figure 6: Location of experimental sites.

(1) The Bohemian Forest sites

Majority of the investigation were conducted in the watersheds of Plešné and Čertovo Lakes undergoing recovery from anthropogenic acidification (Fig 2., decreasing levels of S and N depositions since 1980's), but still releasing NO_3^- . The watersheds of glacial lakes are situated in mountain area of the Bohemian Forest, in the south-western part of the Czech Republic. These model ecosystems with near-natural and mostly unmanaged forests have been under regular investigation of major elements fluxes on the watershed-lake ecosystem level since 1998 (Kopáček et al., 1998). It was a great opportunity to study microbial N transformation in soil at these sites, because data be directly related

to the history of the sites, as well as to the N fluxes in the ecosystem as a whole.

The watersheds of Plešné (48°77'N, 13°86'E) and Čertovo (49°16', 13°20'E) lakes are situated at elevations from 1030 to 1378 m a.s.l. with north-east and east exposition, respectively. The bedrock of Plešné Lake watershed is formed by granite, while in the Čertovo Lake watershed it is mica-schist. Soils in both watersheds are leptosols, podsols and dystric cambisols. The forest is dominated by Norway spruce (*Picea abies*, 90-160 years old), with a minor contribution of European beech (*Fagus sylvatica*), silver fir (*Abies alba*) and mountain ash (*Sorbus aucuparia*). Understory vegetation is composed mainly of blueberry (*Vaccinium myrtillus*) and bushgrass (*Calamagrostis villosa*). The snow cover lasts usually from November to April, the average annual temperature is 4 - 6 °C and the average annual precipitation is 1400 mm.

(2) *The Načetín site*

The next Czech experimental site called Načetín is located on the ridge of the Ore Mountains, in the north-western part of the Czech Republic. This region was exposed to extremely high sulphur depositions ($\sim 800 \text{ mmol m}^{-2} \text{ yr}^{-1}$ in 1980s; Berge, 1997), five times higher than in the Bohemian Forest sites, and is currently also undergoing recovery after the decreases of deposition. We have started to be interested in this site, because, in contrast to the Bohemian Forest sites, there was a sharp decrease in leachate NO_3^- , with soil water concentrations close to zero, indicating growing importance of microbial immobilization and/or decreases in NO_3^- production.

The Načetín site (50°35'N, 13°15'E) is situated at elevation of 784 m a.s.l. on a gentle slope oriented to the northwest. The bedrock is paragneiss and soils are mostly dystric cambisols. The forest at the site is completely dominated by Norway spruce (~ 63 years old) with the understory vegetation dominated by wavy hair-grass (*Deschampsia flexuosa*), bushgrass, and blueberry. The snow cover lasts usually from November to April, the average annual temperature is 6.3 °C and annual mean precipitation is ~ 1000 mm.

(3) *The Pop Ivan site*

The intention to compare N saturated soils with undisturbed soils, both developed on acid bedrock and under comparable climate, led us to the mountain site located in the Pop Ivan massif in the Carpathian biosphere reserve (on the border between Ukraine and Romania). Forests in this area were, until recently,

considered as unimpacted by human activity, hence being one of the most pristine forests in Europe. However, soon after the site was chosen, our results and as well as other recent studies showed that this area is strongly acidified, most probably due to trans-boundary deposition. Similarly to our Czech sites, the area also seemed N saturated. Despite of this, the Ukrainian soils gave us the opportunity to study N transformation functioning under possible energy constrains of microbial metabolism, as this site, unlike those in the Bohemian Forest, was marked by a lower C availability.

The Ukrainian natural forest site (the Pop Ivan site, 47°57'N, 24°31'E) is situated at 1480 m a.s.l. on a steep slope exposed to the west. The bedrock is composed of crystalline schist and gneiss. Most soils are dystric cambisols and entic podisols. The forest at the site is almost completely dominated by primeval Norway spruce with a minority of European beech and mountain ash. Understory vegetation is composed of alpine lady fern (*Athyrium distentifolium*), bushgrass, and blueberry. The snow cover lasts from October till April, the annual average temperature is 2°C, and the average annual precipitation is 1800 mm.

4. Present investigation

This thesis is based upon five publications or papers. **Paper I** evaluates the relative importance of microbial N biomass in relation to other soil N pools (**h1, 2**). **Papers II and III** present long-term monitoring of N availability, N mineralization potential of soils and investigates possible links between soil chemistry and N transformations (**h3, 4, 9**). **Paper IV** is interested in the significance of microbial N immobilization in preventing N losses from N saturated soils and presents it as a function of C availability (**h 5 – 10**). **Paper V** shows that the restoration of the N retention capacity may be the consequence of reduced S depositions through C availability retrieval due to decomposition of accumulated organic matter (**h1, 9**).

Paper I

Nitrogen transformations and pools in N-saturated mountain spruce forest soils.

Site: Bohemian Forest

The aim of this study was to assess the size and the relative importance of the main soil N pools (microbial biomass N, ammonium, nitrate, dissolved organic N) in N saturated mountain forest soils. In addition, the influence of temperature on the net processes of microbial N transformation (N mineralization, nitrification, N immobilization) was determined. Results showed that microbial N pool was much larger than mineral N pools, and that the net N flux through microbial N pool greatly exceeded total mineral N fluxes. It was an important indication that the examined mountain soils probably maintain high N retention capacity, despite their N saturation. All processes ran under zero temperatures indicating that mineral N can accumulate during winter under the snow cover, and is leached out after snow melt. The possible effect of the shift in fungi to bacteria ratio in N saturated soils (microbial C-to-N ratio decrease) on microbial respiration is discussed.

Paper II

Nitrogen availability in Norway spruce forest floor - the effect of forest defoliation induced by bark beetle infestation.

Site: Bohemian Forest

This paper contains results of the long-term monitoring of N availability within the stand of mountain spruce forest. Its main aim was to elucidate the relationship between available NH_4^+ and NO_3^- measured *in situ* and the potential (net rates) of N mineralization and nitrification determined during laboratory incubations. We found that enhanced N mineralization contributed to high mineral N leaching after forest decline, although reduced immobilization by trees was identified as the primary cause of N leaching. The excess of mineral N release was most probably caused by the elevated input of spruce litter with low C-to-N ratio into the soil and its subsequent decomposition without parallel increases of microbial N immobilization. This was an important indication that soil N retention capacity is related to soil (substrate) C-to-N ratio.

Paper III

Changes in soil chemistry after bark beetle infestation and forest dieback.

Site: Bohemian Forest

This chapter aims to fill the gap in understanding how the chemistry of forest floor changes following natural forest dieback, when elevated litter decomposition occurs in acidified and N saturated soils. It integrates the results of a three-year monitoring in six-week sampling intervals. The forest dieback significantly increased water extractable NH_4^+ , organic N, and P. However, a 2-year delay was observed in available NO_3^- after main litter input through spruce defoliation. We hypothesize that doubled concentrations of water soluble C could support microbial immobilization of excess N. Besides, the study shows that soil conditions can be partly improved by increased base saturation following the decomposition of the litter.

Paper IV

Microbial N immobilization is of great importance in acidified mountain spruce forest soils.

Site: Bohemian Forest, Pop Ivan

Main goal of presented study was to determine whether microbial N immobilization can be a primary process affecting N leaching from N saturated mountain forest soils. It assesses with the immobilization of different ^{15}N sources (ammonium, nitrate, glycine) by microbial biomass and describes the transformation of added N sources during 48 hours of aerobic soil incubation in relation to C availability and microbial community composition.

Very fast and simultaneous immobilization of all added N forms into microbial biomass was found with clear preference for organic over inorganic N sources. Total N flux to the microbial pool exceeded N flux into mineral N pools. Nitrogen transformation pattern was different under reduced C availability (Pop Ivan site). Microbial N pool and associated N flux were smaller than the mineral N pools and fluxes under unsuitable C conditions. In most cases, NO_3^- was not immobilized into microbial biomass, while gross nitrification was pronounced.

The composition of bacterial community was related to DOC content and C and N in microbial biomass. When fungi were more abundant in soils, more glycine was taken into microbial biomass (regardless soil C availability), but with higher subsequent release of NH_4^+ to the soil.

Abiotic immobilization into dissolved organic matter is also discussed.

Paper V

Major changes in forest carbon and nitrogen cycling caused by declining sulphur deposition.

Site: Načetín

The main goal of the study was to evaluate changes in C and N pools and cycling in a mountain spruce forest over the 16 year period of declining acid input. A significant decrease of total C and N soil pools, potential N mineralization, and N leaching was observed, corresponding to the decline in S deposition. Moreover, an increase in litterfall and forest floor C-to-N ratio and DOC leaching were found. We assumed that the reduction in S loadings have stimulated the decomposition of organic matter, which, in turn, supported microbial N immobilization. This led to the reversal effect on the N saturation of the soil, demonstrated by the disappearance of NO_3^- leaching from the soil.

5. Conclusions and future prospects

Microbial N transformations in mountain forest ecosystems were examined in this thesis, with emphasis on the immobilization process. Various aspects and approaches were used to describe N cycle in acidified and N saturated soils. We combined the relatively standard techniques of measuring soil N transformations (soil extractions to know soil N pools, fumigation-extraction method for measuring microbial biomass, soil incubation for measuring net rates of processes) with more specific methods involving field measurements of N availability (ion resin bags), stable isotope labelling experiments and also molecular biology methods. Despite the different approaches used, all studies produced results that can be generalized and summarized within a broader ecological context, which is the aim of this last chapter of the thesis.

We revealed based on a major part of the results that biogeochemical cycles of C and N are closely coupled in forest soils. We showed that upper soil horizons comprised a majority of total N (~70%) in the soil profile. Similarly, the activity of microorganisms was the highest in the litter horizon (**I**), which confirms the generally observed trend of decreasing microbial activity with decreasing C content down to the soil profile (e.g. Šantrůčková et al., 2010). In agreement with our expectations, soil C-to-N ratio and organic C availability were linked to the ability of microorganisms to immobilize mineral N (**II-V**). It is closely linked to the hypothesis by Goodale et al. (2005), which says that observed decrease in stream NO_3^- concentrations may be linked to recent DOC increases in ecosystems undergoing recovery from acidification. In other words, the supply of DOC to microorganisms can promote microbial N immobilization and lead to the reduction of NO_3^- leaching from soils. Our papers highlight the importance of microbial biomass as the biggest N pool in the microbial-soil system. Microbial N pool was up to five times higher than both mineral pools and the N flux into it exceeded the N fluxes into both mineral pools (NH_4^+ and NO_3^- , **I**, **IV**). It is highly probable that any small disturbance of the large microbial N pool would cause a decrease of N immobilization of organic and mineral N and, consequently, lead to an increased risk of N leaching. We found a strong negative correlation between DOC and NO_3^- in examined soils (**IV**) and a lack of microbial immobilization of mineral N in low C Ukrainian soil during manipulative experiments with stable isotopes (**IV**). This indicates that the heterotrophic part of the microbial community in acidified soils may be restricted by the lack of available organic C substrate. If it occurs, nitrification is

enhanced by the NH_4^+ concentration increases through organic matter mineralization, and microbial capacity to immobilize it is insufficient. As a result, the concentration of NO_3^- in soils increases.

According to Lovett and Goodale (2011) N immobilization into microbial biomass is not expected to contribute to N sinks evaluated over an annual time scale or in a longer perspective. Microbial biomass is indeed a small pool compared to the total soil organic matter (1-5%, Jenkinson and Ladd, 1981). However, it is also very labile pool that responds very quickly to changing soil conditions (moisture disturbances, substrate input etc.). Our results showed that microorganisms immobilize various N sources without any delay and processes of N transformations occur simultaneously in soil (IV). We further showed that although microorganisms preferred organic N, both forms of mineral N were also used (IV). The turnover time of microbial biomass was estimated to be from days to a year (e.g. Raubuch and Joergensen, 2002; Schmidt et al., 2007; Kreutzer et al. 2009; Rousk and Bååth, 2011) and released organic N can be quickly recycled by living microorganisms (cryptic growth, Lynch and Whipps, 1990) without releasing it into the soil. This explains the long life-time of N containing compounds in the soil, which can be as long as 50 years (proteins, amino-acids, chitin, Gleixner et al., 2002). Simpson et al. (2007) found that almost 80% of the microbial biomass N constituents can input soil organic matter (SOM). **We suggest, based on our own results and literature findings that microbial immobilization plays a key role in soil N transformation.** Significant amount of N is turned over by microbial biomass even in N saturated soils and NO_3^- losses depend on actual C availability for microorganisms and their ability to grow. Microbial biomass could also be an important N sink from the long-term perspective. Once immobilized, N enters the organic N pool (is built into organic compounds in cells) and can be recycled after the death of microbial cells when the majority of microbial N may enter SOM.

Leaching of NO_3^- is usually observed not only after increased N loading, but also after forest disturbances such as clear cutting or dieback caused by pest infections (e.g. Stevens and Hornung 1990, Grenon et al. 2004, Huber, 2005). The ecosystems in the Bohemian Forest have been invaded by bark beetle during our long term monitoring and some trees died off. Although the presented thesis was primarily focused on the effect of acidification and N saturation on microbial N transformations, we made the best of the situation and evaluated the effect of forest dieback and recovery on soil N transformations and chemistry as

a side goal.

There is a lack of data about changes in soil nutrient cycling after natural forest dieback. The existing studies examined the long term effects of dieback with individual year to year samplings (e.g. Morehouse et al., 2008; Clow et al., 2011; Griffin et al., 2011). In our studies we examined continual changes of soil chemistry after major litter input to soil from dead trees killed by bark beetle infection, and compared it with non-invaded forest sites (**II**, **III**). We found that excess NO_3^- leaching didn't immediately appear after forest dieback, but with a considerable time delay, long after the soil NH_4^+ availability was increased (1- 2 years, **II**, **III**). In our first forest dieback study (**II**) we attributed the delay to the effect of tree physiology of N uptake and, consequently, the increased N availability to enhanced decomposition of low C-to-N litter with no parallel increase of microbial N immobilization. However, based on the following studies (**III**, **IV**) it seems that changes in microbial N transformations play a crucial role. When organic matter N mineralization is in progress and surplus NH_4^+ is released, microorganisms can most probably keep NO_3^- concentrations low, as long as they have enough DOC. This supports findings of Stark and Hart (1997) who claim that microbial NO_3^- immobilization is suppressed in the conditions of high NH_4^+ and low C availability. On the ecosystem level, mineral N export from the impacted Plešné Lake watershed to freshwaters was increased from $83 \text{ mmol m}^{-2}\text{yr}^{-1}$ (years 2000–2004, before forest dieback) to $150 \text{ mmol m}^{-2}\text{yr}^{-1}$ (years 2005–2006, after forest dieback) (Kopáček et al., 2007). However, this increase did not correspond fully with a calculated decline of total inorganic N uptake by forest vegetation that was much pronounced (Kopáček et al., 2007). This means that a part of the remaining N and additional N produced through elevated litter mineralization had to be accumulated in soil. We are currently continuing in our six-week sampling and analyses to see the effect of future exhaustion of available DOC on N transformations and we expect that NO_3^- concentrations in soil solution will probably increase.

Decomposition of litter in the infested study site elevated concentrations of soil BC (**III**). The base saturation increased from 40 to 70% and from 30 to 45% in O and A horizons, respectively. However, ionic Al in soil decreased as it was replaced from soil sorption complex, and simultaneously complexed with DOC. Such DOC-Al complexes are not toxic for biota and do not negatively affect roots and organisms in soil but, being mobile, can influence the recipient freshwaters. In lakes, the liberation of ionic Al from DOC-Al complexes may occur by photochemical reactions (Kopáček et al., 2005a). Its subsequent

hydrolysis under higher pH produces Al hydroxides with large specific surfaces and with a strong affinity for P. Increased concentrations of Al hydroxides in sediments may thus cause reduction of P release from sediments even under hypolimnetic anoxia when metal hydroxides would normally dissolve and P would be released into the water column. This may lead to oligotrophication affecting further the primary production and cycling of organic C in lakes (Kopáček et al., 2000; Kopáček et al. 2005b). This is another confirmation that element cycles in the soil-watershed-lake systems are not isolated, but closely associated.

The mean half decay time of Norway spruce needles was determined to be approximately three years (Šantrůčková et al., 2006). Thus we expect that the gradual liberation of elements to soil will continue probably for several more years and may further influence nutrient transformations in the watershed-lake ecosystems. However, the future progress will also depend on the restoration of vegetation cover and climatic factors.

For further understanding of the N retention mechanisms in N saturated ecosystems many questions still need to be addressed. Why is there a difference in total soil C-to-N ratio and C availability between Bohemian Forest and Ukrainian soils? Could it be a consequence of different S deposition history? In the last paper (V) we showed that declined S deposition corresponded to the increase of DOC concentrations and to the decreases in total C and N soil pools, potential N mineralization, and N leaching. Soils in the Ukrainian forest site were exposed to higher S deposition than Bohemian Forest sites, which could possibly have induced the stabilization of soil organic matter and changes in soil C quality.

In the future work we would like to focus on linkages between microbial N and C transformations and cycling in more detail. We would also like to look at the effect of P availability, which definitively plays an important role in microbial N transformations, but its role has so far been neglected. We are continuing with the studies of microbial C, N and P transformations and biochemical processes during post-disturbance period in the Plešné Lake watershed, including wood decay, soil microclimate and vegetation cover changes, and forest regeneration. In addition, we are planning to compare obtained results with the pre-disturbance period (running project GACR P504/12/1218). We are preparing a long-term manipulative S and N addition experiment in the Ore Mountains to understand the combined effects of S and N deposition on C and N cycling in forest soils (project application in 2012).

References

- Aber, J.D., Nadelhoffer, K., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. *Bioscience* 39 (6), 378-386.
- Aber, J.D., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen saturation in temperate forest ecosystems – Hypothesis revisited. *Bioscience* 48, 921-934.
- Agren, G.I., Bosatta, E., 1988. Nitrogen saturation of terrestrial ecosystems. *Environmental Pollution* 54 (3-4), 185-197.
- Alewel, C., Paul, S., Lischeid, G., Küsel, K., Gehre, M., 2006. Characterizing the redox status in three different forested wetlands with geochemical data. *Environmental Science & Technology* 40, 7609-7615.
- Augusto, L., Ranger, J., 2001. Impact of tree species on soil solutions in acidic conditions. *Annals of Forest Science* 58, 47-58.
- Bárta, J., Applová, M., Vaněk, D., Křišťůvková, M., Šantrůčková, H., 2010. Effect of available P and phenolics on mineral N release in acidified spruce forest: connection with lignin-degrading enzymes and bacterial and fungal communities. *Biogeochemistry* 97, 71-87.
- Barton, L.L., Northup, D.E. 2011. *Microbial Ecology*. Wiley-Blackwell, Hoboken, New Jersey.
- Bengtson, P., Bengtsson, G., 2005. Bacterial immobilization and remineralization of N at different growth rates and N concentrations. *FEMS Microbiology Ecology* 54 (1), 13-19.
- Bengtsson, G., Bergwall, C., 2000. Fate of ¹⁵N labelled nitrate and ammonium in a fertilized forest soil. *Soil Biology & Biochemistry* 32, 545-557.
- Bengtsson, G., Bengtson, P., Månsson, K.F., 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology & Biochemistry* 35, 143-154.
- Berg, B., Matzner, E., 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environmental Reviews* 5, 1-25.
- Berge, E., 1997. Transboundary air pollution in Europe, part I. EMEP MSC-W Report 1/97. Norwegian meteorological Institute, Oslo.
- Binkley, D., 1995. The influence of tree species on forest soils: processes and patterns. In: Mead, D.J., Cornforth, I.S., Eds. 1995. Proceedings of the trees and soil workshop. Lincoln University 28 February – 2 March 1994. Agronomy Society of New Zealand Special Publication No. 10, Lincoln University Press, Canterbury.
- Boberg, J.B., Finlay, R.D., Stenlid, J., Lindahl, B.D., 2010. Fungal C translocation restricts N-mineralization in heterogeneous environments. *Fungal Ecology* 24 (2), 454-459.
- Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecological Monographs* 75 (2), 139-157.
- Clark, J.M., Chapman, P.J., Heathwaite, A.L., Adamson, J.K., 2006. Suppression of dissolved organic carbon by sulfate induced acidification during simulated droughts. *Environmental Science & Technology* 40, 1776-1783.
- Clow, D.W., Rhoades, C., Briggs, J., Caldwell, M., Lewis, W.M., 2011. Responses of soil and water chemistry to mountain pine beetle induced tree mortality in

- Grand County, Colorado, USA, *Applied Geochemistry* 26, S174-S178.
- Compton, J.E., Boone, R.D., 2002. Soil nitrogen transformations and the role of light fraction organic matter in forest soils. *Soil Biology & Biochemistry* 34 (7), 933-943.
- Compton, J.E., Watrud, L.S., Porteous, L.A., 2004. Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. *Forest Ecology & Management* 196 (1), 143-158.
- Corre, M.D., Beese, F.O., Brumme, R., 2003. Soil nitrogen cycle in high nitrogen deposition forest: Changes under nitrogen saturation and liming. *Ecological Applications* 13, 287-298.
- Corre, M.D., Brumme, R., Veldkamp, E., Beese, F.O., 2007. Changes in nitrogen cycling and retention processes in soils under spruce forests along a nitrogen enrichment gradient in Germany. *Global Change Biology* 13 (7), 1509-1527.
- Curtis, C.J., Emmett, B.A., Grant, H., Kernan, M., Reynolds, B., Shilland, E., 2005. Nitrogen saturation in UK moorlands: the critical role of bryophytes and lichens in determining retention of atmospheric N deposition. *Journal of Applied Ecology* 42, 507-17.
- Curtis, C.J., Evans, C.D., Goodale, C.L., Heaton, T.H.E., 2011. What have stable isotope studies revealed about the nature and mechanisms of N saturation and nitrate leaching from semi-natural catchments? *Ecosystems* 14 (6), 1021-1037.
- Davidson, E.A., Hart, S.C., Shanks, C.A., Firestone, M.K., 1991. Measuring gross nitrogen mineralization, immobilization, and nitrification by ¹⁵N isotopic pool dilution in intact soil cores. *Journal of Soil Science* 42 (3), 335-349.
- Davidson, E.A., Hart, S.C., Firestone, M.K., 1992. Internal cycling of nitrate in soils of a mature coniferous forest. *Ecology* 73 (4), 1148-1156.
- De Boer, W., Kowalchuk, G.A., 2001. Nitrification in acid soils: micro-organisms and mechanisms, *Soil Biology & Biochemistry* 33, 853-866.
- Dise, N.B., Matzner, E., Armbruster, M., MacDonald, J., 2001. Aluminium output fluxes from Forest Ecosystems in Europe: A Regional Assessment. *Journal of Environmental Quality* 30, 1747-1756.
- Dise, N.B., Matzner, E., Forsius, M., 1998. Evaluation of organic horizon C:N ratio as an indicator of nitrate leaching in conifer forests across Europe. *Environmental Pollution* 102 (S1), 453-456.
- Dise, N.B., Rothwell, J.J., Gauci, V., van der Salm, C., de Vries, W., 2009. Predicting dissolved inorganic nitrogen leaching in European forests using two independent databases. *Science of The Total Environment* 407 (5), 1798-1808.
- Drábek, O., Mládková, L., Borůvka, L., Szaková, J., Nikodém, A., Němeček, K., 2005. Comparison of water-soluble and exchangeable forms of Al in acid forest soils. *Journal of Inorganic Biochemistry* 99, 1788-1795.
- Driscoll, C.T., Driscoll, K.M., Roy, K.M., Mitchell, M.J., 2003. Chemical response of lakes in the Adirondack Region of New York to declines in acidic deposition. *Environmental Science and Technology* 37 (10), 2036-2042.
- Dunn, R.M., Mikola, J., Bol, R., Bardgett, R.D., 2006. Influence of microbial activity on plant-microbial competition for organic and inorganic nitrogen. *Plant & Soil* 289, 321-334.
- Emmett, B.A., 2007. Nitrogen Saturation of Terrestrial Ecosystems: Some recent findings and their implications for our conceptual framework. *Water, Air, & Soil Pollution* 7 (1-3), 99-109.
- Evans, C.D., Caporn, S.J.M., Carroll, J.A., Pilkington, M.G., Wilson, D.B., Ray, N.,

- Cresswell, N., 2006a. Modelling nitrogen saturation and carbon accumulation in heathland soils under elevated nitrogen deposition. *Environmental Pollution* 143 (3), 468-478.
- Evans, C.D., Chapman, P.J., Clarke, J.M., Monteheith, D.T., Cresser, M.S. 2006b. Alternative explanations for rising dissolved organic carbon export from organic soils. *Global Change Biology* 12 (11), 2044-2053.
- Evans, C.D., Cullen, J.M., Alewell, C., Kopáček, J., Marchetto, A., Moldan, F., Prechtel, A., Rogora, M., Veselý, J., Wright, R., 2001. Recovery from acidification in European surface waters. *Hydrology & Earth System Sciences Discussions* 5 (3), 283-298.
- Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. *Biological Reviews* 63, 433-462.
- Foy, C.D., 1983. The physiology of plant adaptation to mineral stress. *Iowa State Journal of Research* 57, 355-391.
- Frijlink, M.J., Abee, T., Laanbroek, H.J., Boer, W., Konings, W.N., 1992. The bioenergetics of ammonia and hydroxylamine oxidation in *Nitrosomonas europaea* at acid and alkaline pH. *Archives of Microbiology* 157 (2), 194-199.
- Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms - A review. *Soil Biology & Biochemistry* 42 (12), 2058-2067.
- Gleixner, G., Poirier, N., Bol, R., Balesdent, J., 2002. Molecular dynamics of organic matter in a cultivated soil. *Organic Geochemistry* 33 (3), 357-366.
- Gonod, V.L., Jones, D.L., Chenu, C., 2006. Sorption regulates the fate of amino acids lysine and leucine in soil aggregates. *European Journal of Soil Science* 57, 320-329.
- González, P.J., Correia, C., Moura, J., Bronido, C.D., Moura, J.J.G., 2006. Bacterial nitrate reductases: Molecular and biological aspects of nitrate reduction. *Journal of Inorganic Biochemistry* 100, 1015-1023.
- Goodale, C.L., Aber, J.D., Vitousek, P.M., McDowell, W.H., 2005. Long-term decreases in stream nitrate: Successional causes unlikely; Possible links to DOC? *Ecosystems* 8 (3), 334-337.
- Green, C.J., Blackmer, A.M., Horton, R., 1995. Nitrogen effect on conservation of carbon during corn residue decomposition in soil. *Soil Science Society of America Journal* 59, 453-459.
- Grenon, F., Bradley, R.L., Joannis, G., Titus, B.D., Prescott, C.E., 2004. Mineral N availability for conifer growth following clear cutting: responsive *versus* non-responsive ecosystems. *Forest Ecology & Management* 188, 305-316.
- Griffin, J.M., Turner, M.G., Simard, M., 2011. Nitrogen cycling following mountain pine beetle disturbance in lodgepole pine forests of Greater Yellowstone, *Forest Ecology and Management* 261 (6), 1077-1089.
- Gundersen, P., Emmet, B.A., Kjønnaas, O.J., Koopmans, C.J., Tietama, A., 1998. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Ecological Management* 101, 37-55.
- Hagedorn, F., Mohn, J., Schleppei, P., Flüher, H., 1999. The role of rapid flow paths for nitrogen transformation in a forest soil: A field study with micro suction cups. *Soil Science Society of America Journal* 63, 1915-1923.
- Hagedorn, F., Spinnler, D., Siegwolf, R., 2003. Increased N deposition retards mineralization of old soil organic matter. *Soil Biology & Biochemistry* 35, 1683-1692.

- Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994. Dynamics of Gross Nitrogen Transformations in an Old-Growth Forest: The Carbon Connection. *Ecology* 75, 880-891.
- Heim, A., Luster, J., Brunner, I., Frey, B., Frossard, E., 2000. Effects of aluminium treatment on Norway spruce roots: Aluminium binding forms, element distribution, and release of organic substances. *Plant & Soil* 216, 103-116.
- Hobbie, S.E., Reich, P.B., Oleksyn, J., Ogdahl, M., Zytkowski, R., Hale, C., Karolewski, P., 2006. Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology* 87 (9), 2288-2297.
- Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree belowground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist* 187, 485-493.
- Högberg, M.N., Chen, Y., Högberg, P., 2007. Gross nitrogen mineralisation and fungito-bacteria ratios are negatively correlated in boreal forests. *Biology & Fertility of Soils* 44 (2), 363-366.
- Hruška, J., Cienciala, E., 2005. Dlouhodobá acidifikace a nutriční degradace lesních půd - limitující faktor současného lesnictví [in Czech], Česká geologická služba, Praha, 153p.
- Huber, C., 2005. Long lasting nitrate leaching after bark beetle attack in the highlands of the Bavarian Forest National Park. *Journal of Environmental Quality* 34, 1772-1779.
- Huygens, D., Boeckx, P., Templer, P.H., Paulino, L., van Cleemput, O., Oyarzun, C., Mueller, C., Godoy, R., 2008. Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils. *Nature Geoscience* 1 (8), 543-548.
- Illmer, P., Marschall, K., Schinner, F., 1995. Influence of available aluminium on soil microorganisms. *Letters in Applied Microbiology* 21, 393-397.
- Illmer, P., Obertegger, U., Schinner, F., 2003. Microbiological properties in acidic forest soils with special consideration of KCl extractable Al. *Water, Air & Soil Pollution* 148, 3-14.
- Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soil: measurement and turnover. In *Soil Biochemistry* 5, E.A. Paul, J.N. Ladd, Eds., Dekker, New York, 415-471p.
- Johnson, D.W., Cheng, W., Burke, I.C., 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. *Soil Science Society of America Journal* 64 (4), 1503-1514.
- Joner, E.J., Eldhuset, T.D., Lange, H., Frostegård, A. 2005. Changes in the microbial community in a forest soil amended with aluminium *in situ*. *Plant & Soil* 275, 295-304.
- Kämäri, J., Jeffries, D.S., Hessen, D.O., Henriksen, A., Posch, M., Forsius, M., 1992. In: Grennfelt, P., Thörnelö, F. E., Eds., Nitrogen critical loads and their exceedance for surface waters. Report from a workshop held at Lökeborg, Sweden, 6-10 April 1992, Nord 1992:41. Copenhagen: Nordic Council of Ministers, 161-200p.
- Keeney, D., 1980. Prediction of soil nitrogen availability in forest ecosystems: A literature review. *Forestry Science* 26, 159-171.
- Keetle, H., Kopáček, J., Hejzlar, J., 2003. Modelling air temperature at Čertovo Lake back to 1781, *Silva Gabreta* 9, 15-23.

- Killham, K., 1994. *Soil Ecology*. Cambridge University Press, Cambridge, 242p.
- Kochian, L.V., Pineros, M.A., Hoekenga, O.A., 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant & Soil* 274 (1-2), 175-195.
- Kopáček, J., Hejzlar, J., 1998. Water chemistry of surface tributaries to the acidified mountain lakes in Bohemian Forest. *Silva Gabreta* 2, 175-197.
- Kopáček, J., Hruška J., 2010. Reconstruction of acidic deposition in the catchments of Plešné and Čertovo lakes (the Bohemian Forest). *Silva Gabreta* 16 (3), 149-163.
- Kopáček, J., Posch, M., 2011. Anthropogenic nitrogen emissions during the Holocene and their possible effects on remote ecosystems. *Global Biogeochemical Cycles*, 25 (GB2017), 1-16.
- Kopáček, J., Veselý, J., 2005. Sulfur and nitrogen emissions in the Czech Republic and Slovakia from 1850 till 2000. *Atmospheric Environment* 39, 2179-2188.
- Kopáček, J., Borovec, J., Hejzlar, J., Ulrich K.U., Norton, S.A., Amirbahman, A., 2005b. Aluminum control of phosphorus sorption by lake sediments. *Environmental Science & Technology* 39, 8784-8789.
- Kopáček, J., Hejzlar, J., Borovec, J., Porcal, P., Kotorová, I., 2000. Phosphorus inactivation by aluminum in the water column and sediments: Lowering of in-lake phosphorus availability in an acidified watershed-lake ecosystem. *Limnology & Oceanography* 45 (1), 212-225.
- Kopáček, J., Hejzlar, J., Stuchlík, E., Fott, J., Veselý, J., 1998. Reversibility of acidification of mountain lakes after reduction in nitrogen and sulphur emissions in Central Europe. *Limnology & Oceanography* 43 (2), 357-361.
- Kopáček, J., Kaňa, J., Šantrůčková, H., Porcal, P., Hejzlar, J., Píček, T., Veselý, J., 2002a. Physical, chemical, and biochemical characteristics of soils in watersheds of the Bohemian Forest lakes: I. Plešné Lake. *Silva Gabreta* 8, 43-62.
- Kopáček, J., Kaňa, J., Šantrůčková, H., Porcal, P., Hejzlar, J., Píček, T., Veselý, J., 2002b. Physical, chemical, and biochemical characteristics of soils in watersheds of the Bohemian Forest lakes: II. Čertovo and Černé Lakes. *Silva Gabreta* 8, 63-93.
- Kopáček, J., Klementová, Š., Norton, S.A., 2005a. Photochemical Production of Ionic and Particulate Aluminum and Iron in Lakes. *Environmental Science & Technology* 39 (10), 3656-3662.
- Kopáček, J., Procházková, L., Hejzlar, J., Blažka, P., 1997. Trends and seasonal patterns of bulk deposition of nutrients in the Czech Republic. *Atmospheric Environment* 31, 797-808.
- Kopáček, J., Šantrůčková, H., Svoboda, M., Tahovská, K., Matějka, K., Cudlín, P., 2007. Nitrogen pools and fluxes in the catchment-lake ecosystem of Plešné Lake (Czech Republic) prior to and after a bark beetle infestation. In: *Gordon Research Conference - Catchment Science: Interactions of Hydrology, Biology & Geochemistry*. Colby-Sawyer College, New London, NH, United States, 13.7.2007, New London, NH, United States.
- Kopáček, J., Turek, J., Hejzlar, J., Porcal, P., 2011. Bulk deposition and throughfall fluxes of elements in the Bohemian Forest (central Europe) from 1998 to 2009. *Boreal Environment Research* 16, 495-508.
- Kortelainen, P., Saukkonen, A., Mattsson, T., 1997. Leaching of nitrogen from forested catchments in Finland. *Global Biogeochemical Cycles* 11, 627- 638.
- Kreutzer, K., Butterbach-Bahl, K., Rennenberg, H., Papen, H., 2009. The complete

- nitrogen cycle of an N-saturated spruce forest ecosystem. *Plant Biology* 11 (5), 643-9.
- Lovett, G. M., Goodale, C.L., 2011. A New Conceptual Model of Nitrogen Saturation Based on Experimental Nitrogen Addition to an Oak Forest. *Ecosystems* 14 (4), 615-631.
- Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. *Plant & Soil* 129 (1), 1-10.
- Magill, A.H., Aber, J.D., 1998. Long term effect of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. *Plant & Soil* 203, 301-311.
- Majer, V., Cosby, B.J., Kopáček, J., Veselý, J., 2003. Modelling reversibility of Central European mountain lakes from acidification: Part I. - The Bohemian Forest. *Hydrology & Earth System Sciences* 7, 494-509.
- Manzoni, S., Porporato, A., 2007. Theoretical analysis of nonlinearities and feedbacks in soil carbon and nitrogen cycle. *Soil Biology & Biochemistry* 39 (7), 1542-1556.
- McNulty, S.G., Aber, J.D., Newman, S.D., 1996. Nitrogen saturation in a high elevation New England spruce–fir stand. *Forest Ecology & Management* 84, 109-121.
- Melin, J., Nömmik, H., Lohm, U., Flower-Ellis, J., 1983. Fertilizer nitrogen budget in a Scots pine ecosystem attained by using root-isolated plots and ¹⁵N tracer technique. *Plant & Soil* 74, 249-263.
- Merrick, M.J., Edwards, R.A., 1995. Nitrogen control in bacteria. *Microbiological Reviews* 59 (4), 604-622.
- Moir, J.W.B., Wood, N.J., 2001. Nitrate and nitrite transport in bacteria. *Cellular & Molecular Life Sciences* 58, 215-224.
- Moldan, B., Černý, J., Eds., 1994. *Biogeochemistry of Small Catchments: A Tool for Environmental Research*, SCOPE 51, John Wiley & Sons, Chichester, 450p.
- Monteith, D. T., Stoddard, J. L., Evans, C. D., de Wit, H.A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopáček, J., Veselý, J., 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450, 537-540.
- Morehouse, K., Johns, T., Kaye, J., Kaye, M., 2008. Carbon and nitrogen cycling immediately following bark beetle outbreaks in south-western ponderosa pine forests. *Forest Ecology & Management* 255 (7), 2698-2708.
- Morier, I., Schlegel, P., Siegwolf, R., Knicker, H., Guenat, C., 2008. ¹⁵N immobilization in forest soil: a sterilization experiment coupled with (15)CPMAS NMR spectroscopy. *European Journal of Soil Science* 59 (3), 467-475.
- Mulder, J., Cresser, M.S., 1994. Soil and soil solution chemistry. In: Moldan, B., Černý, J., Eds., *Biogeochemistry of small catchments*. Wiley & Sons, Chichester, 107-131p.
- Mulder, J., De Wit, H.A., Boonen, H.W.J., Bakken, L.R. 2001. Increased levels of aluminium in forest soils: Effects on the stores of soil organic carbon. *Water Air and Soil Pollution*, 130, 989-994.
- Murdoch, P.S., Burns, D.A., Lawrence, G.B., 1998. Relation of climate change to the acidification of surface waters by nitrogen deposition. *Environmental Science & Technology* 32, 1642-1647.
- Nadelhoffer, K.J., Coleman, B.P., Currie, W.S., Magill, A.H., Aber, J.D., 2004. Decadal scale fates of ¹⁵N labelled tracers in oak and pine forest plots under ambient and chronically elevated N inputs at the Harvard forest (USA). *Forest Ecology & Management* 196, 89-107.

- Nadelhoffer, K., Downs, M., Fry, B., Magill, A., Aber, J., 1999. Controls on N retention and exports in a forested watershed. *Environmental Monitoring & Assessment* 55, 187-210.
- Nedbalová, L., Vrba, J., Fott, J., Kohout, L., Kopáček, J., Macek, M., Soldán, T., 2006. Biological recovery of the Bohemian Forest lakes from acidification. *Biologia* 61 (S20), S453-S465.
- Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental Microbiology* 10 (11), 2966-2978.
- Nordin, A., Högberg, P., Näsholm, T., 2001. Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* 129, 125-132.
- Norton, S.A., Veselý, J., 2003. Acidification and acid rain. In: Lollar, B.S., Ed., *Environmental Geochemistry, Treatise on Geochemistry*, 9, Elsevier-Pergamon, Oxford, 367-406p.
- Oulehle, F., Cosby, B.J., Wright, R.F., Hruška, J., Kopáček, J., Krám, P., Evans, C.D., Moldan, F., 2012. Modelling soil nitrogen: The MAGIC model with nitrogen retention linked to carbon turnover using decomposer dynamics, *Environmental Pollution* 165, 158-166.
- Oulehle, F., Hofmeister, J., Hruška, J., 2007. Modeling of the long-term effect of tree species (Norway spruce and European beech) on soil acidification in the Ore Mountains, *Ecological Modelling* 204 (3-4), 359-371.
- Oulehle, F., McDowell, W.H., Aitkenhead-Peterson, J.A., Krám, P., Hruška, J., Navrátil, T., Buzek, F., Fottová, D., 2008. Long-term trends in stream nitrate concentrations and losses across watersheds undergoing recovery from acidification in the Czech Republic. *Ecosystems* 11 (3), 410-425.
- Oulehle, F., Hofmeister, J., Cudlín, P., Hruška, J., 2006. The effect of reduced atmospheric deposition on soil and soil solution chemistry at a site subjected to long-term acidification, Načetín, Czech Republic. *Science of the Total Environment* 370 (2-3), 532-544.
- Paavolainen, L., Kitunen, V., Smolander, A., 1998. Inhibition of nitrification in forest soil by monoterpenes. *Plant & Soil* 205 (2), 147-154.
- Pennanen, T., Fritze, H., Pekka, V., Kiiikkilä, O., Neuvonen, S., Bååth, E., 1998. Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. *Applied & Environmental Microbiology* 64 (6), 2173-2180.
- Perakis, S.S., Hedin, L.O., 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. *Ecology* 82 (8), 2245-2260.
- Perelo, L.W., Jimenez, M., Munch, J.C., 2006. Microbial immobilisation and turnover of ¹⁵N labelled substrates in two arable soils under field and laboratory conditions. *Soil Biology & Biochemistry* 38, 912-922.
- Pardo, L.H., Kendall, C., Pett-Ridge, J., Chang, C.C.Y., 2004. Evaluating the source of streamwater nitrate using d¹⁵N and d¹⁸O in nitrate in two watersheds in New Hampshire, USA. *Hydrological Processes* 18 (2), 699-712.
- Pierzynski, G.M., Sims, J.T., Vance, G.F., 2000. *Soils and environmental quality*. 2nd Ed. CRC Press LLC, Boca Raton, 584p.
- Pitter, P., 2009. *Hydrochemie [in Czech]*, 4th Ed. Praha, VŠCHT Praha, 568p.
- Potthast, A., Schiene, R., Fischer, K., 1996. Structural investigations of N-modified lignins by ¹⁵N-NMR spectroscopy and possible pathways for formation of nitrogen containing compounds related to lignin. *Holzforschung* 50 (6), 554-

- Puhe, J., Ulrich, B., 2001. Global climate change and human impacts on forest ecosystems. *Ecological Studies* 143, 593p.
- Raubuch, M., Joergensen, R.G., 2002. C and net N mineralisation in a coniferous forest soil: the contribution of the temporal variability of microbial biomass C and N. *Soil Biology & Biochemistry* 34 (6), 841-849.
- Reich, P.B., Oleksyn, J., Modrzynski, J., Mrozinski, P., Hobbie, S.E., Eissenstat, D.M., Chorover, J., Chadwick, O.A., Hale, C.M., Tjoelker, M.G., 2005. Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. *Ecology Letters* 8 (8), 811-818.
- Rice, C.W., Tiedje, J.M., 1989. Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. *Soil Biology & Biochemistry* 21 (4), 597-602.
- Rosswall, T., Schnurer, J., Soderlund, S., 1986. Interactions of acidity, aluminium ions and microorganism. In: Jensen, V., Kjoller, A., Sørensen, L.H., Eds., *Microbial communities in soil*. FEMS Symposium 33, Elsevier Applied Science Publishers, London
- Rothwell, J.J., Fitter, M.N., Dise, N.B., 2008. A classification and regression tree model of controls on dissolved inorganic nitrogen leaching from European forests. *Environmental Pollution* 156 (2), 544-552.
- Rousk, J., Bååth, E., 2011. Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiology Ecology* 78 (1), 17-30.
- Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied & Environmental Microbiology* 75, 1589-1596.
- Šantrůčková, H., Kaštovská, E., Kozlov, D., Kurbatová, J., Livečková, M., Shibistova, O., Tatarinov, F., Lloyd, J., 2010. Vertical and horizontal variation of carbon pools and fluxes in soil profile of wet southern taiga in European Russia. *Boreal Environment Research* 15, 357-369.
- Šantrůčková, H., Křišťůvková, M., Vaněk, D., 2006. Decomposition rate and nutrient release from plant litter of Norway spruce forest in Bohemian Forest. *Biologia Bratislava* 61 (S20), 499-508.
- Šantrůčková, H., Šantrůček, J., Šetlík, J., Svoboda, M., Kopáček, J., 2007. Carbon isotopes in tree rings of Norway spruce exposed to atmospheric pollution. *Environmental Science & Technology* 41, (16), 5778-5782.
- Scheel, T., Dörfner, C., Kalbitz, K., 2007. Precipitation of dissolved organic matter by aluminium stabilizes carbon in acidic forest soils. *Soil Science Society of America Journal* 71, 64-74.
- Scheel, T., Jansen, B., van Wijk, A.J., Verstraten, J.M., Kalbitz, K., 2008. Stabilization of dissolved organic matter by aluminium: a toxic effect or stabilization through precipitation? *Soil Science Society of America Journal* 59, 1122-1132.
- Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591-602.
- Schimel, J.P., Firestone, M.K., 1989. Nitrogen incorporation and flow through a coniferous forest soil-profile. *Soil Science Society of America Journal* 53, 779-784.
- Schleppi, P., Muller, N., Feyen, H., Papritz, A., Bucher, J.B., Flühler, H., 1998. Nitrogen budgets of two small experimental forested catchments at Alptal, Switzerland. *Forest Ecology & Management* 101, 177-85.

- Schmidt, S.K., Lipson, D.A., Ley, R.E., Fisk, M.C., West, A.E., 2004. Impacts of chronic nitrogen additions vary seasonally and by microbial functional group in tundra soils. *Biogeochemistry* 69 (1), 1-17.
- Schmidt, S.K., Costello, E.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C., Weintraub, M.N., Meyer, A.F., Martin, A.M., 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* 88 (6), 1379-1385.
- Simpson, A.J., Simpson, M.J., Smith, E., Kelleher, B.P., 2007. Microbially derived inputs to soil organic matter: are current estimates too low? *Environmental Science and Technology* 41(23), 8070-8076.
- Sinsabaugh, R.L., 2010. Phenoloxidase, peroxidase and organic matter dynamics of soil. *Soil Biology & Biochemistry* 42 (3), 391-404.
- Smith, S. J., van Aardenne, J., Klimont, Z., Andres, R., Volke, A., Delgado Arias, S., 2011. Anthropogenic sulfur dioxide emissions: 1850–2005, *Atmospheric Chemistry & Physics* 10, 16111-16151.
- Sposito, G., 1996. *The environmental chemistry of aluminium*. 2nd edition, CRC Press LLC, Boca Raton, 480p.
- Sprent, J.I., 1987. *The ecology of nitrogen cycle*. Cambridge studies in ecology, Cambridge University Press, Cambridge, Great Britain, 151p.
- Stark, J.M., Hart, S.C., 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385 (6611), 61-64.
- Stevens, P.A., Hornung M., 1990. Effect of harvest intensity and ground flora establishment on mineral-N leaching from Sitka spruce plantations in North Wales UK. *Biogeochemistry* 10, 53-65.
- Stoddard, J.L., 1994. Long-term changes in watershed retention of nitrogen - its causes and aquatic consequences. *Environmental chemistry of lakes and reservoirs*. Advances in Chemistry Series 237, 223-284.
- Strauss, E.A., Lamberti, G.A., 2000. Regulation of nitrification in aquatic sediments by organic carbon. *Limnology & Oceanography* 45 (8), 1854-1859.
- Strauss, E.A., Lamberti, G.A., 2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. *Freshwater Biology* 47 (1), 65-74.
- Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications, *Soil Biology & Biochemistry* 42 (9), 1385-1395.
- Sverdrup, H.U., 1990. *The kinetics of base cation release due to chemical weathering*. Lund: Lund University Press, 245p.
- Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A., 2005. *Principles and applications of soil microbiology*. 2nd edition, Pearson Education Inc., New Jersey, 672p.
- Taylor P., Townsend, A.R., 2011. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. *Nature* 464 (7292), 1178-1181.
- Tiedje, J.M., 1989. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder A.J.B., Ed.: *Biology of Anaerobic microorganisms*. John Wiley & Sons, New York, 179-244.
- Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* 11 (10), 1111-1120.
- Vance, G.F., Stevenson, F.J., Sikora, F.J., 1996. Environmental chemistry of aluminorganic complexes. In: Sposito, G., Ed., *The environmental chemistry of aluminum*. 2nd edition, Lewis Publishers, New York, 480p.

- Venterea, R.T., Groffman, P.M., Verchot, L.V., Magill, A.H., Aber, J.D., 2004. Gross nitrogen process rates in temperate forest soils exhibiting symptoms of nitrogen saturation. *Forest Ecology & Management* 196 (1), 129-142.
- Veselý, J., 1994. Investigation of the nature of the Šumava lakes: a review. *Časopis Národního Muzea* 163, 103-120.
- Veselý, J., Majer, V., Kopáček, J., Norton, S.A., 2003. Increasing temperature decreases aluminum concentrations in central European lakes recovering from acidification. *Limnology & Oceanography* 48 (6), 2346-2354.
- Vrba, J., Kopáček, J., Bittl, T., Nedoma, J., Štrojsová, A., Nedbalová, L., Kohout, L., Fott, J., 2006. A key role of aluminium in phosphorus availability, food web structure, and plankton dynamics in strongly acidified lakes. *Biologia* 60 (S20), S441-S451.
- Waldrop, P.M., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications* 14 (4), 1172-1177.
- Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J., Schlesinger, W.H., 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology & Management* 222, 459-468.
- Zak, D.R., Pregitzer, K.S., Holmes, W.E., Burton, A.J., Zogg, G.P., 2004. Anthropogenic N deposition and the fate of $^{15}\text{NO}_3^-$ in a northern hardwood ecosystem. *Biogeochemistry* 69 (2), 143-157.
- Zhang, L.M., Hu, W.H., Shen, J.P., He, J.Z., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *The ISME Journal* 6, 1032-1045.
- Zogg, G.P., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology* 81, 1858-1866.

RESEARCH ARTICLES

PAPER I

**Nitrogen transformations and pools in N-saturated
mountain spruce forest soils**

Šantrůčková, H., Tahovská, K., Kopáček, J. (2009)
Biology and Fertility of Soils 45 (4), 395 – 404

Abstract

Nitrogen leaching persists in mountain forests of Europe even in the presence of decreasing N depositions. We have hypothesized that this leaching is linked to soil N transformations occurring over the whole year, even at 0°C temperatures. The aims were to estimate (1) the effect of temperature on N transformations and (2) N pools and fluxes. The study sites are situated in the Bohemian Forest (Czech Republic). Litter, humus, and 0–10-cm mineral layers were sampled in early spring, and the effect of temperature on net nitrification, net ammonification, and microbial N immobilization were measured in a short-term incubation experiment without substrate addition. Nitrogen pools were calculated from the concentrations of N forms in the soil and soil pool weights, while daily N fluxes were calculated from daily net rates of processes and soil pool weights. Relationships between temperature and net nitrification, net ammonification, and microbial N immobilization did not follow the Arrhenius type equation; all processes were active close to 0°C, indicating that microbial N transformations occur over the whole year. Microbial N immobilization rate was generally greater than N mineralization rate. The microbial N pool was significantly larger than mineral N pools. Organic layers containing tens of grams of available N per square meter contributed more than 70% to the available N in the soil profile. Daily N fluxes were related to N pools. On average, N fluxes represented daily mineral and microbial N pool changes of 1.14 and 1.95%, respectively. The effect of microbial composition on the C/N ratio of microbial biomass and respiration is discussed.

Following 10 pages of the thesis include copyrights. Full version original of the thesis is archived only in the Academic Library of University of South Bohemia in České Budějovice.

Contribution of the author: 60%

Copyright © 2008 Springer-Verlag

*The original publication is available at
<http://www.springer.com/life+sciences/agriculture/journal/374>
DOI 10.1007/s00374-008-0349-4*

Abstract [in Czech]

Vyplavování dusíku přetrvává v horských lesích Evropy i přes pokles depozic dusíku. Předpokládali jsme, že toto vyplavování souvisí s přeměnami N v půdě probíhajícími během roku dokonce i při teplotách 0°C. Cílem bylo (1) stanovit vliv teploty na přeměny dusíku a (2) stanovit zásobníky N a jeho toky. Studijní plochy se nacházely v Národním Parku Šumava (Česká republika). Časně z jara byly odebrány vzorky opadu, humusu a 0-10 cm z minerální vrstvy a byl stanoven vliv teploty na čistou nitrifikaci, čistou amonifikaci a mikrobiální imobilizaci N během inkubačního pokusu bez přídavku substrátu. Zásobníky dusíku byly spočítány z koncentrací forem N v půdě a objemové hmotnosti půdy, zatímco denní toky N byly spočítány z denních rychlostí čistých procesů a objemové hmotnosti půdy. Vztah mezi teplotou a čistou nitrifikací, čistou amonifikací a mikrobiální imobilizací N nebyl Arrheniova typu; všechny procesy byly aktivní i při 0°C, což značí, že mikrobiální přeměny mohou probíhat po celý rok. Rychlost mikrobiální imobilizace N byla celkově vyšší než rychlost mineralizace N. Mikrobiální zásobník dusíku byl významně větší než pooly minerálního dusíku. Organické vrstvy, obsahující desítky gramů dostupného dusíku na metr čtvereční, odpovídaly více než 70% dostupného dusíku v půdním profilu. Denní toky N souvisely s velikostmi zásobníků. V průměru reprezentovaly toky N denní změnu 1.14 a 1.95 % v minerálním a mikrobiálním zásobníku, v tomto pořadí. Je diskutován vliv složení mikrobiálního společenstva na C/N poměr mikrobiální biomasy a respiraci.

Následující pasáž o rozsahu 10 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena pouze v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Podíl studenta na publikaci: 60%

Copyright © 2008 Springer-Verlag

Originál publikace je dostupný na:

<http://www.springer.com/life+sciences/agriculture/journal/374>

DOI 10.1007/s00374-008-0349-4

PAPER II

Nitrogen availability in Norway spruce forest floor - the effect of forest defoliation induced by bark beetle infestation

Tahovská, K., Kopáček, J., Šantrůčková, H. (2010)
Boreal Environment Research 15 (6), 553 – 554

Abstract

The objective was to evaluate whether lower nitrogen (N) immobilization by spruce trees (*Picea abies*) or higher microbial N mineralization in the soil is the main mechanism changing the soil N balance after forest defoliation caused by bark beetle. We measured *in situ* mineral N availability using ion exchangers, net N mineralization (N_{miner} , ammonification and nitrification) and N content in microbial biomass (N_{microb}) in the forest floor of infested and control plots in an unmanaged area of The Bohemian Forest National Park. *In situ* N availability already increased before the defoliation culminated, which affirms the primary effect of reduced N immobilization by vegetation. N mineralization was enhanced after maximum forest defoliation (2 vs. 30 $\mu\text{g N g}^{-1}\text{d}^{-1}$). The contribution of N_{miner} to *in situ* N availability was supported by the correlations found between *in situ* mineral N availability and $N_{\text{miner}}/N_{\text{microb}}$ and $N_{\text{miner}}/C_{\text{miner}}$ ratios. The influence of litter input with low C/N ratio on N mineralization is discussed.

Following 12 pages of the thesis include copyrights. Full version original of the thesis is archived only in the Academic Library of University of South Bohemia in České Budějovice.

Contribution of the author 85%

Copyright © 2010 Boreal Environment Research Publishing Board

The original publication is available at <http://www.borenv.net/>

Abstract [in Czech]

Cílem bylo stanovit, zda snížená imobilizace dusíku (N) smrky (*Picea abies*) nebo zvýšená mikrobiální mineralizace N v půdách jsou hlavními mechanismy měnícími rovnováhu N v půdě po defoliaci lesa vlivem lýkožrouta. Měřili jsme *in situ* dostupnost minerálního N za použití iontoměničů, čistou mineralizací N (N_{miner} , amonifikace a nitrifikace) a N v biomase mikroorganismů (N_{microb}) ve svrchní vrstvě lesní půdy na napadené a kontrolní lokalitě v bezzásahovém území Národního Parku Šumava. *In situ* dostupný N vzrostl již před tím, než vrcholila defoliace, což potvrzuje hlavní vliv sníženého odběru N vegetací. N mineralizace se zvýšila až po maximum defoliace (2 vs. 30 $\mu\text{g N g}^{-1}\text{d}^{-1}$). To, že N_{miner} přispěla k *in situ* dostupnému N bylo podpořeno i zjištěnou korelací mezi *in situ* dostupným minerálním N a poměry $N_{\text{miner}}/N_{\text{microb}}$ a $N_{\text{miner}}/C_{\text{miner}}$. Je diskutován vliv vstupu opadu s nízkým C/N poměrem na mineralizaci N.

Následující pasáž o rozsahu 12 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena pouze v archivovaném originále disertační práce uložené na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Podíl studenta na publikaci: 85%

Copyright © 2010 Boreal Environment Research Publishing Board

Originál publikace dostupný na: <http://www.borenv.net/>

PAPER III

**Changes in soil chemistry after bark beetle infestation and
forest dieback**

Kaňa, J., Tahovská, K., Kopáček, J.
Biogeochemistry, *in press*

Abstract

We evaluated changes in the chemistry of the uppermost soil horizons in an unmanaged spruce forest (National Park Bohemian Forest, Czech Republic) for three years after dieback caused by a bark beetle infestation, and compared these changes with a similar undisturbed forest area. The soils below the disturbed forest received 2–6 times more elements via litter fall compared to the unaffected plot. The subsequent decomposition of litter and reduced nutrient uptake by trees resulted in a steep increase in soil concentrations of soluble N ($\text{NH}_4\text{-N}$, organic-bound N) and P forms in the disturbed plot. The average concentrations of $\text{NH}_4\text{-N}$ and soluble reactive P increased from 0.8 to 4.4 mmol kg^{-1} and from 0.04 to 0.9 mmol kg^{-1} , respectively, in the uppermost soil horizon. Decomposition of litter at the disturbed plot elevated soil concentrations of Ca^{2+} , Mg^{2+} and K^+ , which replaced Al^{3+} and H^+ ions from the soil sorption complex. Consequently, soil concentrations of exchangeable base cations increased from 120 to 200 meq kg^{-1} , while exchangeable Al^{3+} and H^+ decreased 66% and 50%, respectively, and soil base saturation increased from 40% to 70%. The Al^{3+} liberation did not elevate concentrations of ionic Al in the soil solution, because most of the liberated Al^{3+} was rapidly complexed by dissolved organic carbon (DOC) and transformed to DOC-Al complexes. The chemical parameters investigated at the unaffected plot remained stable during the study.

Following 15 pages of the thesis include copyrights. Full version original of the thesis is archived only in the Academic Library of University of South Bohemia in České Budějovice.

Contribution of the author 40%

Copyright © 2012 Springer Science+Business Media B.V.

*The original publication is available at
<http://www.springerlink.com/content/g2x8751pw1u47540/>
DOI 10.1007/s10533-012-9765-5*

Abstract [in Czech]

Vyhodnotili jsme změny v chemismu ve svrchních půdních horizontech v bezzásahovém území smrkového lesa (Národní Park Šumava, Česká republika) během tří let od odumření lesa způsobeného lýkožroutem a porovnali jsme tyto změny s územím, které zůstalo nepostiženo. Do půd v narušeném lese vstoupilo s opadem 2-6 krát více živin v porovnání s nepostiženou plochou. Následný rozklad opadu a snížený odběr živin stromy vedl k prudkému nárůstu koncentrací rozpustného půdního N ($\text{NH}_4\text{-N}$, organicky vázaný N) a P na narušené ploše. Průměrné koncentrace N- NH_4 a rozpustného reaktivního P vzrostly z 0.8 na 4.4 mmol kg^{-1} a z 0.04 na 0.9 mmol kg^{-1} , v tomto pořadí ve svrchním půdním horizontu. Rozklad opadu na narušené ploše vedl ke zvýšení koncentrací půdních bazických kationtů (Ca^{2+} , Mg^{2+} a K^+), které nahradily Al^{3+} a H^+ ionty na půdním sorpčním komplexu. Následkem toho koncentrace výměnných bazických kationtů vzrostla ze 120 na 200 meq kg^{-1} , zatímco výměnný Al^{3+} a H^+ klesly o 66% a 50%, v tomto pořadí a saturace bázemi vzrostla z 40% na 70%. Uvolnění Al^{3+} nezvýšilo koncentrace iontového hliníku v půdním roztoku, protože většina uvolněného Al^{3+} byla rychle vázána rozpuštěným organickým uhlíkem (DOC) a přeměněna na DOC-Al komplexy. Chemické parametry se na nenarušené ploše během studie nezměnily.

Následující pasáž o rozsahu 15 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena pouze v archivovaném originále disertační práce uložené na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Podíl studenta na publikaci 40%

Copyright © 2012 Springer Science+Business Media B.V.

Originál publikace je dostupný na:

<http://www.springerlink.com/content/g2x8751pw1u47540/>

DOI 10.1007/s10533-012-9765-5

PAPER IV

**Microbial N immobilization is of great importance in
acidified mountain spruce forest soils**

Tahovská, K., Kaňa, J., Bárta, J., Oulehle, F., Richter, A., Šantrůčková, H.,
submitted

Abstract

Prevailing N saturation paradigm still considers microbial N immobilization as less important process of ecosystem N retention. In contrast, we hypothesize that it can even be a primary process affecting N leaching from N saturated soils. We studied N transformations in soils of acidified near-natural and primeval forests in the Bohemian Forest (watersheds of Plešné and Čertovo Lakes, Czech Republic) and Pop Ivan Massif (Ukraine). We ran laboratory experiments in which mixtures of different N sources (N-NH₄, N-NO₃ and glycine) were added to the soil with only one source labelled by ¹⁵N. We followed ¹⁵N partitioning within soil N pools and analysed the composition of microbial community (16SrDNA-DGGE fingerprint of bacteria, ergosterol analyses, qPCR of fungal 18S rDNA gene). The microbial N pool was always three to five times higher than the total soluble N pool. We found fast (15min) and simultaneous immobilization of all added N forms into microbial biomass with clear preferences for organic N over inorganic sources. Total N flux to the microbial pool always exceeded N flux into mineral N pools. The pattern of N transformation in the C limited Ukraine soil was different from the two Czech soils. The microbial pool and N flux into it has been smaller compared to the mineral N pools and fluxes. The contribution of N-NO₃ to microbial immobilization was negligible, while nitrification was almost equal to N mineralization. Total N flux through soluble N pools was greater than total N flux to insoluble pools (residual and microbial N); this was accompanied by lower microbial N uptake efficiency and shorter residence time of N in microbial pool than in soils with higher C availability. The composition of bacterial community was related to DOC content and C and N in microbial biomass. In soils with higher fungi abundance, more glycine was taken up regardless soil C availability, but with higher deamination (~50 vs. 20 %) and subsequent release of N-NH₄ back to the soil. Our study emphasized the role of microbial N immobilization in preventing N-NO₃ loss from N saturated ecosystems as a function of C availability. Nitrification was favoured when enough N-NH₄ was available in C limited soil. The produced N-NO₃ was not immobilized by the microbes and could be, if not taken up by plants, leached out. C limitation plays an important role in the susceptibility of ecosystems to N leaching and could partly explain the observed differences in some N saturated ecosystems.

Following 37 pages of the thesis include copyrights. Full version original of the thesis is archived only in the Academic Library of University of South Bohemia in České Budějovice.

Contribution of the author 80%

Abstract [in Czech]

Převažující paradigma N saturace stále považuje mikrobiální imobilizaci N za méně významný proces zadržování N v ekosystému. My se naopak domníváme, že je to základní proces ovlivňující vyplavování N z půd saturovaných N. Studovali jsme přeměny N v acidifikovaných půdách přírodě blízkého lesa v NP Šumava (povodí Plešného a Čertova jezera, Česká republika) a v půdách původního lesa v pohoří Pop Ivan (Ukrajina). Provedli jsme laboratorní pokusy, při kterých byla do půdy přidána směs různých zdrojů dusíku (N-NH₄, N-NO₃ a glycin) kdy pouze jeden ze zdrojů byl značen ¹⁵N. Sledovali jsme distribuci ¹⁵N mezi půdními zásobníky a analyzovali jsme složení mikrobiálního společenstva (16SrDNA-DGGE otisk bakterií, analýza ergosterolu, qPCR houbového 18S rDNA genu). Mikrobiální N byl vždy 3 až 5-krát vyšší než celkový rozpustný N. Imobilizace N byla rychlá (15min) a všechny formy N byly imobilizovány současně. Organický N byl imobilizován přednostně vzhledem k minerálním zdrojům N. Celkový tok N do mikrobiálního zásobníku byl vždy vyšší než tok N do minerálních zásobníků. Charakter procesů přeměn N v ukrajinské půdě, která byla limitována C, byl odlišný od přeměn N v českých půdách. Mikrobiální N a tok N do tohoto zásobníku byli menší v porovnání s minerálním N a tokem minerálního N do tohoto zásobníku. Mikrobiální imobilizace N-NO₃ byla zanedbatelná, zatímco nitrifikace byla téměř rovna mineralizaci N. Celkový tok N rozpustnými zásobníky byl v C limitové půdě, větší než celkový tok nerozpustnými zásobníky (reziduální a mikrobiální N), což bylo doprovázeno nižší účinností příjmu N mikroorganismy a kratší dobou zdržení N v mikrobiálním zásobníku. Složení bakteriálního společenstva souviselo s DOC, C a N v mikrobiální biomase. V půdách s větším výskytem hub mikroorganismy přijímali více glycinu nehlédě na dostupnost C, ale docházelo k jeho vyšší deaminaci (~50 vs. 20 %) a následnému uvolnění N-NH₄ do půdy. Studie vyzdvihla roli mikrobiální imobilizace N jako funkce dostupnosti C. Pokud byl v půdě, která byla limitována C, dostatek N-NH₄, byla nitrifikace podpořena. Dusičnan nebyl imobilizován mikroorganismy a mohl by být, pokud by nebyl přijat rostlinami, vyplaven z půdy. Limitace C hraje důležitou roli v náchylnosti ekosystému k vyplavování N a může částečně vysvětlit rozdíly ve vyplavování N, které byly pozorovány mezi některými ekosystémy saturovanými N.

Následující pasáž o rozsahu 37 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena pouze v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Podíl autora na publikaci: 80%

PAPER V

Major changes in forest carbon and nitrogen cycling caused by declining sulphur deposition

Oulehle, F., Evans, C., Hofmeister, J., Krejčí, R., Tahovská, K., Persson, T.,
Cudlín, P., Hruška, J. (2011)
Global Change Biology, 17 (10), 3115 – 3129

Abstract

Sulphur (S) and nitrogen (N) deposition are important drivers of the terrestrial carbon (C) and N cycling. We analyzed changes in C and N pools in soil and tree biomass at a highly acidified spruce site in the Czech Republic during a 15 year period. Total S deposition decreased from 5 to 1.1 g m⁻² yr⁻¹ between 1995 and 2009, whereas bulk N deposition did not change. Over the same period, C and N pools in the Oa horizon declined by 116 g C and 4.2 g N m⁻² yr⁻¹, a total decrease of 47% and 42%, respectively. This loss of C and N probably originated from organic matter (OM) that had accumulated during the period of high acid deposition when litter decomposition was suppressed. The loss of OM from the Oa horizon coincided with a substantial leaching (1.3 g N m⁻² yr⁻¹ at 90 cm) in the 1990s to almost no leaching (<0.02 g N m⁻² yr⁻¹) since 2006. Forest floor net N mineralization also decreased. This had consequences for spruce needle N concentration (from 17.1 to 11.4 mg kg⁻¹ in current needles), an increase in litterfall C/N ratio (from 51 to 63), and a significant increase in the Oi + Oe horizon C/N ratio (from 23.4 to 27.3) between 1994 and 2009/2010. Higher forest growth and lower canopy defoliation was observed in the 2000s compared to the 1990s. Our results demonstrate that reducing S deposition has had a profound impact on forest organic matter cycling, leading to a reversal of historic ecosystem N enrichment, cessation of nitrate leaching, and a major loss of accumulated organic soil C and N stocks. These results have major implications for our understanding of the controls on both N saturation and C sequestration in forests, and other ecosystems, subjected to current or historic S deposition.

Following 15 pages of the thesis include copyrights. Full version original of the thesis is archived only in the Academic Library of University of South Bohemia in České Budějovice.

Contribution of the author 15%

Copyright © 2011 Blackwell Publishing Ltd

*The original publication is available at
<http://onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291365-2486>
DOI: 10.1111/j.1365-2486.2011.02468.x*

Abstract [in Czech]

Suchozemské cykly uhlíku a dusíku jsou řízeny depozicí síry a dusíku. Studovali jsme změny v zásobnících C a N v půdě a biomase stromů na vysoce acidifikovaných smrkových plochách v České republice za posledních 15 let. Mezi lety 1995 a 2009 klesla celková depozice síry z 5 na 1.1 g m⁻² rok⁻¹, zatímco depozice dusíku na otevřené ploše se nezměnila. Za stejné období, se snížila zásoba C a N v Oa horizontu o 116 g C a 4.2 g N m⁻² rok⁻¹, což odpovídá celkovému poklesu o 47% a 42%, v tomto pořadí. Ztráty C a N pravděpodobně pochází z organické hmoty, která se naakumulovala v období vysoké kyselé depozice, kdy byl rozklad opadu potlačen. Ztráta organické hmoty z Oa horizontu proběhla současně s vymizením odtoku N (z 1.3 g N m⁻² rok⁻¹ v 90cm v letech 1990 na méně než 0.02 g N m⁻² yr⁻¹ od roku 2006). Mineralizace N ve svrchní vrstvě lesní půdy také poklesla. To způsobilo pokles koncentrace N v jehličí (z 17.1 na 11.4 mg kg⁻¹), vzestup C/N poměru v opadu (z 51 na 63) a významný nárůst C/N poměru v Oi a Oe horizontu (z 23.4 na 27.3) mezi lety 1994 a 2009/2010. V první dekádě 21. století v porovnání s devadesátými léty 20. století byl pozorován vyšší růst lesa společně s nižší defoliací. Naše výsledky ukazují, že pokles depozice síry měl významný vliv na cyklus organické hmoty v lese, což vedlo ke zvratu v historickém obohacení ekosystému N, vymizení vyplavování dusičnanů a ztrátě naakumulovaného organického C a N. Tyto výsledky jsou velmi důležité z hlediska pochopení toho, co řídí saturaci N a ukládání C v lesních a jiných ekosystémech vystavených S depozici.

Následující pasáž o rozsahu 15 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena pouze v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Podíl autora na publikaci: 15%

Copyright © 2011 Blackwell Publishing Ltd

Originál publikace je dostupný na:

<http://onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291365-2486>

DOI: 10.1111/j.1365-2486.2011.02468.x

Curriculum Vitae

Karolina Tahovská (*1982, Benešov), maiden name: Skopcová

Academic achievements

2005-2012 Ph.D. at Department of Ecosystem Biology, Faculty of Science, University of South Bohemia in České Budějovice, *broken in 2009-2011 (maternity leave)*

Theme of dissertation thesis: N availability and transformation in soils of acidified and nitrogen saturated mountain forest ecosystems

2000-2005 M.Sc. at Department of Ecosystem Biology, Faculty of Science, University of South Bohemia in České Budějovice

Professional experience

2009 – up to now part time and from March 2011 full time researcher with the Department of Ecosystem Biology, Faculty of Science, University of South Bohemia in České Budějovice

2007 - 2009 part-time researcher with the Institute of Hydrobiology, Biology Centre of the AS CR in České Budějovice

2006 3 month residency and research at Department of Chemical Ecology and Ecosystem Research, University of Vienna, Austria

2005 International summer school “Land-Use, land-use change and forest history; Impacts on the environment”, Nancy, France

2005 part time job - chemical laboratory assistant (Bosch s.r.o. České Budějovice)

Participation in research projects

2012 – up to now member of the research team in project “The effect of natural dieback of mountain spruce forest on microclimate” (P504/12/1218, Grant Agency of the Czech Republic)

2009 – 2011 Co-investigator of project “What are the main mechanisms affecting N flow through soil N pools in N saturated mountain soils?” (KJB600960907, Grant Agency of AS CR)

Participation in conferences, workshops (in the 5 previous years)

- 2012 Biogeomon (talk): Microbial N immobilization is of great importance in acidified and N-saturated mountain forest soils if carbon is available, USA (Maine)
- 2011 Ecology of Soil Microorganisms - Microbes as Important Drivers of Soil Processes (talk): „Are microorganisms adapted for mineral N assimilation in acidified soils, Czech Republic (Prague)
- 2011 Czech Ecological Society (CSPE) conference (poster): „Microbial N assimilation in primeval and N saturated forest soils (in Czech), Czech Republic (Kostelec nad Č. Lesy)
- 2010 EGU conference (2 posters): „Aluminum availability in forest floor of two acidified mountain watersheds“ (Kaňa and Tahovská) and „Nitrogen availability in mountain spruce forest floor after forest defoliation“ (Tahovská et al.), Austria (Vienna)
- 2009 Biogeomon (poster): “Microbial N transformations in N loaded spruce forest soils: immobilization of different ¹⁵N forms into microbial biomass”, Finland (Helsinki)
- 2008 Mountain soils under a changing climate and land-use (poster & short presentation):
Microbial assimilation of nitrogen in N saturated mountain forest soils - ¹⁵N tracer study, Switzerland (Zürich)
- 2008 CSPE conference (poster): “N availability in mountain forest soils after forest defoliation” (in Czech), Czech Republic (Třeboň)
- 2007 Soil processes under extreme meteorological conditions (2x poster & 1x short presentation): „Nitrogen availability and fluxes in acidified mountain forest soils – *in situ* measurement“ (Tahovská et al.) and “N pools and seasonal fluxes in acidified mountain spruce forest soil” (Šantrůčková & Tahovská), Germany (Bayreuth)
- 2007 GORDON conference (poster): “Nitrogen pools and fluxes in the catchment-lake ecosystem of Plešné Lake (Czech Republic) prior to and after a bark beetle infestation“ (Kopáček et al.), USA (New Hampshire)

Other professional activities

Reviewer for: Oecologia

Member of CSPE: Czech Ecological Society

© for non-published parts Karolina Tahovská

tahovska@centrum.cz

Nitrogen availability and transformation in soils of acidified and nitrogen saturated
mountain forest ecosystems

Ph.D. Thesis Series, 2012, No. 5

All rights reserved

For non-commercial use only

Printed in the Czech Republic by Vlastimil Johanus

Edition of 20 copies

University of South Bohemia in České Budějovice

Faculty of Science

Branišovská 31

CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 772 244

www.prf.jcu.cz, e-mail: sekret@prf.jcu.cz