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Effects of two-year nutrient loading on microbial community and N transformations in mineral and organic soils of wet meadows

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Annotation: This study observes an influence of two-year application of NPK fertilizer on the amount of soluble nitrogen, microbial N transformations, and microbial biomass and the composition of microbial community in mineral and organic soils of two wet meadows. This study is the first version of manuscript, supplemented with a wider literature review, which will be submitted in 2010.

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Mach Jiří

This study is a part of project of Grant Agency of the Czech Republic (No. 526/06/0276), solving the eutrophication of wet meadows: Effect on soil-plant interactions in respect to C and N transformations. This project continues on Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

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Content

Abstract

The biomass and composition of microbial community, the amount and forms of soluble N and microbial N transformations were studied in two types of soils from wet meadows. Both study sites are situated in the protected biosphere reservation Třeboňsko in the Czech Republic: (1) a mesotrophic sedge-sweet grass meadow on mineral soil and (2) a nutrient poor sedge meadow on organic soil. A eutrophication of the meadows was simulated by the NPK fertilizer addition to selected permanent plots in 2006 and 2007 in amounts of 9 kg N + 4 kg P ha^{-1} $year^{-1}$ (low dose) and 45 kg N + 20 kg P ha^{-1} $year^{-1}$ (high dose). Two-year nutrient loading was too short for affect measured soil characteristics significantly. We observed no change in microbial biomass N, but its C:N ratio significantly decreased. The relative abundance of gram-positive bacteria and actinomycetes within microbial community increased and the ratio of bacteria to fungi tended to be higher in fertilized soils. Each application of fertilizer caused a short-term (few days lasting) increase in the amount of total soluble nitrogen, given by the increased amounts of ammonium and soluble N. Measured N transformations – nitrification, N mineralization and N assimilation were not significantly affected by fertilization, but we detected some trends as increasing nitrification and decreased N assimilation in fertilized organic soil, which could indicate an acceleration of soil N cycle after a longer-term nutrient loading.

1. Introduction

1.1. Wetlands and wet meadows

Wetland ecosystems sore approximately 5 % of world landscape (Aselman and Crutzen, 1989). In Czech Republic, wetlands ecosystems are represented by alluvial forests, fishponds, peat-bogs, freshwater marches, freshwater lakes with their littorals and also by wet meadows. Wet meadows, ecotonal habitats on the edge between grasslands and wetlands, are typically waterlogged during the whole year or for certain parts of the year. Wet meadows as a part of wetlands are known as possible stabilizing factor of global cycles of carbon, nitrogen and sulfate (Mitsch and Gosseling, 2000). Wet meadows are also key biotopes for survival of many endangered species of plants, fungi and animals (Chytil et al., 1999). Humans can affect marshes by changing water levels with drainage ditches, canals, dams, or levees (Keddy, 2008). Other human impacts can arise from a pollution by added nutrients and this excessive nutrient loading (eutrophication) leads to an increase of biomass production (Kruk, 2003) and decreased species diversity (Prach, 1996; Bollens et al., 2001; Aerts et al., 1990). Eutrophication also affected structural and functional traits of ecosystem (Craft and Richardson, 1998), including changes in plant and animal species composition (Galatowitsch et al., 2000). Later, many of wet meadows ecosystems were left without any management. Due to those processes, wet meadows became one of the most endangered type of ecosystems in the Czech Republic nowadays (Moravec et al., 1995).

1.2. Aims and hypothesis

The purpose of the study of two wet meadow ecosystems was to determine the effect of two-year nutrient loading (NPK fertilizer) on soil microbial processes, with the stress on soil N transformations in either mineral or peaty soils of the wet meadows. The aims of the study were to (i) quantify N forms in wet meadow soils (total soil N content, soluble organic nitrogen and inorganic forms of N – ammonium and nitrates) in soils receiving different levels of nutrient inputs (ii) study the changes in microbial community biomass, C:N ratio and its composition (based on the analysis of phospholipid fatty acids, PLFA) under different levels of nutrient inputs into the soils, (iii) quantify N transformations in soil microbial biomass (mineralization, microbial N assimilation, nitrification), (iv) compare the impact of nutrient input on two different wet meadow soils (mineral and organic). Our hypothesis was that increased loading of nutrients, though short-term (occurring for two years), will increase

the amount and availability of N forms in soil, and consequently it will accelerate processes responsible for soil N fluxes and also influence the composition of microbial communities.

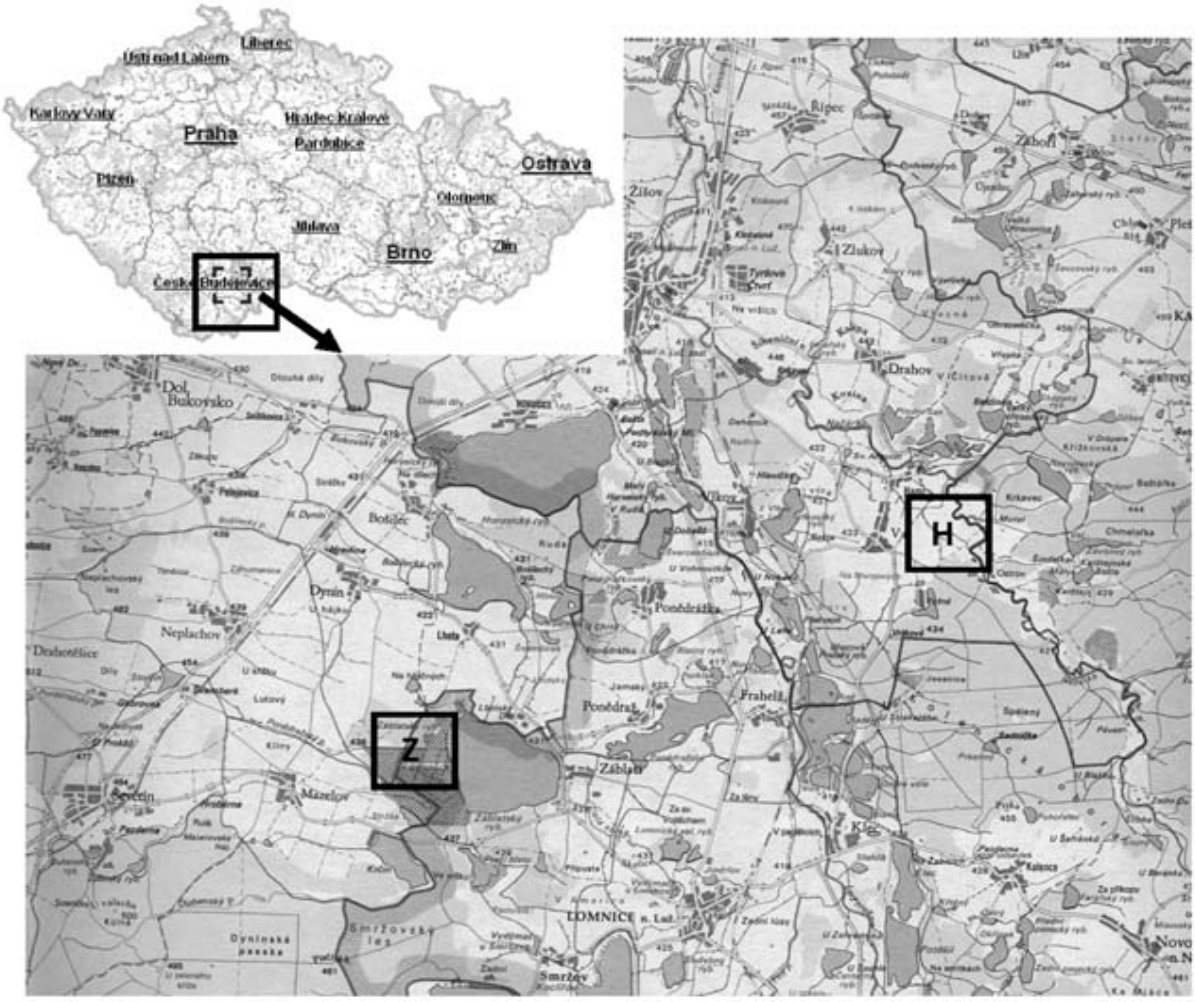

Figure 1. Map of the Třeboň Basin Biosphere Reserve (TBBR) showing the study site locations. Z = Záblatské Louky – peaty organic soil site; H = Hamr – mineral soil site. (adapted from Picek et al., 2008)

2. Literature overview

2.1. Amount and composition of soil nitrogen

Nitrogen is an essential component of proteins, genetic material, chlorophyll, and other key organic molecules (Vitousek et al., 2000). The highest amount of nitrogen in the World is in the lithosphere, which contains 1 636 000 * 10^{14} kg N (Stevenson et al., 1999). While the N concentrations in minerals resources are very low, the top of the lithosphere – the pedosphere is very rich in N. Total soil nitrogen amount is 2,4 * 10^{14} kg N, from that 90% is in organic forms (5 – 10 % in amino sugars, 20 – 40 % in amino acids and proteins, 1 – 5 % in biomass and the rest in hardly decomposable forms of aromatic compounds and mucopeptids (Úlehlová, 1989). Mineral N comprises only a small part of the total N in the soil (Harmsen and Kolenbrander, 1965). It includes gaseous forms (N_2O, NO, NO_2, NH_3), occurred in low concentrations in the soil atmosphere, and NH_4^+, NO_2^-, NO_3^- ions, fixed in organo-mineral complex or in the soil solution. The total N amount in soil differs among different types of ecosystems.

As noted previously, over 90% of the N in most surface soil occurs in organic forms. The forms of organic N in soil can be divided into two broad categories: (i) organic residues, consisting of undecomposed plant and animal residues and products of partial decomposition, and (ii) soil organic matter (SOM) (Kelly and Stevenson, 1995). Within SOM, there are two small labile N pools, which deserve more attention for their higher sensitivity to changes in soil ecosystem. The first, **microbial biomass N**, defined as the living microbial component of the soil, is the primary agent of the soil ecosystem responsible for litter decomposition, nutrient cycling and energy flow (Wardle, 1992). The second pool is **soluble nitrogen**. It is divided to small mineral part (10-30%) and to larger organic part – soluble organic nitrogen (SON). Mineral N cycles very rapidly, because it is supplied by mineralization of soil organic matter, as well as from fertilizer, manure and atmospheric deposition, and is depleted by plant uptake and immobilisation by microorganisms, by denitrification and by leaching (Bremner, 1965; Murphy et al., 1999). The SON fraction is composed of free amino acids (up to 3%), amino sugars and heterocyclic-N bases (15%) and other amino compounds (Murphy et al., 1999). The SON could be a sensitive indicator to evaluate the soil nitrogen status (Mengel et al., 1999) as it significantly correlates with net N mineralization and microbial biomass N (Zhong and Makeschin, 2002). SON has been also identified as a key pool in soil-plant N cycling in forest systems (Qualls and Haines, 1994), arctic tundra (Atkin, 1996) and

subtropical wet heathland (Schmidt and Stewart, 1997). Total amount of SON is usually higher under grasslands and forests and the proportion of amino compounds is greater than in arable soils (Németh et al., 1988; Mengel et al. 1999).

2.2. Soil nitrogen cycle and N transformations

The nitrogen cycle is very complex consecution of transformations, which are all driven by soil microbial community. There are two main pathways of nitrogen in soil, the first became from atmospheric N (N_2). **Biological N fixation**, the conversion of N_2 to NH_4^+, is accomplished by free-living and endosymbiotic prokaryotes (Jakson et al. 2008). The N_2 fixation is influenced by many environmental and edaphic factors, but temperature and available phosphorus (P) are two of the most important ones (Hartwig, 1998; Sinclair and Vadez, 2002). The second pathway of soil nitrogen became from soil organic matter (SOM). The soil nitrogen cycle is driven by SOM, which plays a key role in many soil processes because it affects, among others, soil structure, nutrient dynamics and soil life (Rosswall, 1982; de Vries et al., 2006).

Depolymerization of soil organic matter by extracellular enzymes, produced by fungi and bacteria, release monomers, such as amino acids (Sylvia et al., 1998), which are recycled and reused through microbial metabolism, faunal grazing of microbes, and microbial death and damage that are caused by stress, such as from wet-dry or freezy-thaw cycles (Schimel and Bennett, 2004; Jackson et al., 2008) (Figure 2). Root exudates, root turnover, and mycorrhizal turn over are other sources of compounds that increase the availability of labile C and N (Bais et al., 2006; Högberg and Read, 2006). The C and N cycles are closely intertwined, and the soil C availability from root exudates and soil organic matter can drive the microbial processes that release soil N in plant-available forms (Jackson et al., 2008). Through **mineralization**, heterotrophic microbes break down organic monomers and release NH_4^+, which can be used as an energy source by ammonia-oxidizing microbes to produce NO_2^- that usually readily converted to NO_3^- (nitrification), and also to nitric oxide (NO) and nitrous oxide (N_2O) (Gödde and Conrad, 2000) (Figure 2).

The process of **nitrification** exists in autotrophic and heterotrophic forms, which depends on microbial composition. This process is limited by soil aeration, microbial community size and ammonium availability. Nitrification is inhibited in very low moisture, but increase with soil moisture up to -0,01 MPa, and then declines as the soil becomes saturated (Smith et al., 2003). Nitrification is more prevalent in tilled soils than in undisturbed soils (Gödde and Conrad, 2000).

In the process of **microbial N immobilization (assimilation)**, N is taken up, metabolized by soil microbes and built into microbial biomass. This non-symbiotic microbial process competes with plant N uptake (Schimel and Bennet, 2004).

Denitrification takes place when heterotrophic bacteria under oxygen limitation use NO_3^- as an alternative electron acceptor to produce N_2O and N_2 (Jakson et al., 2008). The N_2: N_2O ratio decrease with O_2 availability, and it often decrease under high NO_3^- availability (Firestone and Davidson, 1989). Emissions of ammonia (NH_3) (process of **volatization**) gas begins to increase at soil with pH > 8 and ultimately contributes to N deposition elsewhere in the landscape (Jakson et al., 2008). Another N output from the ecosystem is **NO_3^- leaching**, which contaminates groundwater as well as surroundings ecosystems.

The N cycle in wet meadows is changing with a periodicity of flushing. Unstable water level, which is dependent on seasonal conditions and actual weather, caused periodic changes in soil aeration and in soil water saturation. In summer, when water level is lower and soil aeration is higher, aerobic processes (nitrification) dominate, whereas in spring and autumn time, when is water level higher and aeration lower, anaerobic processes (denitrification) occur in larger scale (Golterman, 1995).

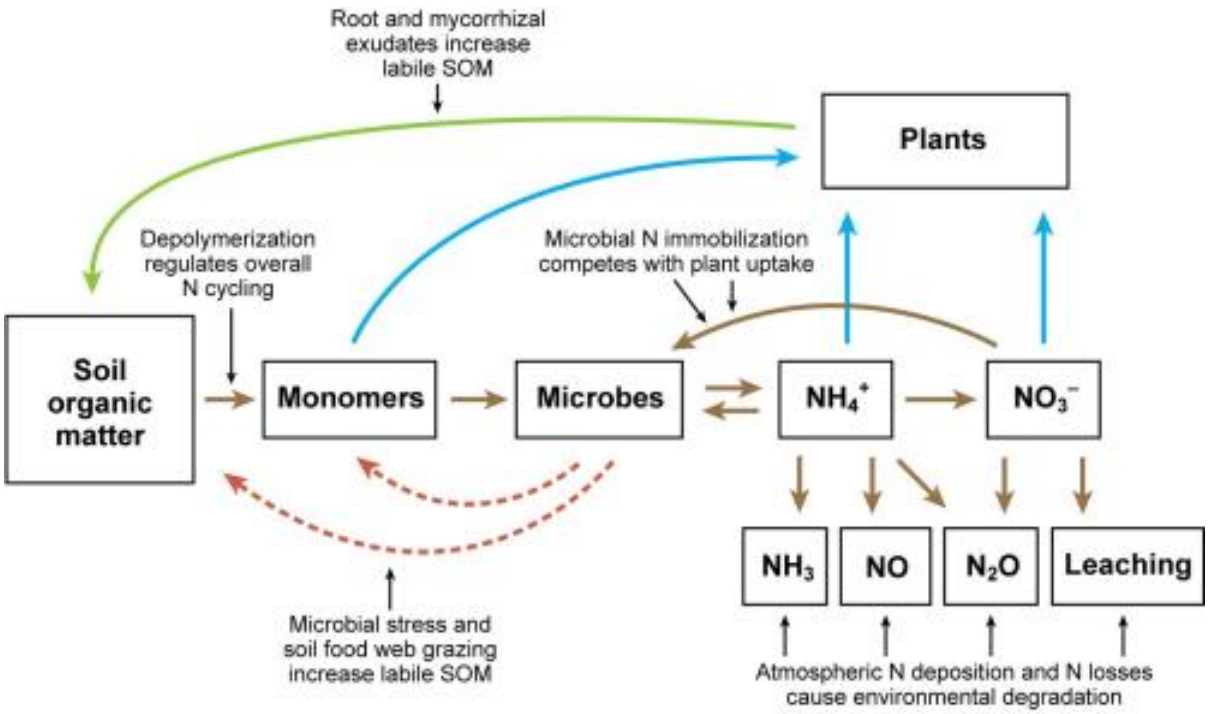

Figure 2. The soil nitrogen cycle, adapted from Schimel, J. P., and J. Bennett. (2004).

2.3. Influence of eutrophication on soil N cycle

Global trends of increased emissions of reactive N are of concern due to the role of oxidized and reduced forms of N in acidification and eutrophication of terrestrial and aquatic ecosystems (Vitousek et al., 1997; Bobbink et al., 1998; Fenn et al., 2003; Galloway et al., 2003). Human activities have more than doubled the amount of fixed nitrogen N that enters ecosystems annually (Vitousek et al., 1997). Increased N deposition can have dramatic impacts on ecosystem processes and biological communities (Allison et al., 2007). Increased N depositions leaded to an accumulation of nutrients in soil and to trophy growing – **process of eutrophication**. In general, the main generators of eutrophication are acid deposits, farming and drainage of sewerage water (Barendregt and Beltman, 2005). The main causes of eutrophication of wet meadows soils are fertilizer ablation from arable soils and fertilizing of wet meadows in order to get higher plant production.

Elevated nitrogen deposition can affect soil N cycle in many ways. Raising soil N availability further stimulates plant biomass production (Gough et al., 2000; Shaver et al., 2001; Henry et al., 2005), which is connected with the increased quantity of litter inputs with lower C:N ratio (e.g. Henry et al., 2005; Corstanje, 2007) and higher exudation (Kuzyakov et al., 2002; Peterson et al., 2006) into the soil. Other changes in soil N cycle could be driven by altering of plant community composition towards rapidly growing species and these tend to produce high quality litters which decompose rapidly (Suding et al., 2005).

In spite of relatively predictable effects of eutrophication on plant communities, there is greater uncertainty in regards to the responses of soil organisms and processes to increased nutrient levels. Microbial biomass may increase (Scheu and Schaefer, 1998), decrease (de Vries et al., 2006) or have a variable response to increased N loadings. The composition of microbial community could be also changed but the results again vary. The fungi:bacteria ratio could decrease (de Vrie et al., 2006) or increase (Smolander et al., 1994; Ettema et al., 1999) with increasing N application rate, which has also negative effect on the mycorrhizal fungi (Bradley et al., 2006). Increased N loading leads to faster litter decomposition, increased N mineralization (Lowet and Rueth, 1998) and slower N immobilization (Dijkstra et al., 2004). It also causes higher leaching of nitrates from ecosystems (de Vries et al., 2006), because nitrate forms are vulnerable to leaching from ecosystems or to transformations to gaseous forms of N through denitrification (Subbarao et al., 2006). The process of nitrification is dominating in N overbalanced ecosystems (Subbarao et al., 2006). It could be generalized that eutrophication accelerates soil N cycle and increases N loses from the ecosystems.

3. Materials and methods

3.1. Study sites

Two wet meadows in Trebon Basin Biosphere Reserve (TBBR), South Bohemia, Czech Republic, were chosen as the ecosystems for studying the effect of eutrophication on soil interactions. These two sites are representative of two main temperate wet meadows. The first, Zablatske louky site, (lat. 49°06´, long. 14°39´, alt. 426 m above sea level) (Fig. 1) is a marginal wetland (Prach, 2002) with organic peaty soil (Histosol, FAO-WRB classification), located in the inundantion area of a human-made fishpond. The water level is usually stable here, except long periods of extreme summer drought and a few months period of fishpond drawdown in autumn. This site is dominated by *Carex vesicaria* and *Carex acuta* (values of 2 and 3 on Braun–Blanquet scale). The second, Hamr site, (lat. 49°09´, long. 14°46´, alt. 415 m above sea level) (Fig. 1) , is an alluvial meadow near the Nežárka river. This site has silt-sand mineral substrate (Gleysol, FAO-WRB classification) and the water level is the same as in local drainage pools connected with the river. This site is dominated by *Glyceria maxima* and *Carex acuta* (values of 3 on Brau-Blanquet scale). Both soils in these study sites are classified as silty-loams, more physical and chemical parameters are shown in Table 1. Water level was measured using the STELA system, which consists of a field datalogger M4516 with GSM/GPRS modem MG40 and water sensor (Fiedler, Electronics for ecology, Czech Republic. The water level data measured at the first season of nutrient loading are shown at Figure 3.

Table 1. Physical and chemical parameters of mineral and organic soils of study sites (means ± standard deviations)

Site	Mineral soil	Organic soil
bulk density [g.cm^{-3}]	0,52 ± 0,04	0,21 ± 0,02
clay [%]	12,5	22,5
sand [%]	15,0	0,0
total C [%]	9,63 ± 1,65	22,33 ± 2,25
total N [%]	0,64 ± 0,1	1,18 ± 0,09
C to N ratio	15,0	18,9
pH_{H_2O}	4,9	5,1

3.2. Experimental design and soil sampling

Four blocks were established in each meadow in May 2006. Each block was divided to three treatment plots (3,5 x 3,5 m) and each plot has different treatment of commercial NPK fertilizer (Lovofert 15:15:15 NPK, Lovochemie, a.s.): 1) no fertilizer addition (control, C), 2) 65 kg NPK * ha^{-1} * yr^{-1} (low, L) 3) 300 kg NPK * ha^{-1} * yr^{-1} (high, H). The low treatment simulates current management treatments for Czech farms while the high treatment is the dose recomended by agricultural firms for grasslands of this type (European Environmental Agency, 2007). The fertilizer was always applied in half-doses two times a year, in mid-May and mid-July of 2007 and 2008.

Soils from all treatment plots were sampled three times a year in 2007 and 2008. The first two samplings were always done 6-7 days after fertilization events, the last in the end of the vegetation season (October). From each treatment plot one soil sample was taken, mixed from 10 randomly sampled soil cores (to 20 cm depth). Soil samples were kept at 4°C, sieved through 5-mm mesh next day after sampling and immediately prepared for analyses.

3.3. Soil analyses

For an analysis of total soluble N (TSN) and concentrations of ammonium and nitrates (NH4-N and NO3-N, respectively), a portion of each soil sample was extracted in 0.5 M K2SO4 (soil: extractant 1:4). The extracts were filtered and stored in the freezer. The TSN was measured on a LiquicTOC II (Elementar, Germany) and NH4-N and NO3-N using flow injection analysis (FIA Lachat QC8500, Lachat Instruments, USA). The amount of soluble organic N (SON) was calculated as the difference between TSN and a sum of NH4-N and NO3-N. Soil microbial biomass C and N were measured using the chloroform fumigation-extraction method (Vance et al. 1987) and calculated as the difference between organic C and TSN contents in fumigated and non-fumigated samples. Coefficient of 0,45 (Vance et al. 1987) was used to correct the results for soil microbial biomass C and coefficient of 0,54 (Brookes et al. 1985) was used to correct the results for soil microbial biomass N. The rates of net N-mineralization, nitrification and N-assimilation into microbial biomass were calculated from changes in concentrations in particular N pools (NH_4-N, NO_3-N and soil microbial N) after three weeks of aerobic incubation of soil samples at 20 °C. All measurements were done in three laboratory replications per soil sample. The results in the tables are arithmetic means of three sampling times per year, expressed on an oven-dry soil basis (24 h at 105 °C).

Phospholipid fatty acid (PLFA) analysis was done in soil samples from October 2008. These samples were extracted by a mixture of chloroform-methanol-phosphate buffer

(1:2:0.8) according to (Bligh and Dyer, 1959). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck) and the samples were subjected to mild alkaline methanolysis. The free methyl esters of phospholipid fatty acids were analyzed by gas chromatography–mass spectrometry (Varian 3400; ITS-40, Finnigan). Selected PLFA were used to determine community composition on notional microbial groups; G+ and G- bacteria (Federle 1986; Frostegård et al. 1993), fungi (Federle 1986) and actinomycetes (Kroppenstedt, 1985). Physiological status was detected using the ratio of cyclopropane PLFA to its monoenoic precursor (*cyclo/prec*), which increase indicated a shift from exponential to stationary growth, thus the substrate limitation (Thomas and Batt, 1969; Guckert et al., 1986; Navarrete et al., 2000), and the ratio of monoenoic to saturated PLFA (MUFA/STFA), an indicator of substrate availability (Bossio and Scow, 1998).

3.4. Statistical analyses

Repeated measures ANOVA followed by the Tukey test ($p<0.05$) was used to evaluate the effects of sampling site, fertilization and sampling time on soil properties (Statistica 8.0, StatSoft Inc., USA).

The effect of fertilization and sampling site on microbial community composition (PLFA fingerprint based on mol% of individual PLFA within total PLFA) was tested using Redundancy Analysis (RDA) (CANOCO for Windows 4.0, ter Braak and Šmilauer,1998). The potential differences between differently treated plots were further tested by ANOVA.

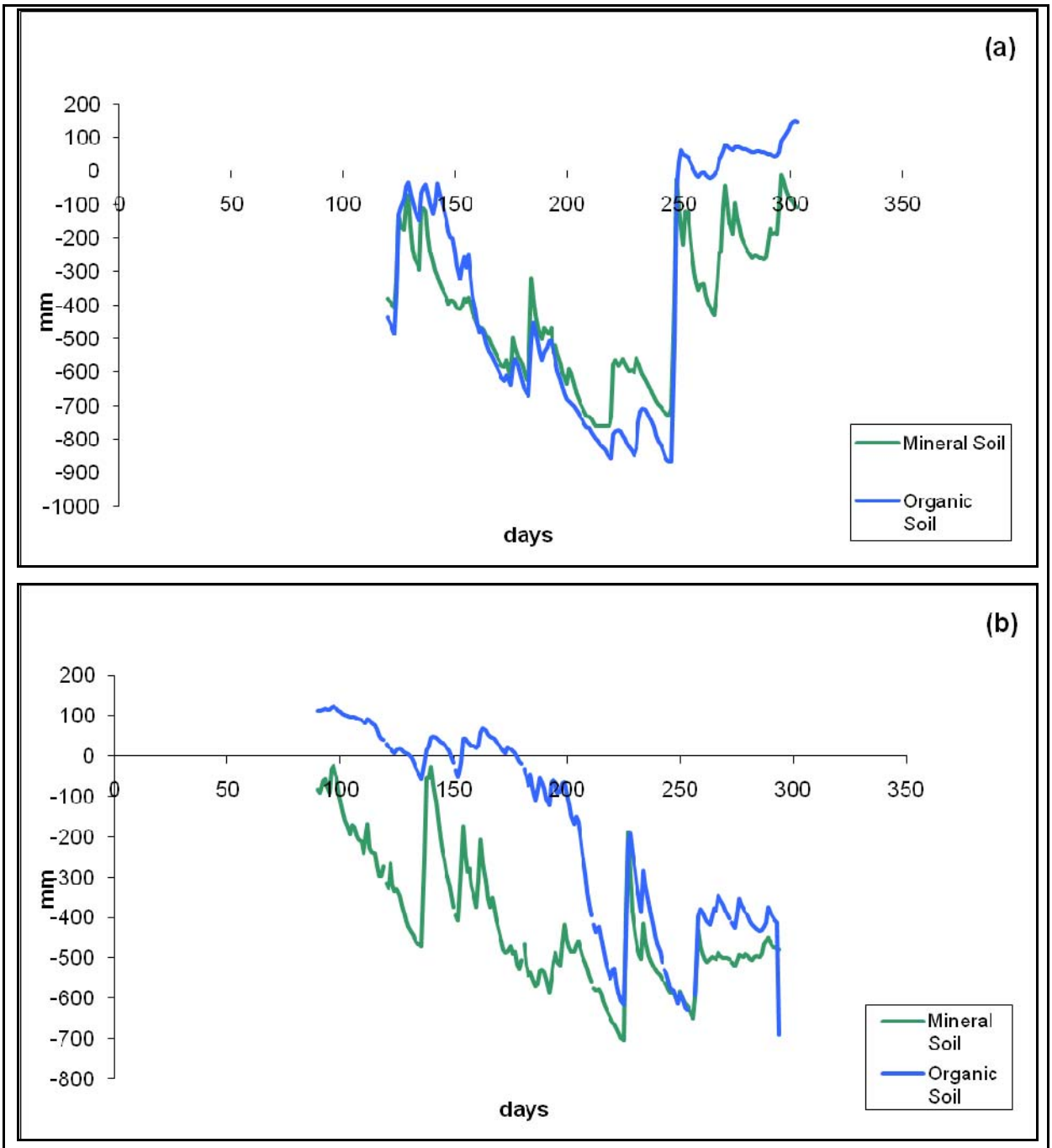

Figure 3. Water levels in years 2007 (a) and 2008 (b), measured during the vegetation season from April to October. Blue line shows data from organic soil and green line shows data from mineral soil (measured with the STELA system).

4. Results

4. 1. Soluble nitrogen in soils

The amounts of TSN as well as SON were significantly higher in organic than mineral soil ($p<0.001$), whereas the concentrations of inorganic N forms were comparable (Table 2). The proportions of SON within TSN were high in both soils, ca 80% and 77% in organic and mineral soils, respectively. The effect of fertilization on soluble N forms was not significant. The amount of TSN tended to increase with increasing amount of applied fertilizer in both samplings done early after fertilization in organic soil (Fig. 4a) and in summer sampling in mineral soil (Fig. 4b). This increase can be ascribed to higher amounts of SON and increasing amounts of ammonium in fertilized treatments as compared to control plots (Fig. 4). In case of ammonium, the increase with fertilization was on the border of significance ($p=0.062$), and the increasing trend could be also seen on the average values (Table 2). However, the potential effect of fertilization on soluble N was only short-term and totally disappeared during autumn sampling in both soils (Fig. 4). The amounts of nitrates were almost regularly lowest in low fertilization treatment and highest in control plots (Table 2, Fig. 4).

Table 2. Concentrations of total soluble N (TSN), soluble organic N (SON), ammonium and nitrates in differently fertilized soils (C, L, H) of two sampling sites in 2008. Means of three sampling times in 2008 (n=12) and standard deviations are given. Different letters in subscripts show significantly different values within a column.

Site	Treatment	TSN	SON	NO3-N	NH4-N
		μg N g^{-1}	μg N g^{-1}	μg N g^{-1}	μg N g^{-1}
Organic soil	C	$34,56^{b}$ ± 4,74	$27,94^{b}$ ± 3,78	$2,55^{b}$ ± 0,78	$4,07^{a}$ ± 2,04
	L	$35,19^{b}$ ± 3,56	$29,19^{b}$ ± 2,85	$1,69^{a}$ ± 0,61	$4,30^{a}$ ± 1,86
	H	$35,63^{b}$ ± 4,24	$28,80^{b}$ ± 3,77	$1,96^{ab}$ ± 0,66	$4,87^{a}$ ± 2,12
Mineral soil	C	$27,51^{a}$ ± 7,16	$20,97^{a}$ ± 4,94	$2,18^{b}$ ± 1,45	$4,36^{a}$ ± 1,95
	L	$25,97^{a}$ ± 3,78	$20,46^{a}$ ± 2,78	$1,14^{a}$ ± 0,42	$4,37^{a}$ ± 1,17
	H	$29,20^{a}$ ± 7,15	$22,51^{a}$ ± 5,97	$1,91^{ab}$ ± 0,95	$4,93^{a}$ ± 1,90

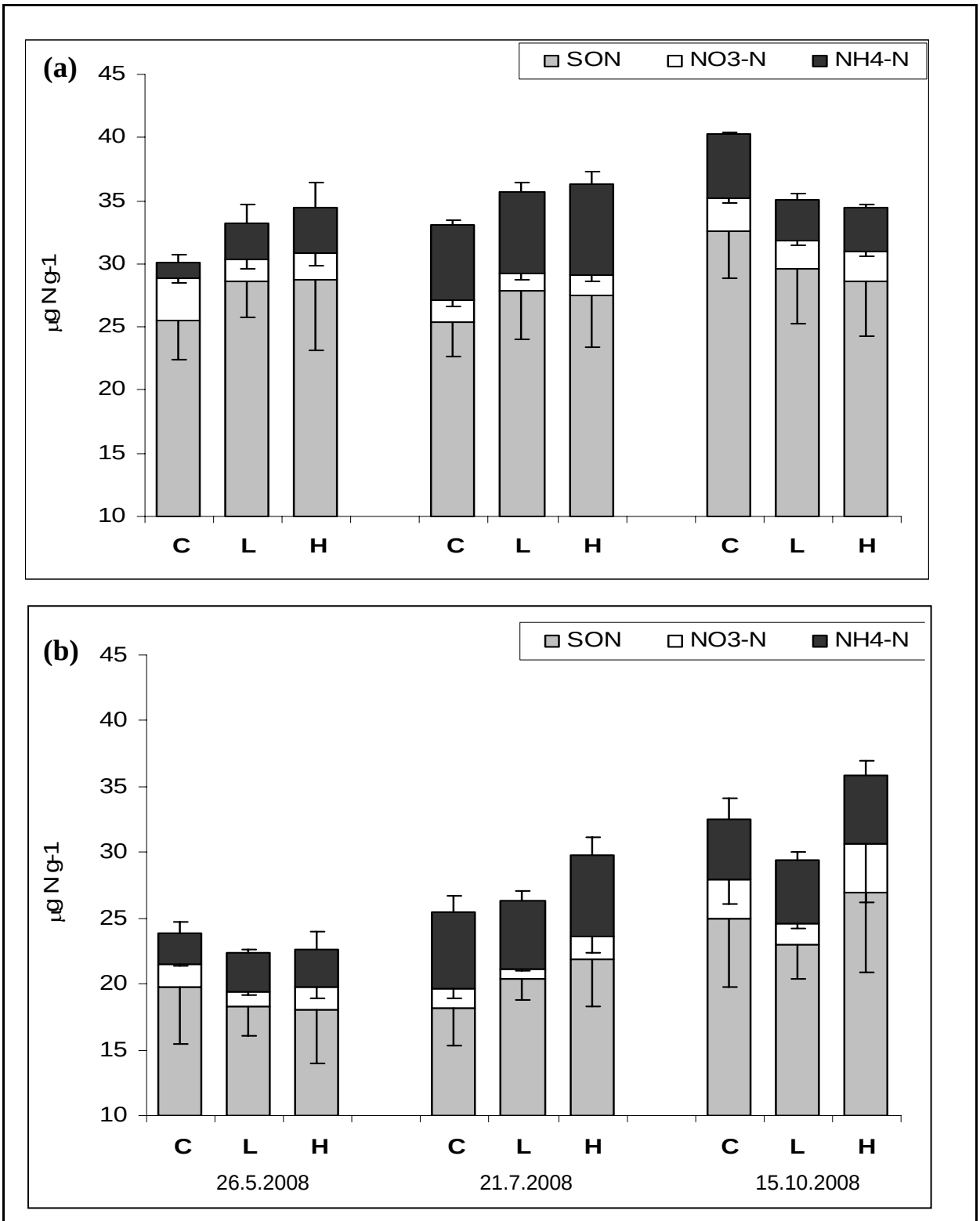

Figure 4. The amounts of SON, NO_3 and NH_4 in organic (a) and mineral (b) soils in three sampling times (spring, summer, autumn) in 2008; (C) means control plot, (L) means low fertilized plot and (H) means high fertilized plot.

4.2. Microbial biomass N and C/N ratio

The amounts of N microbial biomass (Nmic) comprised ca 2.7% of total soil N in organic and 4.4 % in mineral soil. Organic soil contained higher amount of Nmic than mineral one but the difference was significant only in 2008 ($p<0,002$) (Table 3). Fertilization did not affect the Nmic at all (Table 3).

The biomass C/N ratio was comparable between both soils (except 2007, where C and L treatments of organic soil had higher Nmic than the rest of plots), being markedly lower in 2007 than in 2008 (Table 3). In organic soil, the biomass C/N ratio tended to decrease in highly fertilized plots in comparison to C and L treatment in almost all samplings (Fig. 5a), but the effect of fertilization was significant only in 2007 ($p<0,05$) (Table 3). In mineral soil, fertilized plots (H and L) also tended to have lower biomass C/N ratio than control treatment in some sampling times (Fig. 5b) but the differences among treatments were not significant.

Table 3. Microbial biomass nitrogen (Nmic) and biomass C/N ratio in differently fertilized soils (C, L, H) of two sampling sites in 2007 and 2008. Means of three sampling times per year (n=12) and standard deviations are given. Different letters in subscripts show significantly different values within a column.

Site	Treatment	Nmic (μg N g^{-1})		Biomass C/N	
		2007	2008	2007	2008
Organic soil	C	$170.95^{a} \pm 61.10$	$138.69^{b} \pm 45.63$	$15.46^{b} \pm 2.51$	$19.27^{a} \pm 4.13$
	L	$190.41^{a} \pm 90.60$	$133.26^{b} \pm 39.69$	$15.30^{b} \pm 3.57$	$19.54^{a} \pm 3.73$
	H	$193.35^{a} \pm 68.34$	$125.73^{b} \pm 32.70$	$13.20^{a} \pm 2.91$	$18.54^{a} \pm 3.14$
Mineral soil	C	$173.55^{a} \pm 53.72$	$100.16^{a} \pm 39.17$	$13.23^{a} \pm 3.50$	$21.35^{a} \pm 9.30$
	L	$157.22^{a} \pm 44.28$	$103.45^{a} \pm 26.25$	$12.07^{a} \pm 2.75$	$17.43^{a} \pm 4.29$
	H	$176.89^{a} \pm 69.32$	$106.79^{a} \pm 30.67$	$12.23^{a} \pm 3.18$	$18.14^{a} \pm 5.04$

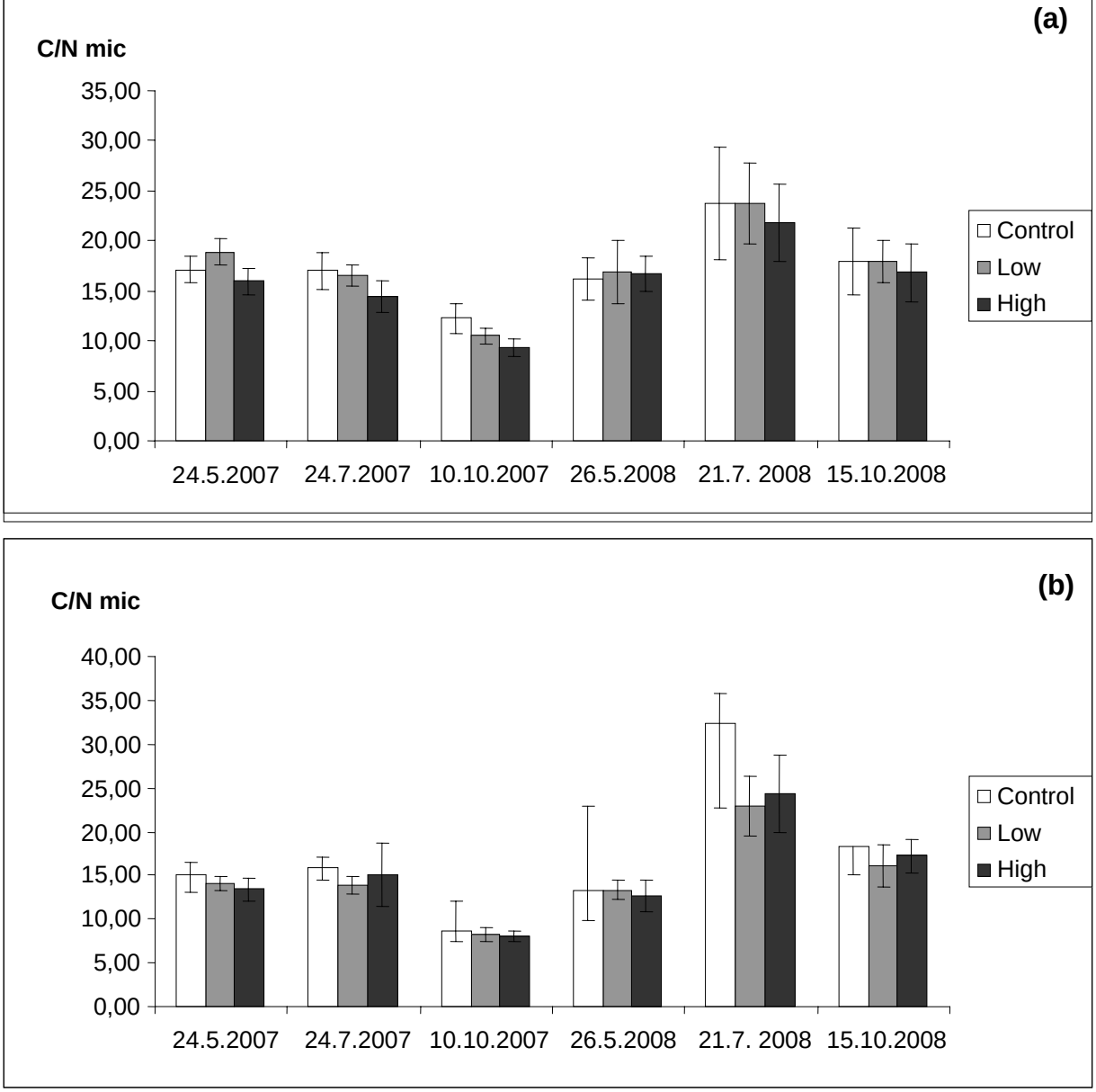

Figure 5. Biomass C:N ratios in organic (a) and mineral (b) soil.

4.3. Composition of soil microbial biomass

In total 19 PLFA were identified with GC-MS in both soils. The total amount of PLFA was non-significantly higher in mineral than organic soil (Fig. 6). The effect of locality explained 60% of variability in the PLFA fingerprint (data not shown) and 29% in the proportion of notional groups within soil microbial communities (Fig. 6). Soil microbial community in mineral soil contained higher proportion of G+ bacteria ($p<0.001$) but lower proportions of G- bacteria and fungi ($p<0.001$ and $p=0.001$, respectively) in comparison to that in organic soil. This led to a significantly higher bacteria to fungi ratio (B/F) in mineral soil ($p=0.010$).

Mineral soil further showed lower MUFA/STFA ratio and higher cyclo/prec ratio than organic soil ($p<0.001$ in both cases).

Fertilization led to a slight decrease in total PLFA amount in soil, but the change was not significant. However, it significantly affected the composition of soil microbial community. Its effect explained 7.3% in the variability of PLFA fingerprint (data not shown) and 7.5% in the proportion of notional groups within soil microbial communities (Fig. 6). Fertilization led to a significant increase in the proportion of actinomycetes ($p=0.031$) and G+ bacteria ($p=0.007$) in microbial community. This was caused mainly by positive effect of fertilization on following PLFA: 10Me-16:0 (actimomycetes), a15:0 and a17:0 (G+ bacteria). The proportion of bacteria in microbial community also tended to increase ($p=0.080$), while fungi and G- bacteria were not affected. Therefore B/F ratio was not changed in fertilized plots. Further, the MUFA/STFA ratio tended to decrease in fertilized plots of organic soil and the cyclo/prec ratio increased significantly ($p=0.02$) in fertilized plots of mineral soil.

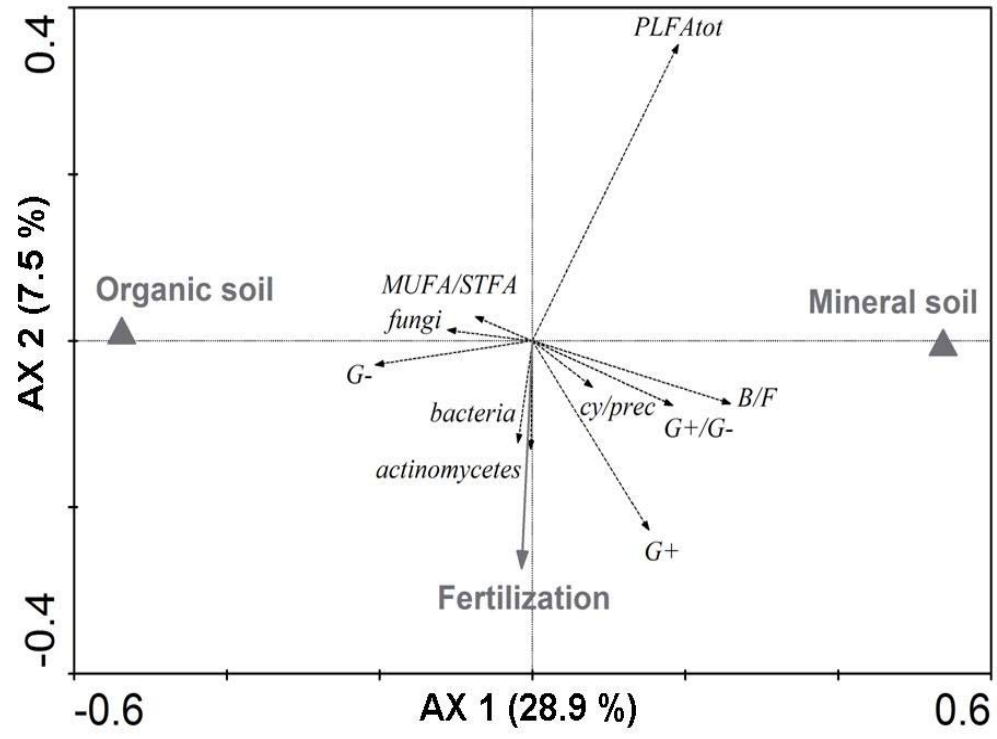

Figure 6. The effect of fertilization and soil type on total PLFA amount and relative abundance of notional groups within soil microbial community (RDA analysis).

4.4. Microbial N transformations

The rates of all measured processes were comparable between both soils, except of higher nitrification rates in organic than in mineral soil in 2008 ($p<0.001$). Both soils showed positive net nitrification rate, with the mean value of ca 1 μg N g^{-1} day^{-1}, and negligible rate of net N mineralization in both sampling years (Table 4). The rate of net N assimilation into microbial biomass differed between both sampling years, being negative with the mean value of ca -2 μg N g^{-1} g^{-1} day^{-1} in 2007 and positive with the mean value of ca 1 μg N g^{-1} g^{-1} day^{-1} in 2008.

Two years of NPK fertilization had no significant effect on N transformation rates in mineral soil (Table 4). In organic soil, net nitrification rate tended to increase in highly fertilized treatment in 2008 ($p=0.09$) (Table 4) and the rate of N assimilation into microbial biomass decreased in fertilized treatments in comparison to control (significantly in 2007, $p<0.05$, non-significantly in 2008) (Table 4). Mineralization of N was not affected by fertilization at al.

Table 4. Microbial N transformations (nitrification, N-mineralization and N-assimilation) in differently fertilized soils (C, L, H) of two sampling sites in 2007 and 2008. Means of three sampling times per year (n=12) and standart deviations are given. Different letters in subscripts show significantly different values within a column.

Site	Treatment	N - nitrification μg N g^{-1} d^{-1}		N - mineralization μg N g^{-1} d^{-1}		N - assimilation μg N g^{-1} d^{-1}	
		2007	2008	2007	2008	2007	2008
Organic soil	C	$0,75^{a} \pm 0,42$	$1,13^{b} \pm 0,25$	$-0,04^{a} \pm 0,08$	$0,03^{a} \pm 0,01$	$-1,62^{a} \pm 2,57$	$1,08^{a} \pm 1,49$
	L	$0,89^{a} \pm 0,53$	$1,14^{b} \pm 0,20$	$-0,07^{a} \pm 0,10$	$0,03^{a} \pm 0,01$	$-3,08^{b} \pm 4,76$	$0,75^{a} \pm 1,72$
	H	$0,47^{a} \pm 1,02$	$1,21^{b} \pm 0,30$	$-0,03^{a} \pm 0,02$	$0,02^{a} \pm 0,04$	$-3,37^{b} \pm 2,48$	$0,38^{a} \pm 1,36$
Mineral soil	C	$1,03^{a} \pm 0,39$	$0,97^{a} \pm 0,27$	$0,00^{a} \pm 0,09$	$0,00^{a} \pm 0,02$	$-2,55^{b} \pm 2,03$	$0,85^{a} \pm 1,89$
	L	$0,72^{a} \pm 0,16$	$0,66^{a} \pm 0,30$	$-0,03^{a} \pm 0,06$	$0,02^{a} \pm 0,04$	$-1,69^{a} \pm 2,96$	$1,21^{a} \pm 1,88$
	H	$1,00^{a} \pm 0,65$	$0,90^{a} \pm 0,29$	$-0,05^{a} \pm 0,08$	$0,00^{a} \pm 0,03$	$-2,59^{b} \pm 3,35$	$0,94^{a} \pm 1,93$

5. Discussion

5.1. Changes in soluble N pool

In our sites, the amounts of total soluble N and also a proportion of organic N within the total soluble N were significantly higher in organic soil, as it contains higher amount of soil organic matter (Brady and Weil, 2002).

In spring and summer samplings, done 6-7 days after the NPK fertilization, we found increased amounts of total soluble N in soils. In autumn sampling, which was not preceded by fertilization, no such trend was found. The short-term effect of fertilization on soluble N pool could be explained by relatively fast cycling of soluble N pool, especially easily mineralizable organic N and inorganic N forms, due to rapid N transformation pathways and plant uptake (Németh et al., 1988; Mengel et al., 1999). The increased amount of soluble N in soils after fertilization was caused by increased amounts of ammonium and soluble organic N, but not nitrates. Nitrogen in the applied fertilizer consisted from NH_4 and NO_3 forms (ca half to half) and contained no organic N form. Therefore, organic N could originate from microbial processes or chemical reactions of fertilizer with components of soil organic matter (Fog, 1988). The added nitrates were depleted from soil solution faster than ammonium due to its higher mobility (Subbarao et al., 2006). Nitrates could be taken up by plants, which were found to prefer the nitrate form as a source of N against the ammonium (unpublished data), transformed by microbes or partly also leaked form the system. The two-year of nutrient loading was too short to affect soluble N pool permanently, but after N saturation of the system this could occur (Emmet, 2007).

5.2. Changes in microbial biomass and composition

We observed that organic soil contained higher microbial biomass N than mineral soil, which again corresponded with higher amount of soil organic matter (Braddy and Weil, 2002). We found no significant changes in the amount of microbial biomass N due to fertilization. According to Yevdokimov et al. (2008), the soil is capable for the retention of high amounts of nitrate with unchanged size of microbial biomass. Therefore, microbial biomass N, the important component of total soil N, is denoted as a robust parameter, which remains unchanged even after long-term management (Hassink and Neetseson, 1991; Bending et al., 2000). This should mean that two years of fertilization likely was too short period for

distinctive changes in its amount. Moreover, Bardgett et al. (1999) found that N addition had no consistent effect on soil microbial biomass.

Contrary to the absence of clear and significant effect of the NPK fertilization on the amount of microbial biomass N in soils, we observed changes in C:N ratio of microbial biomass that could indicate changes in soil microbial community. We observed decrease in biomass C:N ratio in fertilized treatments in both soils (significant in organic soil and non-significant in mineral soil). The decreased C:N ratio of microbial biomass in eutrophied soils could be connected with a shift in composition of microbial community structure towards bacteria, as found by Högberg et al. (2003), de Vries et al. (2007), Alisson et al. (2007) or Yevdokimov et al. (2008). We also found increasing tendency in bacterial proportion within microbial community, but this was non-significant. As fertilization did not affect the proportion of fungi at all, the bacteria/fungi ratio did not change. However, there were significant changes within bacterial community. Fertilization leaded to increases in the relative abundance of gram-positive bacterial PLFAs, as also found by Yevdokimov et al. (2008), Denef et al. (2009), and actinomycetes. It is known that high N supply can negatively affect the mutualistic relationships between plants and arbuscular mycorrhizal fungal communities (Bradley et al., 2006). The PLFA 16:1w5 is commonly used for mycorrhizal detection in PLFA analysis. This PLFA occurs in both our soils but no mycorrhizal infection of plant roots were found by microscopical methods (unpublished results). Therefore, we suggest that using this PLFA for a mycorrhizal detection could be misleading in our case.

In fertilized soils, PLFA indicators of the physiological status (decreased MUFA/STFA ratio, increased cyclo/prec ratio) implied a possible metabolic stress. Decreasing MUFA/STFA ratio, similarly to increasing cyclo/prec ratio are commonly used to indicate a substrate limitation (Thomas and Batt 1969; Guckert et al. 1986; Bossio and Scow, 1998; Navarrete et al. 2000). However, we have no other indices to specify the stressful conditions in fertilized soils.

5.2. Changes in microbial N transformations

We also observed effect of two-year fertilization on the processes of N transformation in the soils. We found increasing tendency of net nitrification rate along with higher N fertilization in organic soil. Increased nitrification potential due to fertilization was also found by Zhong et al. (2007) and Lovett a Rueth (1999) with influences of seasonality and aboveground biomass (Fortuna et al., 2003). The N assimilation decreased with higher amount of fertilizer in organic soil; significantly only in the sampling in 2007, but the same decreasing tendency was

evident also in the sampling in 2008. Decreased N immobilization in microbial biomass could cause a problem in ecosystem N balance in future. It is an important process in maintaining soil fertility and plant nutrition due to the N immobilization-remineralization and retards the loss of N (and also fertilizer N) by leaching or via volatilization of gaseous N compounds. It belongs between important factors controlling the resilience of the ecosystem (Yevdokimov et al., 2008). The above described trends were, however, not visible in mineral soil, which respond to the nutrient loading to much lesser extend in all measured parameters. We found no significant changes in N mineralization due to fertilization. Similarly to microbial biomass N, N transformations probably need a longer period to show significant changes as affected by fertilization. We must also take into account that we observed only rates of net N-transformations- changes in concentrations of soluble nitrates, ammonium, organic N and microbial biomass N. These result from several processes. Observing the rates of gross N-transformations with use of ^{15}N could give more detailed view into the effects of fertilization on soil N transformations.

5.3. Conclusions

Short-term NPK fertilization of wet meadows leaded to several changes in observed soil parameters. The period of nutrient loading was too short to affect significantly soil biomass N and microbial N transformations. In spite of this, fertilization significantly decreased the C:N ratio of microbial biomass and caused marked changes in microbial community composition, especially within bacterial community. There was also short-term effect of added fertilizer on soluble N; the increased amounts of ammonium and soluble organic N were still found a week after fertilizer application. In organic soil, we found indices of an acceleration of soil N cycle (increased nitrification rate and decreased N immobilization in microbial biomass).

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