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Bio-available amino acids and mineral nitrogen forms in soil of moderately mown and abandoned mountain meadows

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Summary. The abandonment of traditional mowing methods of mountain meadows in the Czech Republic at the end of the last century has resulted in secondary re-colonization of these areas. Altered accumulation of plant biomass resulted in a deceleration of N turnover. A mountain meadow may be regarded as a N-limited ecosystem in which plant nutrition is dependent on direct uptake of soil amino acids. The composition and distribution of ammonium ions, nitrate ions and the 16 bio-available proteinaceuous amino acids were investigated in the top 7 cm of the Ah horizon of a Gleyic Luvisol in a long-term moderately mown meadow and an eleven year old, abandoned or uncut meadow. Ammonium N has a dominant role in both ecosystems. The moderately mown meadow showed accelerated N-turnover and higher net ammonization. The plant community showed a dependence on this form. Plant utilization of nitrates and amino acids appeared to be negligible. The uncut or abandoned meadow showed net ammonization from May (start of the experiment) through August, after which plant N-uptake consisted only of amino acids due to microbial immobilization. The release of bio-available nitrogen from spring until the beginning of summer in the Ah horizon was too low to explain total plant N-uptake. Glutamic acid, arginine and aspartic acids had the highest concentrations of any of the amino acids analyzed.

Keywords: Soil bio-available amino acids – Mountain meadows – Abandonment – Mowing – Mineral nitrogen forms

1. Introduction

Socio-economic changes in the Czech Republic towards the end of the last century led to considerable changes in the environmental conservation management of non-forested ecosystems. There has been decreasing human population numbers in mountainous regions, where traditional farming and mowing of meadows was practiced. Meadow abandonment initiated secondary succession with shrubs and trees re-colonizing these areas. Plant coverage in abandoned or uncut meadows is steadily increasing. Root biomass is considered to be a very important indicator of meadow stands, where most of the net primary production is accumulated below ground (Fiala, 1993). Variations in plant coverage associated with the cessation of mowing are associated with changes in the quantity and quality of the root systems. Mowing can result in the root systems alterations in size, depth and/or proportion of living plant parts (Fiala, 1997). The changes in below- and above-ground plant biomass can probably lead to subsequent changes in soil physical-, chemical- and biological properties (Slavíková, 1986).

Abandoned meadows have slower nitrogen cycling (Fiala and Zelená, 1991). In such ecosystems, the amount of available mineral nitrogen can be insufficient to satisfy plant demand, and amino acids can become more important in plant nutrition. Evidence indicating the importance of amino acids in ecosystems nutrition comes from alpine, arctic and boreal areas where availability of mineral nitrogen was found to be low to account for annual plant uptake (Rehder and Schafer, 1978; Fisk and Schmidt, 1995; Kaye and Hart, 1997). Abandonment of unfertilized mown meadows in mountain regions, which are usually N-limited ecosystems, may result in such areas becoming more dependent on direct uptake of bio-available amino acids. Bio-available (plant-available) amino acids are in this sense thought to be individual proteinaceous amino acids dissolved in the soil solution and exchangeably sorbed on soil colloids. Both above-ground litter and dead roots are considered to be the largest source of amino acids in the soil (Jones and Kielland, 2002). Root biomass comprises a major part of total plant biomass in mown or abandoned meadows, and abandonment increases the supply of amino acids from the above-ground litter.

The aim of this study was to investigate: a. how the distribution of soil bio-available nitrogen forms, which supply the local plant community, changed eleven years after abandonment of originally long-term mown mountain meadows and; b. the composition of bio-available amino acids in moderately mown and abandoned meadows.

2. Materials and methods

2.1 Study site

The experimental site ''Bílý Kříž'' is located in the Moravian-Silesian Beskids Mountains in the northeast part of the Czech Republic (N 49°30′17″, E 18°32′28″), on a slope with an elevation of 825–860 m.a.s. and a southeast orientation. The local subcontinental climate in this region is characterised by a mean annual air temperature of 4.9 °C, a mean relative air humidity of 80% and a mean annual precipitation of 1100 mm. There are 160 days with snow cover per year. The experimental meadow (1 ha) was originally mown regularly, the hay removed and stored as feed for livestock. This traditional management practice ceased eleven years ago on one half of the meadow (un-mown meadow), while the remaining half has been moderately mown once a season. For example, mowing was applied there on 28 July for 2004. Before abandonment, the original area of two study plots was uniform with respect to plant populations and soil properties. The moderately mown meadow plant community belongs to the *Nardo-Callunetea* class while the un-mown meadow is characterised by a higher representation of forbs; this community belongs to the *Molinio-Arrhenatheretea* class (Zelená, unpublished). Plant coverage was more uniform in the moderately mown meadow compared to the un-mown counterpart. Still, between-meadows differences in the total (above-ground and below-ground) biomass production were insignificant in 2004 ($1889 \pm 427\ g \cdot m^{-2}$ *versus* $1758 \pm 394\ g \cdot m^{-2}$ un-mown and mown, respectively; mean ± SD; $n = 5$–6) (Holub and Tůma, 2005). Below-ground biomass production was higher in the moderately mown part, while the un-mown part had greater above-ground production. Plant uptake of N, P and K was higher on the un-mown part, with N, P and K concentrations in live above-ground biomass as follows (mown/un-mown): N = 1.14/1.67%, P = 0.18/0.24% and K = 1.47/2.11%. The N:P ratio in the above-ground biomass ranged from 6.8 to 7.9 for both meadows (Holub and Tůma, 2005). Plant cover properties are listed in Table 1.

Table 1. Plant cover on the moderately mown and 11 years un-mown mountain meadows

Mown meadow		Un-mown meadow	
Ec = 95%		Ec = 95%	
E1 = 89–91%		E1 = 95%	
E0 = 10%		E0 = 0%	
Festuca rubra agg.	5	*Rumex acetosa*	4
Veronica officinalis	3	*Hypericum maculatum*	4
Hieracium laevigatum	2b	*Holcus mollis*	4
Nardus stricta	2a	*Achillea millefolium*	3
Luzula campestris	2a	*Potentilla erecta*	2a
Agrostis capillaris	2a	*Veronica chamaedrys*	2a
Potentilla erecta	2a	*Hieracium lachenalii*	2m
Avenella flexuosa	2a	*Cardaminopsis halleri*	2m
Carex pilulifera	1	*Jacea pseudophrygia*	2m
Holcus mollis	1	*Carex ovalis*	1
Hieracium pilosella	1	*Ranunculus repens*	1
Hieracium lachenalii	1	*Festuca rubra* agg.	1
Vaccinium myrtillus	1	*Agrostis capillaris*	1
Anthoxanthum odoratum	+	*Deschampsia cespitosa*	1
Lychnis flos-cuculi	+	*Galeopsis pubescens*	1
Campanula patula	+	*Lychnis flos-cuculi*	1
Hypericum maculatum	+	*Carex nigra*	1
Rumex acetosa	+	*Campanula patula*	+
Carex nigra	+	*Leontodon hispidus*	+
Carex pallescens	+	*Juncus effusus*	+
Gnaphalium sylvaticum	+	*Agrostis stolonifera*	r
Leucanthemum ircutianum	+	*Galeopsis tetrahit*	r
Achillea millefolium	+	*Carex pallescens*	r
Betula pendula	+	*Leontodon autumnalis*	r
Salix caprea	+	*Hieracium aurantiacum*	(r)
Taraxacum sec.Ruderalia	r		
Fragaria vesca	r		

Plant cover was estimated on 25 m^2 plots according to Barkmann: EC-total coverage, E1-herb layer, EO-moss layer; 5 = 75–100% coverage; 4 = 50–75%; 3 = 25–50%; 2b = 15–25%; 2m = 15%; 2a = 5–15%, 1 = <5%; + negligible; r-rare; (r)-rare, out of the plot (Zelená, unpublished)

2.2 Soil sampling

The soil of both meadows is classified as a Gleyic Luvisol (ISSS-ISRIC-FAO, 1998). For the measurement of net release of particular nitrogen forms and their net changes we have adopted procedure of Raison et al. (1987). Soil sampling and incubation were performed throughout the whole 2004 vegetation season starting from mid-May and finishing in mid-October. Two PVC cylinders (15 cm long, 5.9 cm dia) were inserted to a depth of 13 cm at each of five randomly selected replicate points (marked by bars) within each of the two treatment areas (10 PVC cylinders per area; a total of 20 cylinders). The cylinders were inserted close to the selected points throughout the whole experiment to ensure equivalent conditions. One of each pair (A = beginning of incubation, actual concentration) was immediately removed for analysis. Cylinder B (with soil) was removed also, wrapped in a plastic bag, re-inserted into the original hole and left for 30 days, after which it was removed for analysis. This procedure was repeated at 30 day intervals throughout the vegetation season. The soil in the cylinders was undisturbed, plants were not cleared from the incubation tubes and their roots stayed inside the cylinders. This treatment, where roots were cut by cylinder insertion, restricted plant nitrogen uptake, while nitrogen leaching was avoided by wrapping the cylinders into the plastic bags. Cylinders were transported to the laboratory at low temperature. Only the part corresponding to the Ah horizon (from 3 to 10 cm) was used further, prepared by sieving through 5 mm mesh size and stored for 12 h at 4 °C until extraction. The >5 mm fraction constituted 14.9 and 22.9% (w_t/w_t) of the soils in the moderately mown and un-mown meadows, respectively. Mean soil moisture throughout the experiment in A samples ranged from 17.8% in August to 36.2% (w_t/w_t) in October. The water content in B samples was maximally 19.6% (w_t/w_t) higher (36.7 *versus* 17.1) compared to A samples throughout the season, while the water content in B samples, taken from mid-July, was lower by maximally 21.1% (w_t/w_t) (36.2 *versus* 15.1) when related to the beginning of the incubation. Selected physical and chemical properties of Ah horizon are shown in Table 2. Organic horizons L, F and H were not included in this experiment due to their very low thickness after mowing. Every sample was prepared separately; composite samples were not used.

Net microbial release/immobilization of particular nitrogen forms (C) was calculated as the difference between the N form concentration at the end of incubation (B) and the concentration at the beginning (A) of the incubation ($C = B - A$). The net change of total bio-available N forms in uninfluenced soil ($D = A_2 - A_1$) representing net result of all processes

Table 2. Selected physical and chemical properties of the Ah horizon on the moderately mown and 11 years un-mown meadows

Soil properties	Moderately mown	Un-mown
Clay (%)	18.60	19.93
Silt (%)	26.00	27.13
Sand (%)	55.40	52.93
Bulk density ($g \cdot cm^{-3}$)	1.06	0.95
pH (H_2O)/pH 0.01 M $CaCl_2$	4.30/3.80	4.29/3.83
CEC ($mmol_c\ kg^{-1}$)	159.34	184.85
C_t (%)	4.40	5.56
N_t (%)	0.38	0.56
C/N	11.64	9.93
C-humic acids and C-fulvic acids (%)	0.50 and 0.72	0.57 and 0.77

running in the uninfluenced soil, including microbial release/immobilization, root uptake, leaching, gaseous losses and atmospheric deposition, was calculated as the difference between the N form concentration in fresh samples (A_2) from the beginning of the following incubation minus the concentration at the beginning of the previous incubation (A_1). In contrary to the procedure of Raison et al. (1987) we did not separate these processes. For such calculations, it is necessary to interpret the results relevant to bio-available amino acids carefully due to their very short half-life.

2.3 Soil extraction and analysis

According to the best of our knowledge there is no information about extraction efficiency and negative effects of 1% K_2SO_4 on extraction of bio-available amino acids from soil. Ammonium acetate (0.5 M, pH = 6.8) was used in this study because this extractant appeared to be more efficient to extract bio-available soil amino acids when compared with water, and no hydrolytic effect of this extractant was found when tested on sodium caseinate (Formánek et al., 2005). Due to this reason, individual soil samples (50 g) were extracted with 0.5 M ammonium acetate (200 ml) in 500 ml polyethylene bottles. After shaking for 1 h, the samples were filtered through paper and glass fiber GF 30 filters (55 mm, 1 μm, Schleicher & Schuell, Germany) at 6 °C and then were frozen for storage. After storage, the freezed extracts (−18 °C) were reduced to 20 times their original concentration by lyophylization. The lyophylized extracts were dissolved in 0.1 M HCl (500 μl) and filtered through a nylon membrane filter (13 mm, 0.45 μm, Chromatography Research Supplies, Adison, USA). Concentrations of 16 proteinaceous amino acids, namely, aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, valine, methionine, phenylalanine, isoleucine, leucine, lysine and proline were determined using an HP 1100 liquid chromatograph (Hewlett Packard, Wilmington, DE, USA) with an FLD HP 1100 fluorometric detector, operating at 450 nm (Ex = 340 nm), and a Zorbax Exlipse AAA Rapid Resolution (4.6 × 150 mm, 3.5 μm paticle sizes, Agilent Technologies, USA) column. A linear gradient mobile phase profile, consisting of 40 mM Na_2HPO_4, pH 7.8 (solvent A) and ACN/MeOH/water 45:45:10 (v/v) (solvent B), was applied at a flow rate of 2.0 ml min^{-1}. The procedure and gradients produced were as follows: 0% B (1.9 min), 0–57% B (16.2 min), 57–100% B (0.7 min), 100% B (3.5 min), 100–0% B (0.9 min) and 0% B (2.8 min). The column was equilibrated for 5 min under initial conditions prior to injection of each sample. The column temperature was 40 °C. Precolumn derivatization with *o*-phthalaldehyde (OPA) was used for determination of amino acids from soil extracts (Formánek et al., 2005). Mineral nitrogen forms (NH_4^+ and NO_3^-) were extracted by 1% K_2SO_4 (soil/extractant = 10/50 w/v) and concentrations of NH_4^+ were determined by spectrophotometry, in which the ammonium ions react with sodium salicylate and chlorate ions in the presence of sodium nitroprusside (ČSN ISO 7150-1, 1994). NO_3^- was determined by spectrophotometry with sulphosalicylic acid (ČSN ISO 7890-3, 1994).

2.4 Statistical analysis

Nonparametric Kruskal-Wallis ANOVAs *plus* Dannet's *t*-tests were used to analyze the data for every sampling or incubation interval. Oneway Anova was used to compare the total net microbial release of N (Statistica 7.0).

3. Results

Ammonium nitrogen concentrations ranged from 24.7 to 78.3% of the total available nitrogen in the moderately mown meadow soil. Amino acid derived N ranged from 9.5% at the beginning of the vegetation season to 75.3%

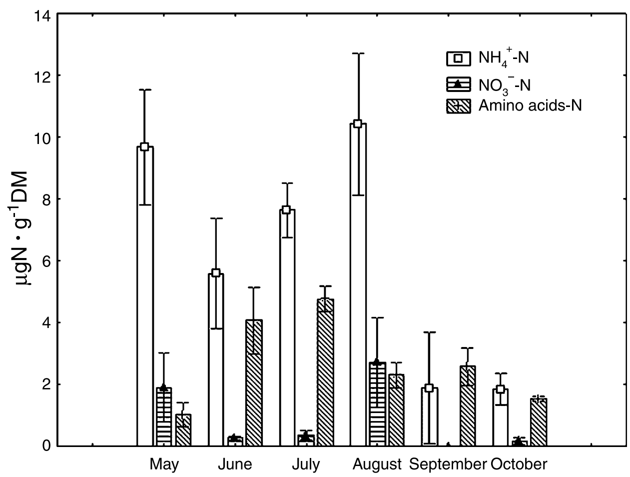

Fig. 1. Seasonal dynamics of concentrations of bio-available nitrogen forms in the Ah horizon in the moderately mown meadow. Mean ± 1SE ($n = 3$–5)

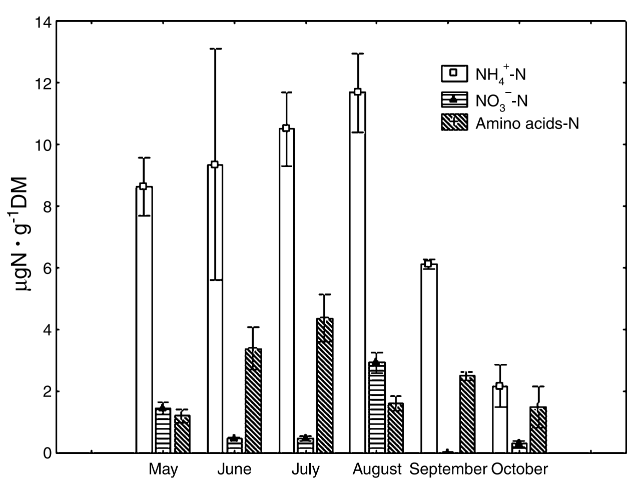

Fig. 2. Seasonal dynamics of concentrations of bio-available nitrogen forms in the Ah horizon in the un-mown meadow. Mean ± 1SE ($n = 3$–5)

in September (Fig. 1). On the un-mown meadow, the ammonium nitrogen concentrations ranged from 56.1 to 75.8% of the total available nitrogen with amino acid derived N increasing from 9.8% in August to 35.5% in October (Fig. 2). Nitrate nitrogen concentrations were the lowest of all N forms. Nonparametrical testings were carried out for the individual sampling periods and each of nitrogen forms: no significant ($P > 0.05$) differences were confirmed for NH_4^+-N, NO_3^--N and amino acid-N when data from the un-mown meadow were compared with those from the moderately mown meadow.

Ammonium was the main N form in the moderately mown meadow. There were much higher levels of NH_4^+-N in the soil due to microbial action, while the amount of NH_4^+-N lost due to sum of undistinquished processes (plant uptake, gaseous losses and/or leaching) was greater than for all other N forms (Fig. 3). The highest total net loses of microbiologically released NH_4^+-N from the Ah horizon were detected at the end of summer. The net release/immobilization of NO_3^--N and amino acid-N on moderately mown meadow was low, the total net losses of these N forms by group of unseparated processes were low, and conversely an input from other sources was evident in the case of these nitrogen forms.

The un-mown meadow exhibited a different pattern of behaviour of N forms. Total net N release by microbial activity was evident only from May to August, while it seems that plant nutrition was dependent on the utilization of ammonium nitrogen in this period (Fig. 4). In several cases, amino acid or nitrate N concentrations were increased by processes other than microbial activity. Total bio-available nitrogen was immobilized by soil micro-organisms from mid-summer to the end of the vegetation period, and it seems that plants could only take up nitrogen from amino acid sources by the end of summer.

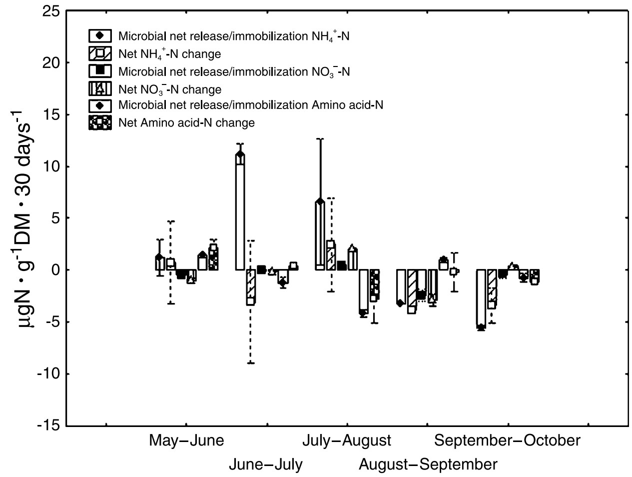

Fig. 4. Net microbial release/immobilization of bio-available N forms and changes to the net total bio-available N forms in the Ah horizon in the un-mown meadow. Mean ± 1SE ($n = 2$–5)

On the moderately mown meadow, the total net microbial release of N was significantly ($P = 0.047$) higher during the studied period (May–October) than for the un-mown meadow (38.1 *versus* 5.13 $\mu g\,N \cdot g^{-1}$ dry soil, respectively). The total seasonal balance of net microbial N release/immobilization was positive only for ammonium N in both the mown and un-mown meadows (41.0 and 9.4 $\mu g\,N \cdot g^{-1}$ dry soil, respectively), while it was negative for nitrate (-2.1 and $-3.3\ \mu g\,N \cdot g^{-1}$ dry soil, respectively)

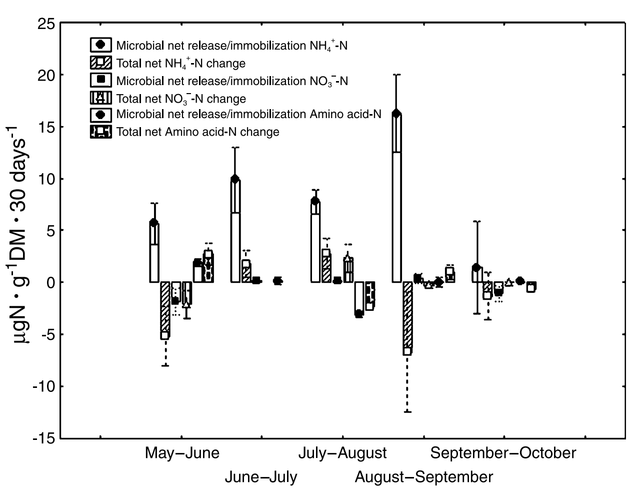

Fig. 3. Net microbial release/immobilization of bio-available N forms and changes to the net total bio-available N forms in the Ah horizon in the moderately mown meadow. Mean ± 1SE ($n = 2$–5)

Table 3. Bio-available amino acid concentrations in the Ah horizon from May to October in the moderately mown and un-mown meadows ($\mu g \cdot g^{-1}$ dry soil; Mean ± SD; $n = 28$)

Amino acid	Mown	Un-mown
Aspartic acid	1.58 ± 0.98	2.02 ± 2.25
Glutamic acid	5.18 ± 3.30	3.98 ± 3.02
Serine	0.62 ± 0.36	0.65 ± 0.44
Histidine	0.10 ± 0.07	0.15 ± 0.24
Glycine	0.73 ± 0.36	0.97 ± 1.09
Threonine	0.51 ± 0.45	0.64 ± 0.61
Arginine	3.02 ± 2.53	2.73 ± 2.48
Alanine	1.25 ± 0.71	0.99 ± 0.67
Tyrosine	0.19 ± 0.29	0.18 ± 0.27
Valine	0.35 ± 0.31	0.32 ± 0.32
Methionine	0.68 ± 0.51	0.64 ± 0.49
Phenylalanine	0.50 ± 0.54	0.48 ± 0.53
Isoleucine	0.84 ± 0.83	1.13 ± 1.00
Leucine	0.25 ± 0.13	0.29 ± 0.16
Lysine	0.28 ± 0.21	0.65 ± 1.13
Proline	0.29 ± 0.30	0.40 ± 0.62

and amino acids N (-0.9 and $-2.7\,\mu g\,N \cdot g^{-1}$ dry soil, respectively). Glutamic acid, arginine and aspartic acid had the highest concentrations of the 16 amino acids analyzed during the course of the season in both meadows (Table 3). The total net release of individual amino acids during the 30-day periods ($n = 27$–33) showed a mean net balance close to zero (from -0.83 to $+0.12\,\mu g \cdot g^{-1}$ dry soil), with the exception of glutamic acid in the moderately mown meadow ($-1.77 \pm 4.35\,\mu g \cdot g^{-1}$ dry soil; mean $\pm$ SD). No significant ($P > 0.05$) differences were found for the concentrations of individual amino acids and/or their release/immobilization by microbial action between both meadows throughout the whole experiment.

4. Discussion

It is evident from the results obtained in this experiment that there are differences in nitrogen turnover between the moderately mown and un-mown meadows. The moderately mown meadow showed a pattern of increased N cycling and, thus, has a plant community that does not need to take up amino acids in the course of the vegetation period. Element cycling was slower in the un-mown meadow. In this site, plant uptake of amino acids in the latter half of the vegetation season occurs as a result of depleted levels of available inorganic forms of nitrogen and increased immobilization of mineral N by microbes. These effects are exacerbated by the likely drier conditions occurring at the end of summer, in which mineral N compounds adsorbed to clay minerals are not likely to be available to the plants. Thus, in this period of elevated climatic and edaphic stress, nitrogen can be taken up in its organic form. This uptake is very short-term and quantitatively of little importance.

Total N uptake by the plant community was 20.0 (moderately mown meadow) and $28.8\,g \cdot m^{-2}$ (un-mown meadow) for the first half of the 2004 vegetation period. Based on the N:P ratio in the total above ground biomass, the plant communities of both meadows were limited by N (Holub and Tůma, 2005). A total of 0.98 and 0.57 g NH_4^+-$N \cdot m^{-2}$ were released from the upper 7 cm of the Ah horizon in the moderately mown and un-mown meadows, respectively, from mid-May to mid-July. This is a much lower amount than what the plant community needs. There was a net microbial release of $<130\,mg \cdot m^{-2}$ of amino acids N and net NO_3^--N immobilization in both meadows during this period. These bio-available amino acids were probably not used by the plant community (Figs. 3 and 4) even when the mineralization rate was not sufficient to cover plant needs; further studies on possible physiological restrictions to plant uptake will be performed. It is generally assumed that amino acids uptake is very important to plants in conditions where the mineralization rate is not sufficient and amino acids are the dominant form of available nitrogen (Kielland, 1995).

There are some disadvantages inherent to procedure used in this study to measure net N release/immobilization by microbial action. Plant roots clipped in the PVC cylinders can still take up mineral or amino acid N (Bloom and Candwell, 1988; Falkengren-Grerup et al., 2000); this uptake may result in an underestimation of net N mineralization. Also, root clipping can cause release of amino acids to soil, and thus overestimate their actual concentrations and mineralization. A 30-day incubation period to assess N release or immobilization by microbial action was used. This is a very long incubation time, especially for amino acids, during which turnover may be very high. Usually, a large part of the N potentially available to plant uptake is present in the soil for a long-time on exchange sites of organic and inorganic colloids (Cresser et al., 1993; Violante et al., 2002). Inorganic colloids are common in both studied meadow ecosystems. In addition, partitioning of the nitrogen pool into the microbial biomass could not be obtained, because tracers were not used. A difference up to 21.1% (w_t/w_t) in moisture content between the studied plots should, due to a greater change in partial oxygen pressure, affect the rate of soil denitrification (Tiedje, 1988; Parkin, 1987). As regards the role of denitrification, the adopted methodology of Raison et al. (1987) does not include such analyses as in their case it was of negligible significance. Being aware of a great diversity within particular grass species, the introduced grass communities of the study site are generally genetically predisposed for nitrate uptake (Larcher, 1988), which was immobilized by microbial processes in the first period of the vegetation season. The organic and >10 cm layers were not considered in this study; if so, they could have improved the calculated N balance between plants and soil. Sieving of soil samples before the extraction should also be considered. Such treatments obviously result in releasing a larger amounts of organic compounds into a reaction mixture. Nevertheless, there has been no other chance for removing both skeletal particles and roots from the samples which were treated afterwards. The roots had to be removed in order to avoiding contamination of the solutions.

The N limitation of plants can be judged only if the preference of the plant community for certain N types is known. It is also important to know which N form is available in the soil. Only when both of these conditions are fulfilled is it possible to think critically about the N/P ratio and its impact on Liebig's Law of the Minimum. It seems that the amount of available N being released in the soil of both meadows is inadequate to provide sufficient plant nutrition. The forbs present on the un-mown meadow have a higher N uptake and strongly prefer nitrogen in the mineral form, compared to grasses, which are more adapted to amino acid uptake (Falkengren-Grerup et al., 2000). Nevertheless, plant preference for nitrates was not taken into account in the study of Falkengren-Grerup et al. (2000). It is necessary to have direct information concerning the preference of particular N forms by individual plant species to understand better the role of different bio-available N forms in plant nutrition of these ecosystems.

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