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SELECTED AMINO ACIDS UPTAKE BY MICROORGANISMS IN SOIL OF DIFFERENTLY MANAGED MOUNTAIN MEADOWS

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Abstract

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This work was aimed at determination of the effect of 13 years abandonment of previously long-term mown mountain meadows on uptake of L-glutamic acid ($^{14}CO_2H[^{14}CH_2]_2[NH_2]^{14}CO_2H$) and L-alanine ($^{14}CH_3{}^{14}CH[NH_2]^{14}CO_2H$) by microbial community of Ap horizon (3–13 cm). The study plots has been located near to the experimental stand "Bílý Kříž" which is located in the Moravian-Silesian Beskids Mountains (N 49°30'17", E 18°32'28"), on a slope with an elevation of 825–860 m a. s. l. and southeast orientation, and soil classified as an Oxyaquic Hapludalf. The study was performed to test method for measurement of ^{14}C-labelled amino acids uptake by soil microbial community and to increase knowledge on particular processes of *N*-transformation in soil of these ecosystems. The results obtained in this work showed that 13 years abandonment of mountain meadow did not significantly ($P > 0.05$) influence rate of glutamic acid or alanine uptake by soil microbial community. Further research including determination of amino acids use by soil microbial biomass with expression of their partitioning between production of new microbial biomass and energy metabolism is necessary.

soil, amino acids, uptake, glutamic acid, alanine, meadow, management

Socio-economic changes in the Czech Republic towards the end of the last century have lead to considerable changes in environmental conservation management of nonforest ecosystems. The local population has moved away from mountainous regions where traditional farming and mowing of meadows was practised. Abandonment of meadows has initiated secondary succession with re-colonizing shrubs and tree species (Formanek *et al.*, 2008a). Plant biomass in abandoned or uncut meadows is steadily increasing. Changes in below- and aboveground plant biomass due to abandonment bring about different physical-, chemical- and biological-soil properties, for example, soil moisture, amount of O_2, temperature and amount of nutrients (Huhta, 2001; Vranova *et al.*, 2007; Formanek *et al.*, 2008 a, b; Vranova *et al.*, 2009 a,b). Some studies have shown that reducing or abandoning management e.g. enhance soil erosion (Paldele, 1994; Körner, 1999; Tasser *et al.*, 1999, 2003; Coppus *et al.*, 2003). Organic material on the soil surface increases while the pH decreases, resulting in a shift from mull to moder humus forms (Seeber and Seeber, 2005). The measured soil chemical variables were linked not only to the natural fertility of the site, but also to long-term management inputs. These relationships between species richness, soil and management were found by Schellberg *et al.* (1999), and Myklestad (2004).

The abandonment of meadows slows down nutrient cycling. In our previous works, we have evaluated the effects of thirteen years abandonment of previously traditionally mown mountain meadows on total carbon and nitrogen content in soil and its stand heterogeneity, activity of selected enzymes, nitrogen mineralization, availability of amino acids in soil, cleavage of proteins or kinetics of heterotrophic

soil respiration (Formánek *et al.*, 2006a,b; Vranová *et al.*, 2007; Formánek *et al.*, 2008a, b; Vranová *et al.*, 2009a, b).

In this study we aim to test a method for measurement of uptake of ^{14}C-labelled amino acids by soil microorganisms and to determine the effect of thirteen years abandonment of previously long-term mown mountain meadows on kinetics of selected amino acids uptake by microbial community from Ap horizon of these ecosystems. As well as comparison of glutamic acid and alanine uptake on differently managed mountain meadows was performed as a new parameter to more understand the processes related to N-transformations in soil of these ecosystems.

MATERIAL AND METHODS

The experimental stand "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. l. and southeast orientation. The local subcontinental climate is characterised by the mean annual air temperature of 4.9 °C, mean relative air humidity of 80% and by mean annual precipitation of 1100 mm. Number of days with snow cover is 160 per year. The experimental meadow (1 ha) was originally mowed regularly once a year, the hay removed and stored as winter feed for livestock. This traditional management ceased 13 years ago on half of the meadow (abandoned meadow), whilst the remaining half has been permanently mown (once a year). Mown meadow plant community belongs to the *Nardo-Callunetea* class PREIZING (1949) (Zelená in Formánek *et al.*, 2008a), mowing treatment in season 2006 was applied there on the 3rd of August. Abandoned meadow is characterised by higher representation of forbs and community belongs to the *Molinio-Arrhenatheretea* class TÜXEN (1937) (Zelená in Formánek *et al.*, 2008a).

The soil is classified as an Oxyaquic Hapludalf with the following sequence of horizons Oi-Oa-Oe-Ap-BAt-Bt1-Bt2-Cg (USDA-NRCS, 2006). Mixed samples (1 sample = 5 sub-samples randomly taken within whole plot forming together 0.5 kg) were taken from both meadows in depth of 3–13 cm (Ap horizon) in August 2006. Sampling was performed using boring bar. After transportation in cool box, the samples were sieved through 5 mm mesh size and stored at 6 °C untill analyses. Selected physical, physical-chemical and chemical properties of Ap horizon of both meadows were calculated from 3–8 repetitions on each of the meadows (expressed as Mean ± SD) are shown in Tab. I.

Amino acids uptake was measured using modified procedure described by Vinolas *et al.* (2001). For these purposes two amino acids, L-glutamic acid ($^{14}CO_2H[^{14}CH_2]_2[NH_2]^{14}CO_2H$) and L-alanine ($^{14}CH_3{}^{14}CH[NH_2]^{14}CO_2H$) were used. One hundred-fifty microlitres of alanine solution (15.7–717 µM) or glutamic acid solution (9.2 to 588.3 µM) was briefly mixed with 0.3 g wet soil in a 15-ml polypropylene tube. Within these ranges, the natural concentrations of "free" L-glutamic acid and L-alanine in Ap horizon of the studied meadows was determined (Formánek *et al.*, 2008a, b). To each sample tube, 1M NaOH (1 ml) was added to capture respired $^{14}CO_2$ and the sample tubes were sealed with a gas-tight stopper and incubated at 14.5 °C for 3 hours. Subsequently, the soils were extracted with 0.5M K_2SO_4 to remove unutilized amino acid (10 min, 200 revolutions min^{-1}) followed by centrifugation (3,550 rpm, 20 min) and counting the ^{14}C label remaining in the supernatant solution.

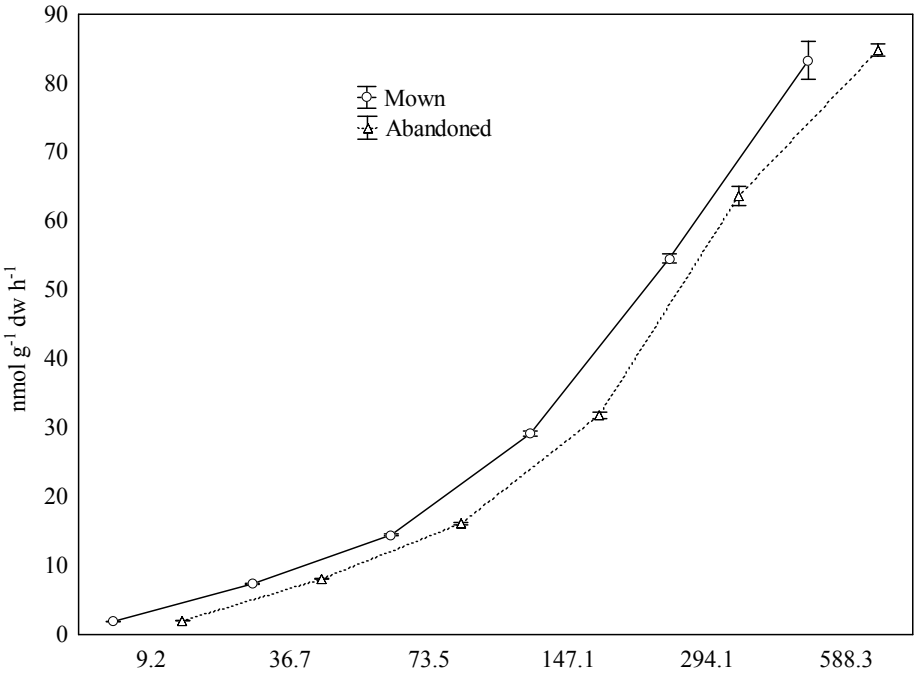
As preconditions for parametric testing (test normality and homogeneity of variance) were not fulfilled to find differences between meadow treatment (mown *versus* abandoned), statistical analysis was performed through the nonparametric Kruskal-Wallis ANOVA and Dannet's *t*-test. All statistical analyses were undertaken using the Statistica 9.0 program.

RESULTS AND DISCUSSION

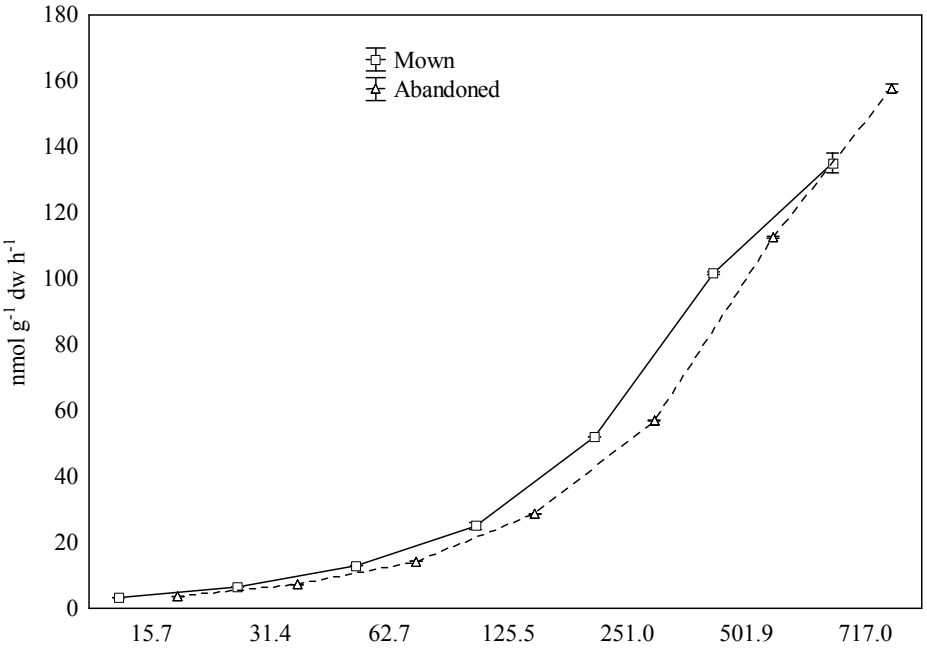
Results obtained in this work showed that 13 years abandonment of the traditional management of mountain meadows did not significantly ($P > 0.05$)

I: *Selected physical, physical-chemical and chemical properties of Ap horizon on moderately mown and abandoned meadow (Mean ± SD; n = 3–8). The different letters mark significant (P < 0.05) differences between the meadows.*

Soil properties	Moderately mown	Abandoned
Clay (%)	18.60 ± 3.4^a	19.93 ± 2.3^a
Silt (%)	26.0 ± 3.7^a	27.1 ± 7.0^a
Sand (%)	55.40 ± 6.5^a	52.93 ± 9.1^a
pH (H_2O)/pH 0.01 M $CaCl_2$	4.30 ± 0.45^a/3.80 ± 0.19^a	4.27 ± 0.42^a/3.78 ± 0.18^a
CEC_{eff} ($mmol_c$ kg^{-1})	159.34 ± 2.28^a	184.85 ± 10.01^b
C_t (%)	4.76 ± 0.76^a	4.77 ± 0.71^a
N_t (%)	0.29 ± 0.06^a	0.33 ± 0.09^a
C/N	16.91 ± 3.58^a	15.24 ± 3.14^a
C-HA and C-FA (%)	0.50 ± 0.05^a and 0.72 ± 0.05^a	0.57 ± 0.11^a and 0.77 ± 0.05^a



1: *Concentration-dependent ^{14}C-labeled glutamic acid uptake by microorganisms of Ap horizon (3–13 cm) of moderately mown and abandoned mountain meadows of the same locality (mean ± SE, n = 6–9)*



2: *Concentration-dependent ^{14}C-labeled alanine uptake by microorganisms of Ap horizon (3–13 cm) of moderately mown and abandoned mountain meadows of the same locality (mean ± SE, n = 3)*

influence rate of glutamic acid or alanine uptake by microbial community of Ap horizon (Fig. I, II).

Soil of both meadows differs significantly ($P < 0.05$) only in cation exchange capacity (CEC). The moderately mown meadow was shown to possess accelerated N-turnover and higher net ammonization whereas increasing immobilization of N was determined in Ap horizon of abandoned meadow (Formánek *et al.*, 2008 a, b). Within the particular processes of N-transformation in soil, proteolytic activity was found to be not significantly ($P > 0.05$) higher in Ap horizon of abandoned meadow (Vranová *et al.*, 2009b). Glutamic acid was found to be dominant in pool of "free" amino acids in soil of both studied meadows and was significantly higher on abandoned plot only when data from season

2004 and 2005 were pooled together (Formánek *et al.*, 2008 b). Alanine concentration when extracted by 0.5 M ammonium acetate was found to be fifth highest of sixteen amino acids under study (Formánek *et al.*, 2008b). Based on summary of these knowledge it is possible to say, the abandonment of mountain meadow for 13 years affects N-cycling in these ecosystems, nevertheless some of the particular processes of N- transformation remins not to be significantly affected.

In previous studies was found higher C-mineralization and microbial biomass on abandoned meadow compared to its moderatelly mown counterpart (Kafka, 2005; Vranová *et al.*, 2009a). On the other hand, the amount of C_{ox} and N_t was quite a similar on both of the meadows including relative stand heterogeneity (Coefficient of Variation in % determined within whole plots) of these soil characteristics in studied Ap horizon; only, slightly favourable C/N ratio was observed on the abandoned meadow horizon (Vranová *et al.*, 2007). Composition of soil microbial biomass on studied meadows may also affect amino acid uptake (Akerblom *et al.*, 2007).

Amino acid uptake in soils may be altered by soil properties. For example, this uptake was approximately twofold lower in eroded soil with approximately twofold lower microbial biomass compared with non-eroded soil (Vinolas *et al.*, 2001). The amino acid uptake rate by soil microorganisms is also dependent on the type of amino acid for example, Jones and Kielland (2002) reported that the order of amino acid type uptake was as follows: glutamate > lysine > glycine. In other study, Jones and Hodge (1999) found that uptake rate of amino acids in soil followed the order glutamate > glycine > lysine.

Individual amino acids differ in their uses in microbial cell metabolism, and their partitioning between use in production of new microbial biomass and energy metabolism. The use of amino acid-C for respiration is reported to range between 10–40 % (Jones, 1999; Jones and Hodge, 1999; Vinolas *et al.*, 2001b; Owen and Jones, 2001; Jones *et al.*, 2005; Kemmitt *et al.*, 2008).

It is possible to conclude that 13-year abandonment of formely mown mountain meadow does not significantly alter amino acid uptake by soil microbial community even total N-cycling was decelerated due to this treatment. More detailed view to these processes, especially comparison of uptake rates for microorganisms versus plants is necessary to better understand amino acid transformation in soils of these ecosystems.

SUMMARY

Results of this work shown that 13 years abandonment of the traditional management of mountain meadows did not significantly ($P > 0.05$) influence rate of glutamic acid or alanine uptake by soil microbial community. This study represents a preliminary study on the uptake of amino acids by soil microbial community of differently managed meadows. Further research when determination of amino acids use by soil microbial biomass with expression of their partitioning between production of new microbial biomass and energy is performed is needed to better understand microbial uptake and utilization of amino acids in soil.

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