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AVAILABLE NITROGEN IN THE SURFACE MINERAL LAYER OF NATURAL MEADOWS

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Abstract

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Based on a greenhouse experiment, we evaluated nitrogen availability in the surface mineral layer of soil under various natural meadow stands by analyzing the following soil characteristics: total organic C, total N, initial content of easily available N inorganic forms, mineralized N content obtained by aerobic and anaerobic incubations and A-value. The experiment was performed on a test plant and through the application of urea enriched with 5.4% ^{15}N. The investigated soils under natural meadows are characterized with comparatively high mineralization intensity and high N availability indices. Contents of mineral N produced by aerobic incubation and the intensity of the mineralization correlate with the total organic C in the soil and the total N in the soil. Correlation of the availability index of the soil N produced by aerobic incubation with the total organic C and the total N in the soil under natural meadows is almost linear (r = 0.9981 and r = 0.9997, respectively). Contents of mineral N produced by anaerobic incubation, as well as the corresponding N availability and mineralization intensity indices correlate poorly with the mentioned parameters. Efficiency of nitrogen utilization from the applied N-fertilizer by the test crop varies within a wide range of values and correlates with the biomass yields of the test crop.

Keywords: nitrogen; incubation; mineralization; nutrient availability; C to N ratio; total organic C; A-value.

INTRODUCTION

Nitrogen (N) is the most important essential nutrient for all living organisms on the Earth. It is present in a great number of complex organic molecules and plays a very important role in their activities. An adequate supply of nitrogen is essential for sustainable productivity of natural meadows. Humidity regimes and nitrogen amount in the soil influenced dry matter yield of meadow phytocenosis. Botanical composition and plant species number depended on agrochemical characteristics of the soil and management activities (Skuodiene *et al.*, 2016). The long lasting storages of available forms of this element cannot be formed in the soil. Potentially available N is released during the vegetation season through mineralization of organic compounds in soil, under the influence of microorganisms. Mineralization, total and mineral nitrogen decreased with depth (Romano *et al.*, 2016).

Ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) are the main nitrogen forms absorbed by plants, beside some organic N compounds (Li *et al.*, 2013). More than 90% of soil N is in organic form, and about 40% of the total soil N is in the form of polymers, like proteins and peptides (Schulten and Schnitzer, 1997). Intermediate products of complex organic nitrogen compounds may be absorbed by plants. Actually, it had been found that the most important source of available N for a possibly wide variety of plant species, including those in natural and in managed ecosystems, are amino acids (Jämtgård *et al.*, 2010). During a vegetation season, an enormous immobilization is hidden by the prevailing mineralization processes. Thereby, the types of vegetation and plant remnants have a strong effect on the microbiological activity of soil.

Although N availability in soils has been the subject of many studies, there is not an generally accepted and completely reliable method for its assessment. Namely, N availability indices, developed for one mode of utilization of the soil surface (e.g. for cultivated land crops), do not give a good interpretation of another mode of soil utilization (e.g. for forest species) (Scott *et al.*, 2005). Obviously, the quantity of available N in soil is an important ecological factor. Soils under meadows and grazing land in Serbia occupy great areas, with moderate productivity, which must be remediated by fertilization (Simić *et al.*, 2016). Additionally, the condition of the meadows and grazing land is unsatisfactory because the productive potentials are utilized insufficiently or not at all (Simić *et al.*, 2016)

The aim of our study, apart from the assessment of available soil N in the surface mineral layer of various types of meadow soils, was to establish the relationship between available N reserves and some agrochemical characteristics of the meadow soils. Thereby, we shall contribute to the information on N availability in various meadow soils of the temperate climatic zone of Europe, useful for understanding of nitrogen dynamics, necessary for the appropriate and adequate management and meadow practice. This is to be achieved by determining the quantity of available N at the beginning of growing season; evaluating potentially available N by different methods; determining nitrogen absorbed by the experimental crop.

MATERIAL AND METHODS

Sites and Soils Used in the Experiment

For this study we collected 5 soil samples from 5 different locations (Tab. I). Soil samples were collected in mid-March from the surface mineral portion of the soil layer, from the depth of 0–20 cm. From the collected soil samples, roots and non-decayed remnants of aboveground grass parts were removed. All the five soils, according to the texture ISSS classification (Baize, 1993) belong to the textural class of light clays. We used the collected soil samples to establish the vegetation experiment and laboratory testing. All research results relate to the features of the so-called fine soil, so that they are comparable with other authors' results. For the determination of agrochemical features and available N, soil samples were air dried, ground and sieved through a 2 mm sieve.

Experimental Design

In our research we employed the A-value method according to the description given by Fried and Dean (1952). According to Fried and Broeshart (1974), the magnitude of the "A" value, indicating the reserve of nitrogen available to plants, does not depend on the dosage of fertilizer; this is a constant value and the nitrogen fertilizer does not have an effect on an increase in the nitrogen mineralization rate in soil. The A-value concept is based on the assumption that major soil elements are absorbed by plants, proportionately to the content of their available forms in the soil. Knowing the dosage of fertilizer applied to the soil and the amount of marked nutrient absorbed by plants, one can determine the value of available nitrogen in the soil (Fried and Dean 1952):

$$A = \frac{Uc \times Ts}{Tc}$$

where:
A ... amount of available nitrogen in the soil (kg ha^{-1});
Ts .. amount of applied fertilizer;
Tc .. amount of nitrogen absorbed from fertilizer in the aboveground part of a plant;

I: *Soils utilized in the experiment and their textural composition*

Soil type (by FAO soil classification)	Location, average annual temperature, average annual rainfall	Granulometric composition (%)			pH in 1M KCl	δ^{15}N (‰)
		Sand 0.02–2.0 mm	Silt 0.002–0.02 mm	Clay <0.002 mm		
Chernozem, formed on loess, carbonated, deeply	Zemun 11.7 °C 669 mm	41.9	29.8	28.3	6.9	+0.26
Eutric cambisol, formed on lake sediments, lessived	Ralja 10.7 °C 649 mm	29.2	29.9	40.9	4.4	+28.76
Calcaric cambisol, typical, shallow	Bileca 12.2 °C 1620 mm	36.0	23.9	40.1	5.3	-27.40
Eutric fluvisol, carbonated, deeply	Obrenovac 11.0 °C 662 mm	30.4	29.8	39.8	5.7	+34.52
Eutric planosol (pseudogley), lowland, deeply	Debrc 11.0 °C 662 mm	31.1	31.6	37.3	5.5	+8.48

Uc . amount of nitrogen absorbed from the soil in the aboveground part of a plant.

For the purpose of the greenhouse experiment, air-dried soil was manually crumbled and the required quantity was weighed (2 kg of soil per pot). Urea, $(NH_2)_2CO$, was applied, enriched with 5.4% ^{15}N. It was applied at the concentration of 50 mg N kg^{-1} soil. Along with urea, 153.4 mg of KH_2PO_4 and 49.8 mg of K_2SO_4 were applied (the ratio of the applied macronutrients, $N:P_2O_5:K_2O$, was 1.0:0.8:0.8). The fertilizers were uniformly applied in the form of a solution before filling the pots with soil. The experiment was carried out in three replications.

The experimental crop was oat (*Avena sativa* L., cv. Kondor). This crop was used for the determination of the A value for two reasons. First, contrary to some perennial grass species, oat does not manifest any ^{15}N discrimination during uptake, distributing it evenly across the plant organs. Second, soils have a wide range of physicochemical characteristics, above all the pH range (Tab. 1), that makes the development of oat possible, but could compromise the growth of other test species. To obtain accurate measurements of ^{15}N uptake by the tested crop, we determined $\delta^{15}N$ values in the investigated soils (‰ deviation of ^{15}N from the standard referent material – air nitrogen; Tab. I).

In each pot, 13 oat seeds were sown. After 7 days, the number of plants was reduced to 10. Soil humidity in vegetation pots was maintained at approximately 0.6–0.8 of maximum water holding capacity for each soil type. Two months after germination, the experiment was terminated. Above-ground parts of the crop (straw) were separated from roots and analysed separately. The roots were carefully separated from soil. According to the recommendations of IAEA for plant material preparations for isotope analyses (Axmann *et al.*, 1990), the plant material was dried in oven for 18–24 h at 70 °C, until constant mass was achieved. The material was ground subsequently.

Analytical Methods and Methods of Nitrogen Availability Evaluation

pH values were determined in 1M KCl (1:2.5 w/v). Total organic C in the soil was determined by soil sample mineralization with boiling dichromate and sulfuric acid mixture and subsequent titration of excessive dichromate with Mohr salt solution (Pansu and Gautheyrou, 2006). Total N in soil and plant samples was determined by semi-micro Kjeldahl method (Pansu and Gautheyrou, 2006).

The content of easily available N forms in soil was determined after extraction with 2M KCl (1:10 ratio) and 30 min shaking using a procedure described by Keeney and Nelson (1982). The suspensions were filtered through Whatman 42 filter paper, and NH_4^+-N and NO_3^--N concentrations were determined by steam distillation, with the addition of MgO and Devarda's alloy, and the released NH_3 was collected in an indicator solution of H_3BO_3. The concentrations of NH_4^+–N and NO_3^-–N were determined by titration with standard H_2SO_4.

Quantities of available N, produced in soil under aerobic and anaerobic conditions, were determined according to the procedures described by Keeney and Bremner (1966). The intensity of mineralization (IM) was calculated from the following formula:

$$IM = \frac{N_{min.} - N_{initial}}{N_{total}}$$

where:

N_{min}.....quantity of mineralized N without subtraction of the initial content of available mineral N (for the anaerobic incubation of NH_4^+–N content);

$N_{initial}$...initial content of available mineral N (for the anaerobic incubation of NH_4^+–N content);

N_{total} ....total content of N in soil.

The nitrogen availability indices (ANI) were calculated from the following formula:

$$ANI = \frac{N_{min} - N_{initial}}{N_{initial}}$$

Determination of Isotope-Labelled Nitrogen in the Experimental Crop

Isotope measurements ($^{14}N:^{15}N$) of the plant material were performed in two replications in full compliance with IAEA methodologies (Axmann *et al.*, 1990, Chen *et al.*, 1990): conversion of sample nitrogen into the ammonium form by an oxidation-reduction reaction, further converting of NH_3 into N_2 by the reaction with alkaline hypo-bromide and subsequent measurement on a CEC 21-620-A California mass spectrometer (Consolidated Engineering Corporation, Pasadena, California). For the nitrite and nitrate nitrogen forms, the classic procedure involves the reduction to NH_3 by Devarda's alloy during steam distillation, after previous removal of NH_3 present in the same sample by alkaline distillation with MgO (Chen *et al.*, 1990). Classic equipment for the process of N_2 preparation for the mass spectrometer includes a system based on degassing of liquid samples under vacuum and their subsequent mixing in Rittenberg Y-tubes.

The data presented in the tables are arithmetic means and standard deviations (SD) of the results obtained from three independent analyses of each investigated parameter of N availability. The results were statistically analysed (ANOVA) using the Tukey's test to check for significant differences between means ($P \leq 0.05$). Coefficients of correlation between nitrogen availability parameters obtained by different methods and statistical significance of the coefficients were determined by Microsoft Excel 2010 for Windows.

RESULTS

Vegetation Experiment in Pots

The highest yield of the aboveground biomass (straw) was obtained on the soil of Chernozem type (19.27 g per pot), and the lowest on Calcaric Cambisol (7.07 g per pot). At the same time, the test crop is characterized with big variations in produced biomasses of the aboveground part (straw mass) and the root (Tab. II). The ratio between aboveground part biomass and the root biomass of the test crop is highest on Fluvisol (11.55), i.e. the aboveground part is significantly more developed than the root on this soil. On the remaining soils, this ratio varies between 3.69 and 7.26. The crop grown on Chernozem produced the highest average straw and root biomasses. The biomass yield of the test crop correlates poorly or not at all with the total C and N contents in the investigated soils.

Nitrogen concentration in the test crop varies within a rather wide range of values (Tab. II). The lowest values were found in oats grown on Planosol and Eutric Cambisol, and the highest on Calcaric Cambisol. The highest uptake of nitrogen by the test crop was found on Chernozem (193.16 mg N per pot), and the lowest on Eutric Cambisol (93.36 mg N per pot). Nitrogen concentrations, both in the aboveground part (straw) and in the whole test crop (straw + root) are in very strong correlation with both organic C in the soil (r = 0.9621 and r = 0.9573) and total N in the soil (r = 0.9672 and r = 0.9615).

Inorganic Forms of Available N in Soil

Although the contents of total C and N in the investigated soils vary (Tab. III) within comparatively wide range between 1.08–4.65 % and 0.105–0.442 % respectively, the correlation between these two parameters is very strong (r = 0.9973). The ratio C/N varies between 9.04 and 10.52, which is a common finding for the soils under natural meadows. Ammonium form is the dominant form of easily available mineral N. The highest contents of the available mineral N were found in Chernozem (41.65 mg N kg^{-1} soil), and the lowest in Fluvisol (14.53 mg N kg^{-1} soil).

In the investigated Calcaric Cambisol the highest amounts of mineralized and nitrified N were obtained by aerobic incubation (Tab. III). Due to this, the mineralization intensity (IM) and N availability index (ANI) are the highest in this soil (2.15 and 2.65, respectively), while in the other investigated soils they are lower. In Chernozem, the highest quantities of NO_3^-–N were obtained by aerobic incubation. However, aerobic incubation in brown Eutric Cambisol yielded very low concentrations of mineralized and nitrified N, which were lower than the initial values before the incubation. Thus, the mineralization intensity and the nitrogen availability index have negative values (–0.77 and –0.23). The contents of mineral N produced by aerobic incubation show high correlation with N concentrations in the test crop straw (r = 0.7963) and in the whole test crop (r = 0.7851). Subsequently, the correlations with mineralization indices obtained for aerobic incubation are strong (r = 0.8133 and r = 0.7949), while the correlations with the indices of N availability (ANI) are very strong (r = 0.9664 and r = 0.9605). Mineral N contents obtained by aerobic incubation are in very strong correlation with the total organic C in the soil (r = 0.9264) and the total N in the soil (r = 0.9105).

II: *Parameters of plant yield and N content in the experimental crop*

Soils	Plant part	Concentration of nitrogen (%)	Crop dry mass (g per pot)	N uptake (mg N per pot)
Chernozem	straw	0.826±0.022	19.27±1.01	159.23±9.37
	root	0.650±0.083	5.22±1.15	33.93±3.04
	total	0.789	24.49	193.16
	straw/root ratio	-	3.69	-
Eutric cambisol	straw	0.692±0.018	11.03±1.27	76.36±8.44
	root	0.720±0.068	2.36±0.73	17.00±5.91
	total	0.697	13.39	93.36
	straw/root ratio	-	4.68	-
Calcaric cambisol	straw	1.650±0.156	7.07±0.47	116.60±14.15
	root	1.089±0.059	0.97±0.06	10.60±1.13
	total	1.582	8.04	127.20
	straw/root ratio	-	7.26	-
Fluvisol	straw	1.180±0.057	9.09±0.44	107.26±6.38
	root	0.768±0.014	0.79±0.02	6.04±0.15
	total	1.147	9.88	113.32
	straw/root ratio	-	11.55	-
Planosol	straw	0.694±0.018	13.35±3.39	92.63±24.28
	root	0.660±0.053	3.10±0.33	20.48±1.83
	total	0.687	16.45	113.11
	straw/root ratio	-	4.30	-

Mineralization intensity (IM) shows strong correlation with the total organic C in the soil (r = 0.8891), and a very strong correlation with the total N in the soil (r = 0.9071). Coefficients of correlation between N availability indices (ANI) and total organic C and total N in the soil are very high (r = 0.9981 and r = 0.9997), so the correlation with these parameters is very strong.

On the other hand, Calcaric Cambisol characterized with lower amount of N (41.7 mg N kg^{-1}) was mineralized by anaerobic incubation (Tab. III). Contrary to this, in the remaining soil types, amounts of mineralized N obtained by anaerobic incubation are almost 5 times higher than those obtained by aerobic incubation. Especially high amounts of mineralized N were obtained by anaerobic incubation in Fluvisol (130.60 mg N kg^{-1} soil). Because of that, high values of mineralization intensity (5.43) and availability index (15.57) were also found in this soil. The interesting finding is that the parameters obtained with anaerobic incubation show a weak correlation with the concentrations of N determined in the straw and the whole test crop. However, the indices of N availability in soil (ANI) obtained at anaerobic incubation show very strong negative correlation with the initial mineral NH_4^+–N content in soil (r = –0.9915), while the correlation with the initial amounts of the available mineral N is strong and negative (r = –0.8421). Also, N mineralization indices in soil (IM) obtained at anaerobic incubation show strong negative correlation with total content of available mineral N in the soil found at aerobic incubation (r = –0.8051).

Contents of initially present mineral N, amounts of mineral N obtained by aerobic and anaerobic incubations, as well as the corresponding indices of N availability and mineralization intensity correlate poorly or not at all with the yields of the test crop biomass on the investigated soils.

Nitrogen Availability Determined by the A-value Method

In the experimental crop grown on Fluvisol and Calcaric Cambisol, the lowest values of nitrogen fraction were obtained (Tab. IV) in fertilizer-derived fraction (N_{dff}), only 25.86 mg N per pot and 26.77 mg N per pot, respectively. In the experimental crop on the remaining investigated soil types, the values of the nitrogen fraction are practically two times higher, which shows that the percentage of fertilizer nitrogen utilization varies between 33.15% and 44.62%. Amounts of nitrogen fraction derived from the applied fertilizer (N_{dff}) show a weak correlation with other parameters of N availability in soil. However, the correlations N_{dff} with test crop biomass yields are very strong: r = 0.9866; r = 0.9912 and r = 0.9855 (Tab. V).

The highest values of nitrogen fraction derived from soil (N_{dfs}) were found in test crop grown on Chernozem: 148.54 mg N per pot. On the other hand, on Eutric Cambisol, the lowest amount of nitrogen derived from soil was found (60.21 mg N per pot). Due to this, the lowest ratio of soil derived N (N_{dfs}) was found in the test crop from Eutric Cambisol (64.5%), while the highest ratio was on Calcaric Cambisol (79.0%). Also, amounts of soil derived nitrogen fraction in the straw and the whole

III: *Total organic C, total N and easily available mineral N in soil*

Parameters	Chernozem	Eutric cambisol	Calcaric cambisol	Fluvisol	Planosol
Total organic C (%)	1.84	1.08	4.65	2.13	1.33
Total N (%)	0.192	0.105	0.442	0.226	0.147
C/N ratio	9.57	10.33	10.52	9.43	9.04
Initial content available mineral N (mg N kg^{-1} of soil):					
NH_4^+–N	26.60	26.60	28.70	7.88	25.90
NO_3^-–N	15.05	8.75	7.18	6.65	2.10
(NH_4^++NO_3^-)–N	41.65	35.35	35.88	14.53	28.00
Obtain of aerobic incubation (mg N kg^{-1} of soil):					
NH_4^+–N	21.4	19.2	124.10	21.70	10.50
NO_3^-–N	42.0	8.1	6.70	4.00	19.60
Σ (NH_4^++NO_3^-)–N	63.4	27.3	130.80	25.70	30.10
IM*	1.13 [a]	–0.77 [c]	2.15 [b]	0.50 [b]	0.14 [b]
ANI**	0.52 [a]	–0.23 [c]	2.65 [c]	0.77 [a]	0.07 [b]
Obtain of anaerobic incubation (mg N kg^{-1} of soil):					
NH_4^+–N	116.4	65.1	41.7	130.60	76.40
IM	4.67 [a]	3.34 [a]	0.29 [c]	5.43 [b]	3.43 [a]
ANI	3.37 [a]	1.31 [a]	0.45 [b]	15.57 [c]	1.95 [a]

*IM – intensity of mineralization; **ANI – N availability index.
Different small letters within the same row indicate significance at 0.05 level.

IV: *Amounts of nitrogen fractions originating from the fertilizer (N_{dff}) and soil (N_{dfs}) in the experimental crop (mg N per pot) and the obtained A-values in the investigated soils (±SD)*

Parameters	Chernozem	Eutric cambisol	Calcaric cambisol	Fluvisol	Planosol
N_{dff}					
– in straw (T_C)*	37.73 ± 3.72	28.03 ± 3.30	24.86 ± 2.81	25.04 ± 1.12	29.93 ± 8.14
– in root (T_R)	6.89 ± 0.45	5.12 ± 1.56	1.91 ± 0.26	0.82 ± 0.02	5.43 ± 0.52
– total ($T_C + T_R$)	44.62	33.15	26.77	25.86	35.36
N_{dfs}					
– in straw (U_C)	121.50 ± 6.12	48.34 ± 5.14	91.73 ± 11.70	82.23 ± 5.28	62.71 ± 16.34
– in root (U_R)	27.04 ± 3.49	11.87 ± 4.36	8.69 ± 0.90	5.23 ± 0.06	15.05 ± 1.30
– total ($U_C + U_R$)	148.54	60.21	100.42	87.46	77.75
% fraction of N in the derived from soil	76.9	64.5	79.0	77.2	68.7
A-value (mg N·kg^{-1} soil)	322,05 ± 20,41	172.48 ± 1.92	368.97 ± 24.85	328.40 ± 6.89	209.54 ± 13.62

*Tc – amount of nitrogen absorbed from fertilizer in the aboveground part of a plant; Tr – amount of nitrogen absorbed from fertilizer to the root of plants; Uc – amount of nitrogen absorbed from the soil in the aboveground part of a plant; Ur – amount of nitrogen absorbed from the soil to the root of plants.

crop show strong and very strong correlation, respectively, with pH values in the investigated soils (r = 0.8972 and r = 0.9217), while their correlations with other investigated parameters of N availability in soil are weak.

The calculated A-value (amount of available N in soil) is highest in Calcaric Cambisol (368.97 mg N kg^{-1} soil), and lowest in Eutric Cambisol (only 172.48 mg N kg^{-1} soil). A-value shows strong correlation with the total organic C in the soil (r = 0.8725; Tab. V) and the total N in the soil (r = 0.8998). Also, A-value shows a very strong correlation with mineralization indices (IM) obtained at aerobic incubation (r = 0.9003), while the correlation with the indices of N availability in soil (ANI) is strong (r = 0.8961). We found that A-value does not correlate either with the initial mineral N content in soil, or with the parameters of N availability obtained at anaerobic incubation of the soil. A-value shows a very strong negative correlation with the yields of the crop biomass (r = –0.8555 and r = –0.8756).

A-value correlates very strongly with N concentrations found in the straw and the whole test crop (r = 0.9561 and r = 0.9549). Negative correlations with the amounts of fertilizer derived nitrogen fraction in the straw and the whole crop are strong and very strong, respectively (r = –0.8750 and r = –0.9046), while positive correlations with the amounts of soil derived nitrogen in the straw and in the whole crop are very strong (r = 0.9892 and r = 0.9494).

DISCUSSION

Pot Experiment and Agrochemical Parameters of Nitrogen Availability

Concentrations of NH_4^+ and NO_3^- (mineral N) in soil and soil solution available to plants depend on the relations between mineralization (ammonification and nitrification), uptake by plants and soil microorganisms, denitrification, volatilization and leaching (Corre *et al.*, 2002; Schimel and Bennett, 2004). Substantial participation of clay fraction in a soil texture may protect organic N from microbiological degradation (Nannipieri and Eldor, 2009). Initial NH_4^+–N contents in the investigated soils, which were higher than the initial contents of NO_3^-–N, may be a consequence of enrichment with plant species and the presence of grasses which reduce NO_3^- concentration in soil (Oelmann *et al.*, 2007). However, seasonal influence was not found on mutual relationship between NO_3^-–N concentration and plant species enrichment. On the other hand, legume presence is in positive correlation with soil NO_3^-–N concentration (Oelmann *et al.*, 2007). The mentioned processes are very variable spatially and temporally and may have different characteristics in different years, especially during growing season, because the activities of plants and soil organisms are controlled by variable water supplies and temperatures (Jamieson *et al.*, 1999; Hook and Burke, 2000).

The ability of plants to absorb nitrogen in both organic and inorganic forms indicate that the concentrations and relative ratios of these nitrogen forms in soil are important determinants of plant nitrogen nutrition, and thus of C/N ratio and interactions (Jämtgård *et al.*, 2010). In highly fertile soils, mineral N uptake is the main mechanism of N uptake by grasses (Weigelt *et al.*, 2003). Direct uptake of organic N in the form of amino acids may be encountered in terrestrial ecosystems, especially those with very limited N supplies, like low-productive grazing lands in the areas with moderate climate (Bardgett *et al.*, 2003; Weigelt *et al.*, 2003; Harrison *et al.*, 2007). For instance, although Eutric Cambisol is characterized with very low pH values, total organic C and total N (Tabs. I and III), biomass produced on this type of soil was higher than on Calcaric Cambisol and Fluvisol, which have more favourable pH values and much higher contents of organic C and total N (Tab. II). This effect was noticeable not only in the uptake and distribution of N in the test crop, but also in biomass distribution in the test crop (Tab. II: aboveground biomass / root biomass ratio).

Our results show that the correlation between the total organic C content and total N content in soils under natural meadows is almost linear (Tab. V). Investigation performed on the same soils under forest stands also revealed a highly significant correlation ($P \leq 0.01$; $r = 0.996$) between the contents of total organic C and total N (Dželetović *et al.*, 2011). However, the correlation with the test crop biomass yield on both soils is either poor or absent. Also, C/N ratio does not correlate with the yield parameters.

Nitrogen Availability Indices Obtained by Incubation Method

Although the rates of overall mineralization are primarily determined by the amount of mineral N that may be accumulated in soils, immobilization and losses may potentially significantly affect the accumulation of mineral N (Wang *et al.*, 2001). Total nitrogen mineralization and immobilization rates are significantly higher in the soils under grass than in arable soils, contrary to total denitrification rates which are higher in arable soils (Lang *et al.*, 2016). Nitrogen mineralization indices in soils under simple stands of perennial grasses may differ significantly and are usually higher than nitrogen mineralization in arable soils (Davis *et al.*, 2013). Long-term fertilizer application increase total rates of mineralization, immobilization and nitrification in comparison with non-fertilizer treatments (Lang *et al.*, 2016). In fungal and bacterial communities, the main determinant of the community structure in rhizosphere is the soil type (Singh *et al.*, 2007). Also, the composition of bacterial communities in rhizosphere soil under different plant species is similar, which reveals a weak influence of plant species on the structure of rhizosphere microbial community (Singh *et al.*, 2007).

It is considered that the mechanisms that underlie the differences in net amount of produced N between anaerobic and aerobic incubations depend on the soil type (Wang *et al.*, 2001). That is the probable cause of the encountered differences between the amount of mineralized N in Calcaric Cambisol and the remaining investigated soils. On the other hand, in Eutric Cambisol under aerobic conditions, the values of mineralized and nitrified N, mineralization intensity and nitrogen availability index were encountered that indirectly indicate a very strong denitrification. Nitrification is fast and it is prevailing under aerobic conditions (Aulakh *et al.*, 2000). However, there are specific situations. For instance, low pH exerts direct impact on nitrification processes in Planosol (Jakovljević *et al.*, 2005). Namely, increased and toxic amounts of nitrites arise spontaneously in Planosols as a consequence of decreased biological nitrification (Kresović *et al.*, 2009, 2010). Namely, nitrites may arise with the introduction of ammoniac nitrogen in fertilizers, as a consequence of chemo-denitrification (Kresović *et al.*, 2009). Nitrites produced in Planosol soils may further be oxidized spontaneously by a non-enzymatic process (chemical nitrification) (Kresović *et al.*, 2009). Our results show that the amounts of mineral N produced by aerobic incubation and the mineralization intensity correlate with the total organic C in the soil and the total N in the soil. Besides, correlation of N availability index at aerobic incubation with the total organic N in the soil and the total N in soil under natural meadows is almost linear ($r = 0.9981$ and $r = 0.9997$ respectively).

Immobilization and mineralization transformations are more noticeable under anaerobic conditions. For that reason, the total amounts of mineralized N are always higher under anaerobic conditions, while the immobilization is higher under aerobic conditions (Wang *et al.*, 2001). The highest N mineralization under anaerobic incubation was obtained in Fluvisol. Fluvisols are characterized with periodic flooding and thus with periodic low oxygen content. Because of that, the native microbes are adapted to anaerobic conditions, which may explain high nitrogen mineralization in these soils, under anaerobic conditions. In accordance to this, negative correlation of N availability index at anaerobic incubation with the initial mineral NH_4^+–N in the soil is almost linear ($r = -0.9915$). Anaerobic incubation is recommended by American association of agronomy as a biological index of N availability (Keeney, 1982). However, contrary to the results obtained at aerobic incubation, which correlate with the total organic C in the soil and the total N in the soil under natural meadows, our results show that the amounts of mineral N obtained at anaerobic incubation, as well as the corresponding N availability indices and mineralization intensities correlate poorly or not at all with the yields of the test crop biomass on the investigated soils and with the other investigated parameters. It follows that our results exclude the method of anaerobic incubation as an estimation of the amounts of available N in the surface layer of mineral soil part under natural meadows.

Nitrogen Availability Obtained by the A-method

Most indices are not a direct measure of quantitative plant uptake of nitrogen. Because of that, the indices of N available to plants may differ significantly from nitrogen uptake by a test plant (Velthof and Oenema, 2010). It is widely accepted that the efficiency of N utilization may be improved when the amount of nitrogen introduced with fertilization is adapted to the requirements of

V: *Correlations between the analyzed parameters of nitrogen accessibility obtained by different methods*

Parameters	Total N	Obtain of aerobic incubation			Obtain of anaerobic incubation N	N in straw	T_C	U_C	A-value
		N	IM	ANI					
Total organic C	0.9973^{a}	0.9264^{a}	0.8891^{b}	0.9981^{a}	–0.4329^{c}	0.9621^{a}	–0.4239^{c}	0.3997^{c}	0.8725^{b}
Total N		0.9105^{a}	0.9071^{a}	0.9997^{a}	–0.3798^{c}	0.9672^{a}	–0.4153^{c}	0.4309^{c}	0.8998^{b}
Obtain of aerobic incubation (NH_4^++NO_3^-)–N			0.8877^{b}	0.9165^{a}	–0.5441^{c}	0.7963^{b}	–0.1281^{c}	0.4909^{c}	0.6890^{c}
– IM				0.9080^{a}	–0.1492^{c}	0.8133^{b}	–0.0222^{c}	0.7416^{c}	0.9003^{a}
– ANI					–0.3816^{c}	0.9664^{a}	–0.4061^{c}	0.4382^{c}	0.8961^{b}
Obtain of anaerobic incubation NH_4^+-N						–0.2766^{c}	0.3664^{c}	0.4040^{c}	0.0788^{c}
N concentration in straw							0.9995^{a}	–0.6530^{c}	0.9562^{a}
Amounts of nitrogen fractions originating from the fertilizer (N_{dff}) in straw (T_C)								0.9859^{a}	–0.8750^{b}
Amounts of nitrogen fractions originating from the soil (N_{dfs}) in straw (U_C)									0.9892^{a}

Correlation intensity – r: (a) very strong, 0.901-1.000; (b) strong, 0.751-0.900; and (c) medium and weak, ≤0.750.

the cultivated crop, corrected for the amount of plant-available N derived from soil (Mosier *et al.*, 2004). Because 100 mg N was introduced in each experimental pot, the obtained values of N fraction derived from the applied fertilizer (N_{dff}) are equal to % of N utilization efficiency. On Fluvisol and Calcaric Cambisol, characterized with higher values of total N content, a comparatively low value of N utilization from the applied fertilizer N was found (<27%). Efficiency of the utilization of this nitrogen is highest on Chernozem (44.6%) and it is close to the values which are found in arable soils. Although the efficiency of utilization of nitrogen from the applied N-fertilizer by the test crop varies within a wide range, it correlates with the yields of the test crop biomass.

Grass species differ in their ability to absorb organic and inorganic nitrogen forms (Bol *et al.*, 2002). Besides, total N uptake is also increased with plant species diversity. More diverse plant communities utilize more efficiently limited sources, like those of nitrogen (Oelmann *et al.*, 2007). Increased immobilization may lead to the decrease of yield under the conditions of low nitrogen supply, considering that under the conditions of high nitrogen supply immobilization of NH_4^+–N in comparison with that of NO_3^-–N often exerts no impact on plant yield (Jerabkova *et al.*, 2006). The lowest soil derived nitrogen (N_{dfs}) was found in the crop on Eutric Cambisol, which is characterized with the lowest contents of organic C and total N, while on Calcaric Cambisol, which is characterized with the highest contents of organic C and total N, the ratio of this N is highest. Also, amounts of soil derived nitrogen (N_{dfs}) correlate with pH values in the investigated soils.

Calculated A-value (amount of available N in the soil) correlates with the total organic C in the soil, total N in the soil, indices of mineralization and N availability in soil obtained at aerobic incubation (Tab. V). According to our investigation, A-value does not correlate with the initial mineral N in the soil, nor with the parameters of N availability obtained at anaerobic incubation of the soil, while the correlation between biomass yields of the test crop and A-values in the investigated soils is strongly negative.

CONCLUSION

The investigated soils under natural meadows are characterized with comparatively high mineralization intensity and high N availability indices. Contents of mineral N produced by aerobic incubation and the intensity of the mineralization correlate with the total organic C in the soil and the total N in the soil. Correlation of the availability index of the soil N produced by aerobic incubation with the total organic C and the total N in the soil under natural meadows is almost linear ($r = 0.9981$ and $r = 0.9997$, respectively). Contents of mineral N produced by anaerobic incubation, as well as the corresponding N availability and mineralization intensity indices correlate poorly with the mentioned parameters. Efficiency of nitrogen utilization from the applied N-fertilizer by the test crop varies within a wide range of values and correlates with the biomass yields of the test crop. A-value correlates with the total organic C in the soil, total N in the soil, mineralization and availability indices of soil N produced by aerobic incubation.

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REFERENCES

AULAKH, M. S., KHERA, T. S. and DORAN, J. W. 2000. Mineralization and denitrification in upland, nearly saturated and flooded subtropical soil. I. Effect of nitrate and ammoniacal nitrogen. *Biology and Fertility of Soils*, 31(2): 162–167.

AXMANN, H., SEBASTIANELLI, A. and ARRILLAGA, J. L. 1990. Sample preparation techniques of biological material for isotope analysis. In: HARDARSON, G. (Ed.) *Use of Nuclear Techniques in Studies of Soil-Plant Relationships*. Vienna: International Atomic Energy Agency, pp. 41–54.

BAIZE, D. 1993. *Soil Science Analyses - A Guide to Current Use*. Chichester: John Willey & Sons.

BARDGETT, R. D., STREETER, T. and BOL, R. 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology*, 84(5): 1277–1287.

BOL, R., OSTLE, N. J. and PETZKE, K. J. 2002. Compound specific plant amino acid $\delta^{15}N$ values differ with functional plant strategies in temperate grassland. *Journal of Plant Nutrition and Soil Science,* 165(6): 661–667.

CHEN, D., CHALK, P. M. and FRENEY, J. R. 1990. Release of Dinitrogen from Nitrite and Sulphamic Acid for Isotope Ratio Analysis of Soil Extracts Containing Nitrogen-15 Labelled Nitrite and Nitrate. *Analyst,* 115(4): 365–370.

CORRE, M. D., SCHNABEL, R. R. and STOUT, W. L. 2002. Spatial and seasonal variation of gross nitrogen transformations and microbial biomass in a northeastern US grassland. *Soil Biology and Biochemistry*, 34(4): 445–457.

DAVIS, M. P., DAVID, M. B. and MITCHELL, C. A. 2013. Nitrogen Mineralization in Soils Used for Biofuel Crops. *Communications in Soil Science and Plant Analysis*, 44(5): 987–995.

DŽELETOVIĆ, Ž. S., PIVIĆ, R. N. and DJUROVIĆ, N. Lj. 2011. Available nitrogen in the surface mineral layer of Serbian forest soils. *Journal of Forest Science,* 57(4): 131–140.

FRIED, M. and BROESHART, H. 1974. Priming effect of nitrogen fertilizers on soil nitrogen. *Soil Science Society of America Proceedings,* 38(5): 858–858.

FRIED, M. and DEAN, L. A. 1952. A concept concerning the measurement of available soil nutrients. *Soil Science,* 73(4): 263–272.

HARRISON, K. A., BOL, R. and BARDGETT, R. D. 2007. Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology*, 88(4): 989–999.

HOOK, P. B. and BURKE, I. C. 2000. Biogeochemistry in a shortgrass landscape: Control by topography, soil texture, and microclimate. *Ecology*, 81(10): 2686–2703.

JAKOVLJEVIĆ, M., KRESOVIĆ, M., BLAGOJEVIĆ, S. and ANTIĆ MLADENOVIĆ, S. 2005. Some negative chemical properties of acid soils. *Journal of Serbian Chemical Society,* 70(5): 765–774.

JAMIESON, N., MONAGHAN, R. and BARRACLOUGH, D. 1999. Seasonal trends of gross N mineralization in a natural calcareous grassland. *Global Change Biology,* 5(4): 423–431.

JÄMTGÅRD, S., NÄSHOLM, T. and HUSS-DANELL, K. 2010. Nitrogen compounds in soil solutions of agricultural land. *Soil Biology and Biochemistry*, 42(12): 2325–2330.

JERABKOVA, L., PRESCOTT, C. E. and KISHCHUK, B. E. 2006. Nitrogen availability in soil and forest floor of contrasting types of boreal mixedwood forests. *Canadian Journal of Forest Research,* 36(1): 112–122.

KEENEY, D. R. 1982. Nitrogen-availability indices. In: PAGE, A. L., MILLER, R. H. and KEENEY, D. R. (Eds.) *Methods of Soil Analysis*. 2nd Edition. Madison, Wisconsin: American Society of Agronomy / Soil Science Society of America. Part 2, pp. 711–733.

KEENEY, D. R. and BREMNER, J. M. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy Journal,* 58(5): 498–503.
KEENEY, D. R. and NELSON, D. W. 1982. Nitrogen – inorganic forms. In: PAGE, A. L., MILLER, R. H. and KEENEY, D. R. (Eds.) *Methods of Soil Analysis*. 2nd Edition. Madison, Wisconsin: American Society of Agronomy / Soil Science Society of America. Part 2, pp. 687–693.
KRESOVIĆ, M., JAKOVLJEVIĆ, M., BLAGOJEVIĆ, S. and MAKSIMOVIĆ, S. 2009. Specific transformations of mineral forms of nitrogen in acid soils. *Journal of Serbian Chemical Society,* 74(1): 93–102.
KRESOVIĆ, M., JAKOVLJEVIĆ, M., BLAGOJEVIĆ, S. and ŽARKOVIĆ, B. 2010. Nitrogen transformation in acid soils subjected to pH value changes. *Archives of Biological Sciences,* 62(1): 129–136.
LANG, M., LI, P., HAN, X., QIAO, Y. and MIAO, S. 2016. Gross nitrogen transformations in black soil under different land uses and management systems. *Biology and Fertility of Soils*, 52(2): 233–241.
LI, S.-X., WANG, Z.-H. and STEWART, B. A. 2013. Responses of Crop Plants to Ammonium and Nitrate N. *Advances in Agronomy*, 118: 205–397.
MOSIER, A. R., SYERS, J. K. and FRENEY, J. R. 2004. *Agriculture and the Nitrogen Cycle: Assessing The Impacts of Fertilizer Use On Food Production and The Environment, (SCOPE 65)*. Washington, DC: Island Press.
NANNIPIERI, P. and ELDOR, P. 2009. The chemical and functional characterization of soil N and its biotic components. *Soil Biology and Biochemistry*, 41(12): 2357–2369.
OELMANN, Y., WILCKE, W., TEMPERTON, V. M., BUCHMANN, N., ROSCHER, C., SCHUMACHER, J., SCHULZE, E.-D. and WEISSER, W. W. 2007. Soil and Plant Nitrogen Pools as Related to Plant Diversity in an Experimental Grassland. *Soil Science Society of America Journal*, 71(3): 720–729.
PANSU, M. and GAUTHEYROU, J. 2006. *Handbook of Soil Analysis*. Berlin: Springer.
ROMANO, N., ALVAREZ, R. and BONO, A. 2016. Modeling nitrogen mineralization at surface and deep layers of sandy soils. *Archives of Agronomy and Soil Science*, 63(6): 870–882.
SCHIMEL, J. P. and BENNETT, J. 2004. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology,* 85(3): 591–602.
SCHULTEN, H. R. and SCHNITZER, M. 1997. The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils*, 26(1): 1–15.
SCOTT, D. A., NORRIS, A. M. and BURGER, J.A. 2005. Rapid indices of potential nitrogen mineralization for intensively managed hardwood plantations. *Communications in Soil Science and Plant Analysis,* 36(11–12): 1421–1434.
SIMIĆ, A., DŽELETOVIĆ, Ž., VUČKOVIĆ, S., KRGA, I. and ANDREJIĆ, G. 2016. Soil fertility of meadows and pastures in Western Serbia [in Croatian: Plodnost tla pod livadama i pašnjacima Zapadne Srbije]. In: POSPIŠIL, M. and VNUČEC, I. (Eds.) *Proceedings of 51st Croatian and 11th International Symposium of Agriculture*. Sveučilište u Zagrebu, Agronomski fakultet, Zagreb, pp. 251–255.
SINGH, B. K., MUNRO, S., POTTS, J. M. and MILLARD, P. 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Applied Soil Ecology,* 36(2–3): 147–155.
SKUODIENE, R., KATUTIS, K., NEKROSIENE, R., REPSIENE, R. and KARCAUSKIENE, D. 2016. Effects of soil properties and humidity regimes on semi-natural meadow productivity. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science,* 66(8): 653–663.
VELTHOF, G. L. and OENEMA, O. 2010.: Estimation of Plant-Available Nitrogen in Soils using Rapid Chemical and Biological Metods. *Communications in Soil Science and Plant Analysis,* 41(1): 52–71.
WANG, W. J., CHALK, P. M., CHEN, D. and SMITH, C. J. 2001. Nitrogen mineralization, immobilization and loss, and their role in determining differences in net nitrogen production during waterlogged and aerobic incubation of soils. *Soil Biology and Biochemistry*, 33(10): 1305–1315.
WEIGELT, A., KING, R., BOL, R. and BARDGETT, R. D. 2003. Inter-specific variability in organic nitrogen uptake of three temperate grassland species. *Journal of Plant Nutrition and Soil Science,* 166(5): 606–611.

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