Available nitrogen in the surface mineral layer of Serbian forest soils

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ABSTRACT: Based on a greenhouse experiment, we evaluated nitrogen availability in the surface mineral layer of soil under various deciduous forest stands by analysing 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 studied forest soils are characterized by high mineralization intensity and high N availability indices. Aerobic incubation appears to be the most appropriate method for evaluating the available N content. The amounts of mineralized and nitrified N, obtained by aerobic incubation, with subtraction of the initial content of available mineral N forms are in correlation ($P \leq 0.05$) with total organic C content ($r = 0.916$) and total soil N ($r = 0.903$) while the correlation with the C/N ratio is poor ($r = 0.645$).

Keywords: A-value; C/N ratio; total organic C; total soil N

An adequate supply of nitrogen is essential for successful establishment and sustainable productivity of forest stands. The long lasting reserves 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. Although N availability in soils has been the subject of many studies, there is not a 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 way of soil utilization (e.g. for forest species) (Scott et al. 2005).

During one vegetation season, an enormous immobilization is hidden under the prevailing mineralization process. Thereby, forest plants utilize not only mineral N from the soil but also soil organic N directly (Bennett, Prescott 2004) and approximately one half or more of N in the soil solution occurs in an organic form in forest ecosystems (Yu et al. 2002).

Tree species can have a strong effect on N cycling in forest ecosystems that appears to be manifested through the quality of soil organic matter, while one cannot explain the mechanisms of this influence by standard evaluation of litter quality (Lovett et al. 2004). Nitrogen concentrations vary in different organic horizons, with the highest concentration found in forest litter, while the concentrations in other layers decrease with the depth (Laverman et al. 2000). Soil N availability is more closely related to litter N content than to the litter decomposition rate (Jerabkova et al. 2006).

The type of vegetation may determine the rate of supplying these ecosystems with nitrogen, and this rate may have a strong effect on the vegetation composition. The variation among forest types is likely attributable to vegetation (Jerabkova et al. 2006). The types of vegetation and plant remnants have a strong effect on the microbiological activity of soil. Thus, coniferous forests have a lower content of mineral N than deciduous forests. Deciduous species tend to facilitate nitrification as compared to coniferous species (Augusto, Ranger 2001). The species identity in plant communities may also directly influence N mineralization and nitrification through specific compounds in their leaf litter, such as pine polyphenols (Hättenschwiler, Vitousek 2000) or root exudates (Subbarao et al. 2007).

On the other hand, forest clear-cutting disrupts the existing balance of the soil organic matter content. By clear-cutting, stopping N uptake by trees and decreasing total deposition, N availability in the soil is increased (Jussy et al. 2004). Also, substituting the old coppice for young stands favours the nitrifying community (Zeller et al. 2007). Microbial immobilization, favoured by organic matter input and temperature increase, was probably the cause of a decrease in the net mineralization of N (Jussy et al. 2004).

Obviously, the quantity of available N in soil is an important ecological factor. The aim of our study, apart from the assessment of available soil N in the surface mineral layer of various types of forest soils, was to establish the relationship between available N reserves and some agrochemical characteristics of forest soils. We shall there by contribute to the information on N availability in various forest soils of the temperate climatic zone of Europe, useful for understanding nitrogen dynamics, necessary for the corresponding and adequate forestry 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 4 locations in Serbia, Southeast Europe (Table 1). The forest stands were more than 25 years old. Soil samples were collected in mid-March from the surface mineral portion of the soil layer, from a depth of 0–20 cm. During the sample collection, remnants of forest litter, fallen leaves, flattened grass, tree and shrub branches, mosses, etc., were removed. 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

We used the A-value method for a greenhouse experiment with test plant and application of N-fertilizers labelled with the stable N isotope (^{15}N). 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, Dean 1952):

$$A = \frac{U_c \times T_s}{T_c} \qquad (1)$$

where:

A – amount of available nitrogen in the soil (kg·ha^{-1});
T_s – amount of applied fertilizer;
T_c – amount of nitrogen absorbed from fertilizer in the aboveground part of a plant;
U_c – 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. Firstly, contrary to tree species, oat does not manifest any ^{15}N discrimination during uptake, distributing it evenly across the plant organs. Secondly, soils have a wide range of physicochemical characteristics, above all the pH range (Table 1), that makes the development of oat possible, but could compromise the growth of other test species.

In each pot, 13 oat seeds were sown. After 7 days, the number of plants was reduced to 10. Soil hu-

Table 1. Soils utilized in the experiment and their textural composition

Soil type (by FAO soil classification)	Location, average annual temperature, average annual rainfall	Main tree species	Granulometric composition (%)			pH in 1M KCl
			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	*Acer pseudoplatanus* L. *Alnus glutinosa* Gaertn.	36.5	31.2	32.3	7.1
Eutric cambisol, formed on lake sediments, lessived	Ralja 10.7°C 649 mm	*Quercus robur* L., *Quercus frainetto* Tenore, *Alnus incana* Moench., *Acer campestre* L.	38.7	33.8	27.5	3.8
Calcaric cambisol, typical, shallow	Bileća 12.2°C 1,620 mm	*Quercus pubescens* Willd.	36.9	27.3	35.8	5.2
Eutric fluvisol, carbonated, deeply	Obrenovac 11.0°C 662 mm	*Robinia pseudoacacia* L. *Fraxinus excelsior* L.	52.5 23.4	19.6 39.5	27.9 37.1	6.6 6.8

midity 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. Aboveground 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). We determined the total amount of organic C using the procedure described by Nikitin (1972): heating of soil samples with a chromium-sulphuric mixture and spectrophotometric measurement of optical densities. Total N in soil and plant samples was determined by semi-micro Kjeldahl digestion (Bremner, Mulvaney 1982).

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:

$$IM = \frac{N_{min} - N_{initial}}{N_{total}} \qquad (2)$$

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:

$$ANI = \frac{N_{min} - N_{initial}}{N_{initial}} \qquad (3)$$

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).

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.

Statistical analysis

Statistical analysis of parameters necessary for N availability assessment included calculations of standard deviation (SD), correlation coefficient (r) and the test of statistical significance of correlation coefficients by Student's test.

RESULTS

Vegetation experiment in pots

The experimental crop is characterized by great variations in aboveground mass (straw mass) and root mass (Table 2). The ratio of the biomass of the aboveground part to the biomass of roots of the experimental crop on Fluvisol under the black locust forest is the highest (6.58), namely, a considerably larger aboveground part of the experimental crop was formed on this soil compared to the roots. This ratio is in the range from 4.37 to 4.88 in the experimental crop on other soils. On Eutric Cambisol, the crop reaches the highest straw and root masses. The concentration of nitrogen in the experimental crop varies within a very wide range (Table 2). The lowest values are found in oats grown on Chernozem, and the highest on Calcaric Cambisol and Fluvisol. The aboveground part of oats on Calcaric Cambisol and Fluvisol is richer in nitrogen by 35–55% than the root part, while on Chernozem and Eutric Cambisol, the percentage of nitrogen in the straw is approximately equal to that in the roots. The highest uptake of nitrogen by the experimental crop was observed on Eutric Cambisol (200.47 mg of N per pot) and the lowest on Chernozem (127.80 mg of N per pot).

Inorganic forms of N availability in soil

The total content of C and N in the examined soils (Table 3) is in the range of these values 1.94–7.60% and 0.181–0.594%, respectively. The values of the C/N ratio are in the range of 10.31–12.79, which is usual for forest soils. Except the soil under the black locust forest, the ammonium form of nitrogen is the dominant form of easily available mineral N. The highest amounts of NH_4^+–N and available mineral N are found in Eutric Cambisol, where the ratio of mineral N to total N is the highest (2.67%).

In the investigated Calcaric Cambisol the highest amounts of mineralized and nitrified N were obtained by aerobic incubation (Table 3). Due to this, the mineralization intensity (IM) and N availability index (ANI) are the highest in this soil (1.576 and 2.710, respectively), while in the other investigated soils they are significantly lower. In Chernozem and Fluvisol, the highest quantities of NO_3^--N were obtained by aerobic incubation. However, a very small amount of mineralized and nitrified N is obtained by aerobic incubation in the soil under the black locust (only 0.40 mg $N \cdot kg^{-1}$ soil), and both IM and ANI values are nearly zero.

A slightly smaller N quantity (Table 3) was mineralized by anaerobic incubation in Calcaric Cambisol. However, in the other investigated soils the quantities of mineralized N by anaerobic incubation were even up to 10 times higher than the quantities of N obtained by aerobic incubation. Especially high amounts of mineralized N (implying also high values of IM and ANI) were obtained in Fluvisol, both under black locust and ash forests (Table 3).

Nitrogen availability determined by the A-value method

In the experimental crop grown on Calcaric Cambisol, the lowest values of nitrogen fraction were obtained (Table 4) in fertilizer-derived fraction (N_{dff}), only 18.48 mg of N/pot; and the highest values were determined in nitrogen fraction derived

Table 2. 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	0.702 ± 0.049	14.72 ± 1.55	102.91 ± 8.40
	root	0.771 ± 0.045	3.26 ± 0.85	24.89 ± 5.39
	total	–	17.98 ± 2.37	127.80 ± 12.47
	straw/root ratio	–	4.51	–
Eutric cambisol	straw	0.944 ± 0.102	17.83 ± 0.31	168.41 ± 19.08
	root	0.878 ± 0.018	3.65 ± 0.94	32.06 ± 8.47
	total	–	21.48 ± 1.20	200.47 ± 26.18
	straw/root ratio	–	4.88	–
Calcaric cambisol	straw	1.700 ± 0.081	9.65 ± 0.32	164.07 ± 13.05
	root	1.196 ± 0.088	2.21 ± 0.15	26.43 ± 2.30
	total	–	11.86 ± 0.47	190.50 ± 15.00
	straw/root ratio	–	4.37	–
Fluvisol (b. locust)	straw	1.595 ± 0.124	10.24 ± 1.89	163.36 ± 16.46
	root	1.189 ± 0.092	1.56 ± 0.27	18.52 ± 3.65
	total	–	11.80 ± 2.15	181.88 ± 20.00
	straw/root ratio	–	6.58	–
Fluvisol (ash)	straw	1.729 ± 0.062	9.47 ± 0.93	163.81 ± 18.74
	root	1.111 ± 0.016	2.09 ± 0.47	23.21 ± 4.96
	total	–	11.56 ± 1.37	187.02 ± 23.60
	straw/root ratio	–	4.52	–

from soil (N_{dfs}): 172.02 mg of N/pot. In the experimental crop on the other investigated soil types, the values of this nitrogen fraction are practically twice higher, which shows that the percentage of fertilizer nitrogen utilization varies between 30.6% and 39.4%. On the other hand, the experimental

Table 3. Total organic C, total N and easily available mineral N in soil

	Chernozem	Eutric cambisol	Calcaric cambisol	Fluvisol (b. locust)	Fluvisol (ash)
Total organic C (%)	1.940	2.240	7.600	3.240	2.960
Total N (%)	0.188	0.181	0.594	0.284	0.273
C/N ratio	10.310	12.400	12.790	11.410	10.840
Initial content available mineral N (mg N·kg^{-1} of soil)					
NH_4^+-N	23.100	29.050	25.550	13.650	15.510
NO_3^--N	8.400	19.250	8.920	14.180	8.720
$(NH_4^+ + NO_3^-)$-N	31.500	48.300	34.470	27.830	24.230
Obtain of aerobic incubation					
NH_4^+-N	20.300	58.800	120.800	8.800	35.000
NO_3^--N	22.800	10.300	7.100	19.400	19.500
$\Sigma\ (NH_4^+ + NO_3^-)$-N	43.100	69.300	127.900	28.200	54.500
IM	0.617	1.160	1.573	0.013	1.109
ANI	0.368	0.435	2.710	0.013	1.249
Obtain of anaerobic incubation					
NH_4^+-N	111.700	193.400	109.200	281.000	301.500
IM	4.713	9.080	1.408	9.414	10.476
ANI	3.835	5.657	3.274	19.586	18.439

IM – mineralization intensity, ANI – N availability index

Table 4. 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 (b. locust)	Fluvisol (ash)
N_{dff}					
In straw (T_c)	30.71 ± 2.50	33.25 ± 3.67	16.71 ± 1.81	32.24 ± 3.63	27.52 ± 5.60
In root (T_r)	6.16 ± 0.32	6.14 ± 0.68	1.77 ± 0.10	2.06 ± 0.92	3.09
Total (T_c+ T_r)	36.87	39.39	18.48	34.29	30.61
N_{dfs}					
In straw (U_c)	72.20 ± 6.03	135.16 ± 15.42	147.36 ± 11.36	131.13 ± 13.25	136.29 ± 13.48
In root (U_r)	18.73 ± 5.28	25.92 ± 8.99	24.66 ± 2.19	16.46 ± 4.38	20.12
Total (U_c + U_r)	90.93	161.08	172.02	147.59	156.41
% fraction of N in the derived from soil	71.20	80.30	90.30	81.10	83.60
A-value (mg N·kg^{-1} soil)	117.56 ± 3.85	203.23 ± 1.05	440.82 ± 20.67	203.38 ± 17.71	247.61 ± 34.97

T_c – amount of nitrogen absorbed from fertilizer in the aboveground part of a plant, T_r – amount of nitrogen absorbed from fertilizer to the root of plants, U_c – amount of nitrogen absorbed from the soil in the aboveground part of a plant, U_r – amount of nitrogen absorbed from the soil to the root of plants.

crop on Chernozem is characterized by the lowest soil-derived nitrogen fraction (90.9 mg N per pot), in comparison with the amounts found in crops on the other investigated soils (between 147.6 and 172.0 mg N per pot). Thus, the lowest ratio of N fraction derived from soil (N_{dfs}) is found in the experimental crop on Chernozem (71.2%) and the highest on Calcaric Cambisol (90.3%). Accordingly, the obtained A-values (the amount of available N in the soil) are the highest in Calcaric Cambisol (440.82 mg of N per kg of soil) and the lowest in Chernozem (only 117.56 mg of N per kg of soil).

DISCUSSION

Soil pH and agrochemical parameters of nitrogen availability

Ste-Marie and Paré (1999) suggested that pH is an important regulator of net nitrification in forest soils. An increase of pH in forest floor has a positive effect on net nitrification, while the acidification provokes a decrease. Net nitrate production in organic horizons is positively related to soil pH and negatively related to the C/N ratio (Persson et al. 2000). Net nitrification in forest floor is not found at pH values below 4.5 (Ste-Marie, Paré 1999). Eutric Cambisol is characterized by low pH, approaching the boundary values (Table 1). However, data presented in Tables 3 and 4 show that the amount of available N is higher in Eutric Cambisol than in Chernozem, which is characterized by neutral pH. In addition, it is interesting that the pH value correlates adversely with the content of easily available mineral N forms in soil ($P \leq 0.05$; $r = -0.909$). Evidently, the effect of pH on the mineralization of nitrogen in the surface mineral layer of forest soils is different from that in the forest floor. Besides, total crop and straw masses are in high correlation with the percentage of easily available mineral N in total soil nitrogen ($P \leq 0.01$; $r = 0.968$ and 0.978, respectively), while their correlations with N concentration in straw are negative ($P \leq 0.05$; $r = -0.892$ and -0.886, respectively).

The soil under the vegetation cover consisting of grown-up trees contains a substantial quantity of organic matter derived from vegetation waste, fallen from the trees or from root metabolism products. Due to such, chronically high N deposition, the C/N ratio is narrowed in many forest ecosystems (Michel, Matzner 2002). In the investigated soils the contents of total organic C and total N show a highly significant mutual correlation ($P \leq 0.01$; $r = 0.996$), while the correlation with yield parameters is weak or nonexistent. There is no correlation between the C/N ratio and yield parameters.

Contrary to the process of nitrification favoured in arable soils of fields, the NH_4^+ form is highly prevalent in forest soils (Laverman et al. 2000). Ammonium can be oxidized to nitrate (NO_3) by chemoautotrophic bacteria using CO_2 as a carbon source, or by heterotrophs using organic matter as C and N sources (De Boer, Kovalchuk 2001). Low

NO_3^- concentrations found in forest soils are a frequent characteristic of low nitrification rates (Zeller et al. 2007). We have found that the sum of easily available nitrogen forms, $\Sigma(NH_4^+\text{-N} + NO_3^-\text{-N})$, is in a better correlation with the analyzed parameters. Individually, the contents of NH_4^+-N and NO_3^--N in the investigated soils are in poor correlation with yield parameters, pH values or actual properties.

Nitrogen availability indices obtained by incubation methods

By aerobic incubation we obtained relatively small quantities of NO_3^--N in the examined soils (Table 4). There are different mechanisms that may limit net nitrification in forest soils. Moreover, soil moisture and temperature are the most important factors influencing the overall mineralization rate (Puri, Ashman 1998). Nitrification potential and nitrate production at field water capacity and 25°C are the highest in forest litter, while they are scarcely detectable in the mineral component of soil (Laverman et al. 2000) from which our samples were taken. In the mineral horizon, the limitation of net nitrification in soils with high C/N ratio probably resulted from low gross NH_4^+ production (Christenson et al. 2009).

Investigations based on the isotope dilution method showed that many ecosystems, including coniferous forests and old forests with low to negative net nitrification rates, have high total nitrate production, accompanied by rapid microbial immobilization (Zeller et al. 2007). Michel and Matzner (2002) found out that N mineralization in the surface horizon of forest soil was not affected by the C/N ratio. However, Gundersen et al. (1998) reported a negative correlation between the net nitrification rate and C/N ratio in forest floor. Christenson et al. (2009) also observed a significant negative relationship between net nitrification and the C/N ratio of soil in both organic and mineral horizons. Net nitrification and nitrate leaching show a strong inverse relationship with the C/N ratio of soil (Gundersen et al. 1998; Lovett et al. 2004). As a consequence of this empirical relationship, soil C/N has been proposed as a criterion to evaluate the susceptibility of a site to N saturation and nitrate leaching (MacDonald et al. 2002). Thereby, the mechanism of the relationship between C/N ratio in soil and net nitrification or nitrate leaching is not clear (Christenson et al. 2009).

We found out that the amounts of mineralized and nitrified N obtained by aerobic incubation and the corresponding mineralization rates are not in correlation either with biological parameters of plant yield or with N percentage and N fraction absorbed by the crop. pH values were correlated neither with the amount of mineralized and nitrified N obtained by aerobic incubation nor with the corresponding values of IM and ANI. However, the amounts of mineralized and nitrified N obtained by aerobic incubation with subtraction of the initial content of available mineral N forms are in correlation ($P \leq 0.05$) with total organic C content ($r = 0.916$) and total soil N ($r = 0.903$) while the correlation with the C/N ratio is poor ($r = 0.645$).

Total amount of mineralized N is always higher under anaerobic conditions, while immobilization is higher under aerobic conditions (Wang et al. 2001). In addition, it is considered that the mechanisms causing differences in the net amount of produced N between aerobic and anaerobic incubations depend on the soil type (Wang et al. 2001). Nitrification is rapid and prevailing under aerobic conditions (Aulakh et al. 2000), while immobilization-mineralization transformations are increasingly mutually matched under anaerobic conditions (Wang et al. 2001). Although total mineralization rates are primarily determined by the amount of mineral N that can be accumulated in the soils, immobilization and losses have the potential to affect the N mineral accumulation more significantly (Wang et al. 2001). This is a likely explanation for the expressed differences in the mineralized N quantity between Calcaric Cambisol and the other examined types of soil.

Anaerobic incubation is recommended by the American Agronomist Association as a biological index of N availability (Keeney 1982) and it is widely associated with N uptake, especially in forest soils (Keeney, Bremner 1966). The results obtained by Scott et al. (2005) showed that the indices obtained by the process of anaerobic incubation cannot be used to assess mineralizing nitrogen in clearings and arable soils. We also obtained by anaerobic incubation the values of mineralized N (with and without subtraction of the initial mineral N forms) and the corresponding mineralization intensities that were not in correlation with biological parameters of plant yield, pH values, crop N percentage, absorbed N in the crop, parameters of N availability obtained by other methods.

Thus, our results exclude the method of anaerobic incubation for the assessment of available N amounts in the surface layer of mineral forest soils. We drew this conclusion because of the non-correlation of the observed with other features, while aerobic incubation only correlates with C and N. The fact is that the highest mineralization under anaerobic incubation was obtained in Fluvisols. Fluvisols are characterized by periodical flooding, i.e. by periods with a low content of oxygen. Hence, there has been a selection of adapted microorganisms to anaerobic situations which can explain the high N mineralization in these soils under anaerobic conditions. Thus, each method provides different information.

Nitrogen availability obtained by the A-method

The percentage of fertilizer nitrogen utilization in forest soils has a broad range of values (Table 4). Soils on which there are various tree species differ in many key N-cycling characteristics, including net N mineralization and nitrification, microbial N biomass, and retention of added ^{15}N (Templer et al. 2003; Lovett et al. 2004). These differences are shown in the percentage of soil nitrogen fraction in total nitrogen in the experimental crop, which is the lowest in the crop on Chernozem and the highest on Calcaric Cambisol. Small quantities of N_{dff} are accompanied by significantly higher quantities of N_{dfs} (Table 4). For this reason, the percentage of soil nitrogen fraction in total nitrogen in the experimental crop is so high, and the range of A-values obtained by the calculation is wide.

The results of this study show that the values of some indicators are very specific in the case of Calcaric Cambisol. Namely, the obtained values classify this soil as very well supplied with available N. According to Arslan et al. (2010), the high nitrogen mineralisation potential of oak forests is related to the high carbon and nitrogen content in soil.

The observations of Nadelhoffer et al. (1995) suggested that most of the added and retained inorganic N in soil is assimilated by bacteria and fungi during the growing season. This results in small amounts of added N being nitrified and denitrified, at least in well-drained soils (Groffman et al. 1993), to which the investigated surface mineral layer of soil also belongs. An increase in immobilization may lead to yield depression under the conditions of low nitrogen supply, considering that under the conditions of high nitrogen supply, ammonium nitrogen immobilization, contrary to nitrate nitrogen immobilization, does not often affect the plant yield (Jerabkova et al. 2006). In our experiment, contrary to the yield obtained on Chernozem and Eutric Cambisol, a lower yield of the experimental crop was found out on Calcaric Cambisol and Fluvisol (Table 2), which are characterized by high total N and total organic C contents. Evidently, total N and organic C contents did not affect the level of experimental crop yield. Besides, neither the content of easily available mineral forms of N nor the total quantities of the N fraction derived from the soil in the crop and in the straw express any correlation with the analyzed parameters of biological yield.

Forest soils have high rates of total mineralization and consumption of NH_4^+-N, indicating the fast circulation of NH_4^+-N (Christenson et al. 2009). Establishing the biological immobilization by ammonium-nitrogen addition, the crop yield may be decreased by ammonium-nitrogen fixation through organic matter and by ammonium-nitrogen fixation in clay minerals. In natural environments where concentrations of mineral nitrogen forms in soils are usually low, ammonium nitrogen also decreases the microbiological nitrate uptake. Abiotic immobilization of inorganic N may occur in O horizon and mineral component of soils (Dail et al. 2001).

However, the high percentage of soil nitrogen in the crop indicates a great amount of available N (Table 4). Namely, N_{dff} in straw and in the whole crop is negatively correlated with total organic C content ($P \le 0.05$; $r = -0.939$ and $r = -0.958$), total soil N ($P \le 0.05$; $r = -0.949$ and $P \le 0.01$; $r = -0.976$) and with the amount of mineralized and nitrified N obtained by aerobic incubation ($P \le 0.01$; $r = -0.959$ and $P \le 0.05$; $r = -0.966$). On the other hand, N_{dfs} in the crop and in straw is highly significantly correlated ($P \le 0.01$) with total N content in the crop ($r = 0.969$ and $r = 0.962$) and in straw ($r = 0.963$ and $r = 0.975$).

High A values also indicate large quantities of available N. The A-value is correlated with total organic C content in soil ($P \le 0.01$; r = 0.961), total N content in soil ($P \le 0.05$; $r = 0.952$) and with the amounts of mineralized and nitrified N obtained by aerobic incubation ($P \le 0.05$; $r = 0.887$), while they are negatively correlated with N_{dff} in the crop and straw ($P \le 0.05$; $r = -0.932$ and $r = -0.912$). The percentage of organic matter in soil is recommended by Keeney (1982) as a standard of potentially available N. Also, our results indicate a high dependence of the supplies of available N in the surface mineral layer of forest soils on total organic C content as well as on total N, but not on the C:N ratio in them. Furthermore, the method of aerobic incubation appeared to be the most convenient for the assessment of the amount of available N.

CONCLUSIONS

The studied soils under deciduous forest stands (Chernozem, Eutric Cambisol, Calcaric Cambisol and Fluvisol) are characterized by high mineralization intensity and high N availability indices. Aerobic incubation appears to be the most appropriate method for evaluating the available N content. Our results indicate a strong dependence of available N supplies in the surface mineral layer of forest soils on total organic C content as well as on total N content, but not on the C/N ratio.

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