Seasonal variability in soil N mineralization and nitrification as influenced by N fertilization

S. Malý¹, B. Šarapatka², M. Kršková²

¹Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic ²Faculty of Science, Palacký University, Olomouc, Czech Republic

ABSTRACT

Parameters characterizing N mineralization and nitrification were measured in soils of ten monitoring areas of the basal soil monitoring carried out by the Central Institute for Supervising and Testing in Agriculture. A remarkable seasonal cycle was found only for nitrate concentrations that reached their maxima in the spring (April–June), and late summer and/or autumn, starting in August. Ammonium ions were nitrified immediately after fertilizer application. Anaerobic N mineralization represented a variable parameter, which was not directly affected by mineral N fertilizers. Nitrification measured by means of one-week incubation was significantly stimulated by N fertilizers confirming that substrate availability was a limiting factor of this process. Short-term nitrification activity (SNA) showed no remarkable seasonal fluctuations, which meant that the potential nitrification rate remained relatively constant during the season. Urease activity was mostly constant during the year and was only slightly related to N mineralization.

Keywords: soil; N mineralization; nitrification; N fertilizers; seasonal variability

Soil quality is a term often used for complex soil evaluation referring to its ability to function within the scope of an ecosystem and maintain its productivity, contribute to the quality of the environment, and support the healthy development of plants and animals. The bestdefined element of soil quality is its impact on plant production. Moreover, soil may even influence the quality of plants, animal and human health as well as individual environmental components. In practice, however, the problem how to measure and evaluate this quality arises. It is inevitable to select proper quality indicators which have, according to Doran and Parkin (1996), to be correlated with the ecosystem processes, integrate physical, chemical and biological properties of soils and the soil processes and be relatively easy to use and master under field conditions both by professionals and farmers. In addition, they should be sensitive to changes in farming and/or climate.

Microbial parameters belong to early indicators of soil quality changes because they can respond to modified soil conditions sooner than physical and chemical properties (Tscherko and Kandeler 1999). The application of the microbial parameters in the system of soil quality monitoring is rather limited by their natural variability during vegetation, and the routine use is impossible without its knowledge (Gregorich et al. 1994, Brookes 1995). Within the context of nitrogen turnover, soil quality is significantly affected by two parameters, namely N mineralization and nitrification. Mineralizable nitrogen reflects an unstable and easily plant-available soil nitrogen fraction (Gregorich et al. 1994). Nitrification is a process sensitive to soil disturbances, because it is controlled by a narrow range of chemolithotrophic microorganisms (Sparling 1997).

The dynamics of N mineralization and nitrification in the soil cannot be properly understood without repeated estimation during the year because their seasonal pattern is impossible to predict using only a single determination in the beginning of the vegetation period (Franzluebbers et al. 1995). In arable soils, these processes are significantly influenced by cultural practices (Campbell et al. 1999a) apart from natural factors that will be discussed further on.

In general, N mineralization exhibits increased values either late in the spring during maximum root development due to liberation of root exudates into soil, sufficient soil moisture and favourable temperatures, or after harvest in the late summer and in the autumn when organic residues start to enter the soil. Minimum values were observed at the summer period as a result of a water shortage (Van Gestel et al. 1992, Gill et al. 1995, Rohde 1996). On the contrary, Campbell et al. (1999b) revealed no distinct trends in N mineralization in the course of the vegetation period. It seems that N mineralization is rather dependent on the soil cultivation method over several successive years and the accumulation of an easily mineralizable substrate than on an instantaneous input of N fertilizers (Hassink 1992, Gill et al. 1995).

In general, maximum values of nitrification are achieved in the spring and autumn due to optimum temperature and moisture conditions and higher substrate availability that are limiting factors of nitrification (Paul and Clark 1996). Nitrification increase is observed immediately after N fertilizer application (Lovell and Hatch 1998) because of a permanent shortage of a substrate for ammonia oxidisers in most soils (Tate 2000). Woldendorp and Laanbroek (1989) reported that the amounts of nitrification bacteria did not change in the course of a short period. This is in

good agreement with the low variability of short-term nitrification activity (SNA) during the year as described by Bramley and White (1989).

Enzymatic methods offer a suitable tool for soil quality monitoring (Dick 1992). Urease is one of the frequently used enzymes whose activity is related to N mineralization (Kandeler and Eder 1993, Kandeler et al. 1999). On the contrary, Ruppel and Makswitat (1999) found no relationship between these two parameters and no impact of N fertilizers on the activity of the above enzyme. Tscherko and Kandeler (1999) found just a very little urease activity variability during the season.

The goal of the present study was to assess the natural seasonal variability of the parameters characterizing N mineralization and nitrification in the soil and the impact of N fertilization on these processes. The research was performed as part of the monitoring of biological potential of the soils in the Czech Republic organized by the Central Institute of Supervising and Testing in Agriculture, Brno.

MATERIAL AND METHODS

Soil sampling and storage. Soil samples were collected in the years 1997–1998 from ten monitoring plots of arable soils according to guidelines issued by the Central Institute for Supervising and Testing in Agriculture. Thirty-six subsamples from the 0–30 cm depths were taken from an area of 25 × 40 m to prepare an average sample. The sampling was carried out monthly from March to October. Before analysis samples were sieved (2 mm), and stored at 4°C. All microbiological analyses were performed within 30 days after sampling. The urease activity assessment was carried out at the end of the season. The measured samples were stored at -20°C and before the analyses; those were for a week being defrosted at 4°C. The soil samples were then dried at a laboratory temperature to determine pH, total nitrogen and organic carbon by commonly used methods (Malý et al. 2001).

Anaerobic N mineralization (anaerobic ammonification). Five grams of moist soil were weighed out, put in plastic vessels of 23 × 90 mm, and filled with water up to 10 mm below the upper edge of the vessel. The vessels were then closed with a screw cap and incubated at 40°C for a week. Afterwards, the suspension was removed from the vessels and transferred into 125ml bottles; KCl solution was added to achieve a final concentration of 1 mol/1 (soil:extractant = 1:20). The samples were agitated for 1 hr, centrifuged, and the supernatant was analysed to assess the concentration of ammonium ions (Bundy and Meisinger 1994). Anaerobic N mineralization was expressed as a net increase in ammonium ions after the subtraction of the initial concentration. The estimates were carried out in four replicates.

Nitrification measured by means of one week incubation (nitrification-incubation). Ten grams of moist soil were put in 100 ml Erlenmeyer flasks, moistened to reach 60% WHC and incubated at 25°C for a week. Afterwards,

the sample was supplemented with 50 ml 1M KCl, agitated for 1 hr and centrifuged. The supernatant was analysed for nitrate concentration. Nitrification-incubation was expressed as a net increase in the nitrate concentration after deduction of the initial concentration. The analyses were carried out in three replicates.

Short-term nitrification activity (SNA). Twenty-five grams of moist soil were placed in a glass bottle and supplemented with 100 ml media. The bottle was then closed with a lid having a 7 mm slot, and the suspension was agitated on a horizontal shaker at 25°C for 6 hrs. The medium (pH 7.2) was prepared by mixing 10 ml of phosphate buffer and 15 ml 1M NaClO₃ solution with an addition of 0.5 g (NH₄)₂SO₄ and water to make up 1000 ml. The phosphate buffer contained 1.25 g K₂HPO₄ and 0.38 g KH₂PO₄ per 100 ml. After 2 and 6 hours, 5 ml of the suspension were removed, supplemented with 5 ml 4M KCl, shaken vigorously, filtered (MN 619 G, Macherey-Nagel), and the filtrate was analysed for the nitrite content. Nitrification was calculated as an increase in nitrite concentration per unit time. The analyses were carried out in three replicates.

Urease activity. Five grams of soil were weighed in replicates and placed in glass flasks with an addition of 20 ml borate buffer (pH 10) and 2.5 ml urea solution (4.8 g/l). The samples were incubated for 2h at 37°C. After the incubation, 30 ml of Ag₂SO₄ solution were added to stop the reaction. The Ag₂SO₄ solution contained 100 mg Ag₂SO₄ in 1000 ml potassium chloride solution (2.5 mol/l). A blank was prepared as described above, and 2.5 ml urea solution was added at the end of the incubation. The samples were then agitated for 30 minutes, centrifuged, and the supernatant was analysed to assess the concentration of ammonium ions. Urease activity was expressed as a net increase in ammonium ions after subtraction of the ammonium concentration in blank (Alef and Nannipieri 1995).

The assessment of nitrogen mineral forms. The concentrations of ammonium, nitrite and nitrate ions were measured during the microbiological analyses according to commonly used methods (Malý et al. 2001).

Statistical evaluation. Due to the data features, the non-parametric method was used for statistical evaluation. Spearman correlation coefficients were used to find relationships among the studied parameters. To analyze the influence of fertilization on parameters under study, the results were separated into three groups. The first represents the results of soil samples taken before fertilization, the second one immediately after fertilization, and the third samples collected in a later sampling. Both Kruskal-Wallis analysis and Wilcoxon's test were used to distinguish these three groups. SPSS/PC+ was used for all calculations.

RESULTS AND DISCUSSION

Throughout the seasons of 1997–1998, monthly measurements were performed from March until October to

Table 1. Selected soil parameters of experimental plots and crops in the years of 1997 and 1998

Plot No.	Locality	Soil type	pН	C_{org} (%)	N_{tot} (%)	Crop 1997	Crop 1998 spring barley	
602	Lednice (Břeclav)	Chernozem	6.1	1.68	0.16	maize		
603	Ostrožská N. Ves	Cambisol	6.2	1.13	0.13	mixture	maize	
605	Jaroměřice n. Rokytnou	Luvisol	7.0	1.32	0.13	pea	rape	
613	Újezd u Brna	Chernozem	7.3	1.61	0.17	rape	winter wheat	
615	Moravský Žižkov	Chernozem	7.2	1.85	0.16	winter wheat	winter wheat	
628	Kunovice u U. Hradiště	Fluvisol	6.9	1.88	0.20	pea, spinach	spinach	
629	Nedachlebice	Luvisol	6.4	1.20	0.13	winter barley	rape	
632	Švábenice	Chernozem	6.6	1.69	0.18	winter wheat	poppy	
638	Tavíkovice	Luvisol	6.3	1.13	0.13	rape	winter wheat	
645	Nedakonice	Fluvisol	7.0	1.58	0.20	winter wheat	maize	

assess the parameters that are characteristic for N mineralization and nitrification (i.e. concentration of nitrate and ammonium nitrogen, anaerobic N mineralization, SNA, nitrification-incubation, urease activity) in the soils in 10 different areas of basal soil monitoring. Physico-chemical parameters of the soils and a survey of crops grown on these acreages are given in Table 1. Table 2 contains the data on N fertilizer application during the experimental period.

Soil nitrate concentrations were characterized by a remarkable seasonal pattern in both experimental years (Figure 1). In most of the plots, peaks were observed from April until June, followed by minima in June–July and a new increase from the month of August on. Sometimes, the increased nitrate concentrations were obviously due to N fertilizer application in the respective period (Table 2). There were three cases when the measurements confirmed significantly increased nitrate concentrations later than 70 days after N fertilizer application (57–112 kg N/ha) (602/97-V-VII, 613/97-IX-XI, 645/98-IV-VI).

In the course of the whole experimental period, the amounts of ammonium ions in all the soils monitored were remarkably lower compared to the nitrate levels. A significant increase in the concentration of the ammonium ions was mostly observed after N fertilizer application. As a rule, the ammonium nitrogen fell to the original level when measured at the subsequent sampling date, but no later than within 37 days. A long-term increase in NH₄+in the soil due to N fertilization was less frequent (605/98-V-VI). Ammonium ions released from N fertilizers were probably nitrified immediately as demonstrated by the quick increase in the concentration of nitrates in those fields where Amofos and ammonium sulfate were applied (Figure 1, Table 2).

The range of the measured values, means, maxima/minima and variation coefficients of the respective microbial parameters are summarized in Table 3. Kruskal-Wallis' analysis shows significant differences in nitrification-incubation among all three individual groups of samples. No significant differences in other microbial parameters were found among these groups. Non-parametric Wilcoxon's pair test showed significant differences in nitrifica-

tion-incubation between groups 1 and 2 and again between 2 and 3; and even showed differences in the content of mineral nitrogen, both in the form of NO₂ and NH₄⁺.

Anaerobic N mineralization reflecting the amounts of mineralized nitrogen represented a rather variable parameter without clear seasonal trends with the variation coefficients ranging between 14.0-59.0% (Table 3). There was no instantaneous increase in anaerobic N mineralization after N fertilizer application. Correlation analysis did not prove mutual relationship with the ammonium ions concentration (r = -0.1054, p < 0.3173). Higher anaerobic N mineralization can be explained as a result of previous accumulation of nitrogen by soil microorganisms (Sparling 1997). Consequently, an addition of mineral N to the soil could be manifested after mineral nitrogen immobilization, return liberation and transport to the soil. It should be admitted, however, that the period between N fertilization and sampling was comparatively short for these processes, and/or immobilization was limited by lack of mineralizable carbon. N mineralization fluctuation is rather related to changes in the temperature, moisture and organic substrate inputs into soil than due to the application of fertilizers containing mineral N.

SNA reflects maximal nitrification rate at the beginning of incubation because those parameters which influence nitrification (i.e. pH, substrate concentration) were normally at their optimal levels during the assessments and the short measuring period did not allow the nitrifiers to grow. SNA exhibited a remarkably lower variability in contrast to anaerobic N mineralization and nitrification-incubation (Table 3). Variation coefficients ranged between 15.5–31.2%. There was neither a clear seasonal fluctuation nor any impact of N fertilizers demonstrating that the potential nitrification remained relatively constant during the season and N fertilization had no impact on it.

Nitrification-incubation exhibited remarkable seasonal fluctuations without clear trends. A marked increase in ammonium ion concentration leads, as a rule, to a direct significant increase of nitrification-incubation. Following the decrease in the level of ammonium ions was accompanied by a steep decrease in nitrification-incubation activity (Figures 1 and 2). This shows that a limiting fac-

Table 2. A survey of N fertilizers applied in experimental fields during the period of investigation

Plot	Application date	Date of subsequent sampling	Fertlilizer	Nitrogen form	kg N/ha	
602	14. 3.	1. 4.	amofos	NH ₄ ⁺	18	
	21. 4.	2. 5.	ammonium sulfate	$\mathrm{NH_4}^+$	112	
	17. 10.	4. 11.	amofos	NH_4^+	35	
603	6. 10.	4. 11.	amofos	NH_4^+	30	
613	6. 3.	17. 3.	ammonium nitrate	NH ₄ ⁺ , NO ₃ ⁻	55	
	22. 8.	2. 9.	NPK	NH_4^+ , NO_3^-	36	
	24. 8.	2. 9.	ammonium sulfate	$\mathrm{NH_4}^+$	57	
615	9. 4.	2. 5.	LAV	NH ₄ ⁺ , NO ₃ ⁻	40	
	23. 8.	2. 9.	LAV	NH ₄ ⁺ , NO ₃ ⁻	27	
	4. 9.	2. 10.	LAV	NH ₄ ⁺ , NO ₃ ⁻	30	
629	6. 3.	10. 3.	LAV	NH ₄ ⁺ , NO ₃ ⁻	35	
	6. 5.	5. 6.	DAM	NH_4^+ , NO_3^- , $CO(NH_2)_2$	39	
	21. 8.	2. 9.	NPK	NH ₄ ⁺ , NO ₃ ⁻	39	
632	11. 4.	30. 4.	LAV	NH ₄ ⁺ , NO ₃ ⁻	49	
	13. 5.	2. 6.	DAM	NH_4^+ , NO_3^- , $CO(NH_2)_2$	40	
	17. 5.	2. 6.	DAM	NH_4^+ , NO_3^- , $CO(NH_2)_2$	30	
	26. 9.	1. 10.	DAM	NH_4^+ , NO_3^- , $CO(NH_2)_2$	15	
638	12. 3.	17. 3.	LAV	NH ₄ ⁺ , NO ₃ ⁻	40	
	2. 4.	2. 4.	LAV	NH ₄ ⁺ , NO ₃ ⁻	90	
	27. 9.	3. 10.	NPK	NH ₄ ⁺ , NO ₃ ⁻	30	
602/1998	4. 3.	2. 4.	ammonium sulfate	$\mathrm{NH_4}^+$	50	
	27. 8.	1. 9.	amofos	NH_4^+	20	
603	2. 4.	29. 4.	ammonium sulfate	NH_4^+	70	
605	16. 3.	3. 4.	LAV	NH ₄ ⁺ , NO ₃ ⁻	54	
	10. 4.	1. 5.	LAV	NH ₄ ⁺ , NO ₃ ⁻	54	
613	late VIII.	1. 9.	ammonium sulfate	NH_4^+	64	
615	27. 2.	2. 3.	LAV	NH ₄ ⁺ , NO ₃ ⁻	40	
	10. 4.	29. 4.	LAV	NH ₄ ⁺ , NO ₃ ⁻	40	
	4. 8.	1. 9.	LAV	NH ₄ ⁺ , NO ₃ ⁻	27	
	1. 9.	1. 10.	amofos	NH_4^+	12	
628	beginning III.	2. 3.	ammonium nitrate	NH ₄ ⁺ , NO ₃ ⁻	41	
	11. 3.	2. 4.	LAV	NH ₄ ⁺ , NO ₃ ⁻	27	
629	25. 2.	2. 3.	LAV	NH ₄ ⁺ , NO ₃ ⁻	54	
	8. 4.	29. 4.	DAM	NH_4^+ , NO_3^- , $CO(NH_2)_2$	29	
632	23. 4.	29. 4.	LAV	NH ₄ ⁺ , NO ₃ ⁻	35	
638	12. 3.	3. 4.	LAV	NH_4^+ , NO_3^-	30	
	26. 3.	3. 4.	LAV	NH_4^+ , NO_3^-	30	
645	20. 4.	29. 4.	urea	$CO(NH_2)_2$	69	

Amofos, DAM, LAV, and NPK are commercial brands of fertilizers

tor was substrate availability. The fact that the nitrification rate can be limited by the amount of available substrate is supported by correlation between nitrification and $\mathrm{NH_4^+}$ concentration (r = 0.3673, p < 0.0005). The natural concentration of ammonium ions in soils of the studied plots was very low during the observation period. The application of ammonium fertilizers acted as a strong stimulating factor for nitrification-incubation. Levels of nitrification-incubation in the soils without N fertilizer application reflected the N mineralization rate (Šimek 2000). The concentration of nitrate ions gave no evidence of the instantaneous nitrification rate. There was no corre-

lation between the two parameters (r = -0.0209, p < 0.8428). The concentration of soil nitrates is more important for understanding the process of nitrification during a prolonged period.

In most plots, urease represented the most stable parameter during the experimental period with variation coefficients ranging between 12.6–40.4%. The application of nitrogen fertilizers including those fertilizers with urea had no effect on urease activity (629/97-VI, 632/97-VI, X, 629/98/V, 645/98-V). The correlation analysis showed also a weak correlation between the seasonal cycle of urease activity and N mineralization (r = 0.2175, p < 0.0391). This

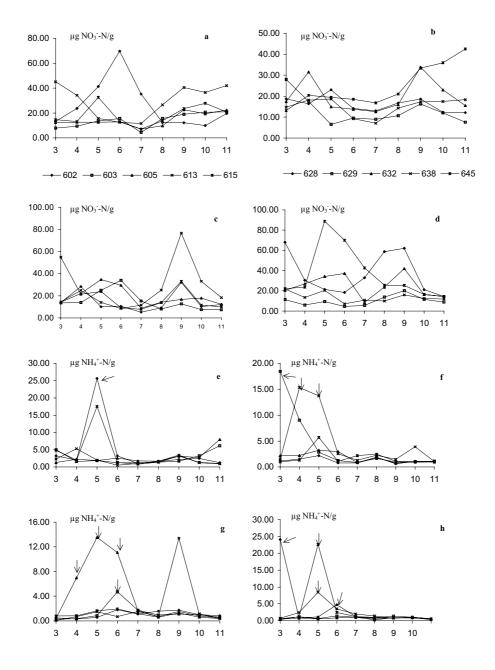


Figure 1. Seasonal variation of the concentrations of ammonium and nitrate ions during the period of 1997 (a, b, e, f) and 1998 (c, d, g, h); the legend at the Figure 1a is valid also for the Figures 1c, 1e, 1g, the legend at the Figure 1b is valid also for the Figures 1d, 1f, 1h; x-axis — month; arrow-heads point at dates of increased nitrification speed due to increased ammonium ions concentration

may be explained by the fact that urease is a very stable enzyme, and an important part of the estimated activity could be of an extracellular origin. For example, Klose and Tabatabai (1999) reported that 67% of the measured soil urease activity was extracellular.

CONCLUSION

Nitrate concentration was the only parameter under study of characteristics for N mineralization and nitrification in soil that exhibited a clear seasonal cycle with maximum in the spring and autumn months. Accumulation of the soil nitrates is proof of the increased N miner-

alization rate in the above-mentioned period. Increased N mineralization in the spring and autumn is probably due to root system development in the spring and as a result of post-harvest residues in the soil in the autumn. On the other hand, anaerobic N mineralization and nitrification-incubation reflected the actual state of the microbial community, which could be a reason why there was not comparable seasonal cycle in these, cases either. The nitrification-incubation method has proven to be a suitable approach to monitor the influence of direct N fertilizer on nitrification in soils with low ammonium concentration status.

It seems necessary to advise repeated samplings during the season when anaerobic N mineralization and ni-

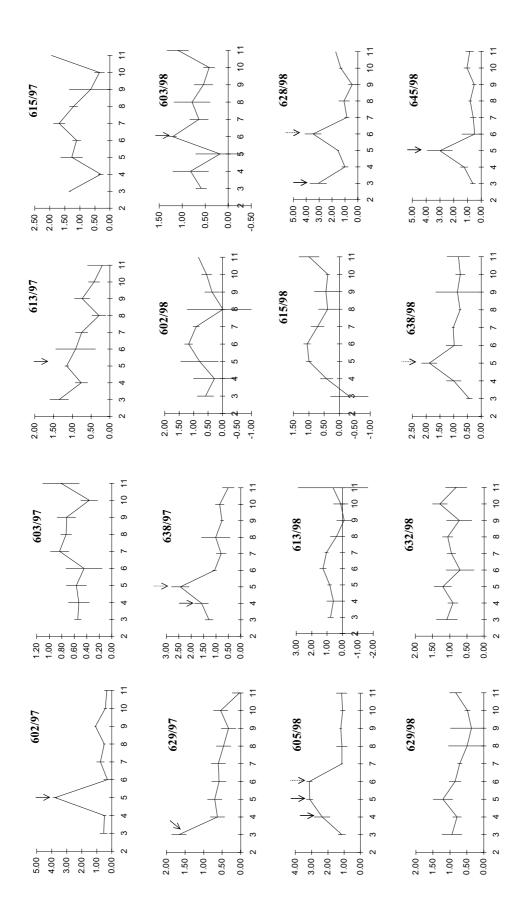


Figure 2. Seasonal patterns of nitrification-incubation in soils of fertilized plots during the period of 1997–1998; bars indicate 95% confidential intervals; x-axis – month; y-axis – μg N-NO₃/g/d; solid arrow-heads point at dates when nitrification of N fertilizers; dotted arrow-heads point at dates when nitrification-incubation rate was found to be higher due to increased ammonium ions concentration which did not immediately follow the application of N fertilizers

Table 3. Descriptive statistics of anaerobic N mineralization, nitrification-incubation, SNA and urease activity in the years of 1997-1998

Plots	Anaerobic N mineralization				SNA			Nitrification-incubation			Urease activity					
	mean	min	max	CV	mean	min	max	CV	mean	min	max	CV	mean	min	max	CV
602	2.70	1.22	3.94	28.5	669	392	883	20.0	0.76	-0.02	3.78	107	23.8	15.5	28.5	14.7
603	1.84	1.33	2.58	19.4	156	101	207	19.9	0.66	0.18	1.21	38.5	7.42	5.13	10.2	16.7
605	3.73	2.04	6.49	35.7	244	144	352	21.4	1.28	-0.15	3.13	64.6	10.7	7.52	15.4	16.3
613	3.64	1.51	5.25	31.0	846	485	1055	15.5	0.67	-0.10	1.35	59.6	30.5	24.2	38.6	13.4
615	2.98	0.83	7.94	59.0	513	332	679	20.2	0.83	-0.32	1.95	67.8	23.4	13.2	46.0	40.4
628	4.17	2.33	7.64	32.5	711	542	1034	16.7	1.71	0.43	3.52	47.6	21.5	15.1	25.2	14.9
629	2.87	2.07	3.71	14.0	413	300	521	16.6	0.68	0.04	1.66	52.5	8.73	4.61	12.3	21.0
632	3.95	2.58	4.96	16.4	524	339	685	19.1	0.93	0.50	1.29	23.2	18.2	13.92	26.3	19.1
638	3.50	2.45	6.08	22.6	247	132	412	31.2	1.04	0.43	2.44	47.0	11.3	7.45	13.5	15.9
645	5.04	2.56	9.21	36.4	1095	760	1500	19.4	1.01	0.01	3.01	65.1	29.5	24.4	36.5	12.6

CV - coefficient of variation

Units: anaerobic N mineralization – μg N-NH₄+/g/d, SNA – ng N-NO₇-/g/h, nitrification-incubation – μg N-NO₃-/g/d,

 $urease \ activity - \mu g \ N\text{-}NH_{_{\!\!4}}^{^{+}}\!/g/h$

trification-incubation are to be used for routine soil monitoring due to a higher natural variability of these parameters. Low natural SNA variability is an advantage in view of the applicability of this method as an indicator of environmental stress because it can reduce the probability of a false warning. Low seasonal variability in soil urease activity may be connected with the extracellular activity of this enzyme. For this reason, the suitability of the application of this parameter in the soil quality check system appears controversial.

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ABSTRAKT

Sezonní variabilita půdní N mineralizace a nitrifikace a vliv N hnojení na tyto procesy

Parametry charakterizující N mineralizaci a nitrifikaci byly měřeny v půdách deseti pozorovacích ploch bazálního půdního monitoringu Ústředního kontrolního a zkušebního ústavu zemědělského. Výrazná sezonní závislost byla nalezena v případě koncentrace nitrátového dusíku, která dosahovala maxim na jaře od dubna do června a v pozdním létě nebo podzimu, počínaje srpnem. Amonné ionty byly nitrifikovány bezprostředně po aplikaci hnojiva. Anaerobní N mineralizace představovala variabilní parametr, na který neměla bezprostřední vliv aplikace minerálních N hnojiv. Nitrifikace měřená jednotýdenní inkubací byla výrazně stimulována N hnojením, což potvrzuje, že limitujícím faktorem tohoto procesu je pravděpodobně dostupnost substrátu. Krátkodobá nitrifikační aktivita (SNA) nevykazovala výrazné sezonní fluktuace, což ukazuje na skutečnost, že potenciální rychlost nitrifikace se během sezony výrazně neměnila. Aktivita ureázy byla ve většině případů konstantní během roku a byla pouze slabě ovlivněna mineralizací N.

Klíčová slova: půda; N mineralizace; nitrifikace; dusíkatá hnojiva; sezonní variabilita

Corresponding author:

Doc. Ing. Bořivoj Šarapatka, CSc., Přírodovědecká fakulta, Univerzita Palackého, tř. Svobody 26, 771 46 Olomouc, Česká republika, tel.: + 420 68 563 45 60, fax: + 420 68 522 57 37, e-mail: sar@risc.upol.cz