

## Some biochemical aspects of *Fragi-Stagnic Albeluvisols*' organic matter

L. Szajdak<sup>1</sup>, P. Kuldkepp<sup>2</sup>, E. Leedu<sup>2</sup>, T. Teesalu<sup>2</sup>, A. Toomsoo<sup>2</sup> and R. Kõlli<sup>2\*</sup>

<sup>1</sup>Research Centre for Agricultural and Forest Environment, Polish Academy of Sciences, ul. Bukowska 19, 60-809 Poznań, Poland; e-mail: szajlech@man.poznan.pl

<sup>2</sup>Institute of Agricultural and Environmental Sciences, Estonian Agricultural University, Kreutzwaldi 64, 54014 Tartu, Estonia; e-mail: raimo@eau.ee

\*Author for correspondence

**Abstract.** The concentrations of  $\text{N-NH}_4^+$ ,  $\text{N-NO}_3^-$ ,  $\text{N}_{\text{total}}$ , total (TOC) and water extractable (DOC) organic carbon, P, K, Ca, Mg, S, the activity of nitrate reductase, the activity of urease activity as well as indole-3-acetic acid (IAA) were investigated in *Fragi-Stagnic Albeluvisols*' organic matter under different organic and mineral fertiliser regimes from a long-term soil formation of a field experiment with three-field crop rotation (potato – spring wheat – spring barley) at Eerika near Tartu, Estonia, and also in the forest of Tiksoja. The concentrations of  $\text{N}_{\text{total}}$ , IAA, the activity of urease and also C/N ratios were generally highest in the forest soil of Tiksoja.

Consequently, characteristics, distributions and conversions in organic matter were found in the proportions of chemical and biochemical factors, particularly after mineral and organic fertilisers. The following kinds of fertilisers increased the activity of urease in the order: WOM/N-120 < FYM/N-120 < RS/N-120 (accordingly, WOM – without organic manure, FYM – farmyard manure, RS – recultivation substance), and increased the activity of nitrate reductase: WOM/N-120 > RS/N-120 > FYM/N-120. The highest increase of IAA was determined in the soil fertilised with RS/N-120.

The experiment was characterised by the highest activity of urease, and the highest increase of IAA and also TOC in soils fertilised with RS/N-120. The various fertiliser regimes were different both for their biochemical factors as well as the crop yields. WOM/N-120 as a mineral fertiliser and RS/N-120 as an organic one revealed the highest crop yield of potato, spring wheat, and spring barley.

**Key words:** *Albeluvisols*, organic matter, long-term field experiment, organic and mineral fertilisation, urease and nitrate reductase, auxine, crop yields

### INTRODUCTION

*Fragi-Stagnic Albeluvisols* and closely with them associated soils, i.e. *Albeluvisols* (*glossic*, *fragic*, *stagnic*), and their endogleyic analogues form Estonian soil cover totally 9.5% or 21.3% of the arable and 3.6% of forest land (Kokk, 1995). On the area of our experiments (Tartu County), their role is very significant as they form more than half of the arable soils of the county. For the named soils, the presence of *albeluvic* tonguing (*glossalbic*) properties and the formation of a *fragic* (or in limited cases *argillic*) horizon are characteristic. In low parts of these soil profiles there

may occur temporal reducing conditions, which are reflected in the presence of *stagnic* or/and *gleyic* colour pattern (FAO, ISRIC & ISSS, 1998).

The importance of organic matter, widely presented in arable agricultural soils by the participation in biogeochemical conversions and pathways, is well established. Its dynamics are affected by humification, mineralisation, immobilisation, leaching, plant uptake, and root exudates. The quality and quantity of soil organic carbon has been suggested as a main factor having an impact on the soil nitrogen dynamics, because of the importance of labile carbon in the microbial immobilisation (Hart et al., 1994). Nitrogen included in organic matter rings is more constant and stable than that of mineral forms. Schimel (1986) indicated that nitrogen immobilisation was lower in cropland than in native grassland, suggesting that microbial activity in the cropland was limited by carbon substrate availability. Degradation of available carbon in cultivated fields may suggest a more rapid nitrogen turnover and losses than in forest or native grassland. Organic matter has also a significant function in regulating root metabolism of cultivated plants (Vaughan & Malcolm, 1985). Low molecular humic compounds may be released from organic and mineral-organic complexes in the process by low-weight carboxylic acid present in root exudates (Albuzio & Ferrari, 1989). These compounds enter the plant root and affect the plant metabolism, by inducing or inhibiting the mechanism of protein, sugars, and fats synthesis. These processes lead to changes in plant root cells, resulting in morpho-functional properties of the plants (Ladd & Butler, 1971; Vaughan & Malcolm, 1979; Piccolo et al., 1992). Albuzio and Ferrari (1989) reported also the impact of some humic fractions on the  $\text{N-NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{K}^+$  uptake.

The goal of this study was to estimate differences in the content and activity of biologically active substances found in the organic matter of arable soils under continuous crop rotation and in forest soils in their natural state. Additionally, the results obtained from all the experiments should give a better insight into changes taking place in organic matter during long-term cultivation.

## MATERIALS AND METHODS

### *Field trials, experimental details*

An international long-term experiment on the organic nitrogen or IOSDV- (Internationale Organische Stickstoffdüngungs Dauer Versuchreihe) with three-field crop rotation (potato – spring wheat – spring barley) was established on a *Fragi-Stagnic Albeluvisol* at Eerika near Tartu (N 58° 22.5'; E 26° 39.8') in 1989. The design of this field experiment is similar to other European network of IOSDV experiments (Boguslawski, 1995).

Each field of the experiment was divided according to the three backgrounds of organic manure:

- 1) without any organic manure from the beginning of the experiment (WOM),
- 2) farmyard manure 40 Mg ha<sup>-1</sup> for potato (FYM),
- 3) alternative organic fertilisers in autumn before ploughing (beet leaves until 1995, straw since 1996).

In autumn 2001, recultivation substance (RS – highly alkaline oil shale semi-coke mixed with acidic sphagnum peat at a voluminous ratio of 1:1) at a rate of 20–60 Mg ha<sup>-1</sup>, was applied. In autumn 2002, composts (40 Mg ha<sup>-1</sup>) formed from RS and solid

fraction of pig slurry for potato or created from RS with sewage sludge for spring barley were applied.

Each background of organic manure (totally 3) was divided into five mineral fertiliser treatments (0, 40, 80, 120 and 160 kg N ha<sup>-1</sup>), of which two application rates of mineral fertiliser (0 and 120 kg ha<sup>-1</sup>) were used in three replications in the actual work. The combined fertilisers OptiCrop<sup>®</sup> 21-08-12 and ProMagna<sup>®</sup> 11-11-21 SG from NORSK HYDRO ASA were used in the field experiment.

The soil samples described in the presented work and taken from different fertiliser backgrounds of arable soils were marked by using the following numeration and nomination:

S1 – without any organic and mineral fertilisers –WOM/N-0,

S2 – direct or after-effect of farmyard manure without additional mineral fertiliser – FYM/N-0,

S3 – after-effect of RS or direct effect of compost made from RS with solid fraction of pig slurry or sewage sludge without additional mineral fertiliser – RS/N-0,

S4 – without organic manure, but using mineral fertiliser 120 kg N ha<sup>-1</sup> – WOM/N-120,

S5 – farmyard manure with mineral fertiliser 120 kg N ha<sup>-1</sup> - FYM/N-120,

S6 – composts from RS with mineral fertiliser 120 kg N ha<sup>-1</sup> - RS/N-120,

S7 – A horizon of forest *Fragi-Stagnic Albeluvisol*.

#### *History of land use and statistical analyses*

The area used for the foundation of the field experiment has the following land use history. Before foundation of the experiment in 1989, it was in a set-aside state (5–6 years) as field-grass fallow. In the period 1957–83, it was used as arable land in the condition of state farm. As arable land it was used for more than one century. For calculation of means and the analyses of variance, the PC program MS Excel was used.

#### *Chemical and biochemical analysis*

Soil samples were taken with soil core from the upper 20 cm (humus horizon). Samples were air-dried and crushed to pass a 1 mm-mesh sieve. For determination of pH (in 1M KCl), P, K, Ca, Mg, S, dissolved organic carbon (DOC), total organic carbon (TOC), total nitrogen (N<sub>total</sub>), N-NO<sub>3</sub><sup>-</sup>, N-NH<sub>4</sub><sup>+</sup>, the activity of urease and nitrate reductase and also indole-3-acetic acid, “mean samples” were taken.

According to the ISA 10390 standard, pH values were measured in 1M KCl, and the contents of the plant available P, K, Ca, Mg, S were determined from one soil extraction by the Mehlich-3 method (Rowel, 1994).

Twice distilled water from silica glass equipment was used. For the estimation of DOC, soil samples in redistilled water were heated in 100°C for two hours under a reflux condenser. The extracts were separated by the mean filter paper and analysed on TOC 5050A (Shimadzu, Japan) (Smolander & Kitunen, 2002). Total organic carbon on TOC 5050A and N<sub>total</sub> were determined by the semimicro-Kjeldahl methods (ISRIC; cit. by Reeuwijk, 1995).

The concentrations of N-NO<sub>3</sub><sup>-</sup> and N-NH<sub>4</sub><sup>+</sup> in the soils were calculated from the early prepared analytical curve estimated on the basis of the Beer - Walter equation:

$$A = a \cdot c \cdot l \quad (1)$$

where: A – absorbance; a – molar absorption coefficient  $\text{dm}^3 \text{ cm mol}^{-1}$ ; c – concentration  $\text{mol dm}^{-3}$ ; l – thickness of layer in cm.

The mean absorbance of  $\text{N-NO}_3^-$  or  $\text{N-NH}_4^+$  is linear according to the Beer's-Walter law. The activity of nitrate reductase in field-moist soil samples was measured by the Kandeler (1996) and the urease activity by Hoffman and Teicher technique (Szajdak & Matuszewska, 2000). The investigations of IAA have been performed by the fluorimetric method (Szajdak, unpublished data).

All the experiments were run in triplicate, and the results were averaged. All the chemicals used in this study were of analytical grade. The precisions based on replicate analyses were  $\pm 0.01$  for pH measurements,  $\pm 4\%$  for P,  $\pm 4\%$  for K,  $\pm 4\%$  for Ca,  $\pm 4\%$  for Mg,  $\pm 4\%$  for S,  $\pm 3\%$  for TOC,  $\pm 3\%$  for DOC,  $\pm 4\%$  for  $\text{N}_{\text{total}}$ ,  $\pm 3\%$  for  $\text{N-NO}_3^-$ ,  $\pm 3\%$  for  $\text{N-NH}_4^+$ ,  $\pm 4\%$  for nitrate reductase activity,  $\pm 4\%$  for urease activity and  $\pm 4\%$  for IAA.

## RESULTS AND DISCUSSION

In order to determine agrochemical properties of humus horizons from the experimental plots, the samples were taken in spring before applying mineral fertilisers (Table 1). Applying high levels of fertilisers in the form of WOM/N-120, FYM/N-120, RS/N-120 had no effect on the concentrations of P, K, Ca, Mg, S and humus and also on pH values between the fertilisers representing low contents of N: such as WOM/N-0, FYM/N-0 and RS/N-0. The chemical parameters of different forms of soil nitrogen and carbon, IAA, the activity of urease and the activity of nitrate reductase were determined in samples taken in autumn after harvesting of crops (Table 2). The contents of  $\text{N}_{\text{total}}$  in different fertiliser backgrounds of the field experiment ranged from 0.90 to 1.02  $\text{g kg}^{-1}$ . However, the application of FYM/N-120 decreased the concentrations of  $\text{N}_{\text{total}}$ . The application of RS/N-120 did not reveal any significant effect on the content of total nitrogen, while RS/N-0 was poor in nitrogen. Mineral fertiliser (WOM/N-120) increased the total nitrogen content only in comparison with RS/N-0. Due to the application of the mineral fertiliser WOM/N-120  $\text{kg N per ha}$  in comparison with WOM/N-0, a decrease in the content of total nitrogen was observed. Moreover, the application of WOM/N-120, increasing the crop yield the highest, has been found to be closely related to crop yield, irrespective of the cultivated plants potato, spring barley and spring wheat (Table 3).

The lowest content of TOC was measured in the treatment with WOM/N-0 where additional organic manure had not been used for 14 years. The treatment with farmyard manure during the investigated period led to an increase in the content of TOC. Thus, the present results support the previous findings from IOSDV (Kuldkepp, 1997). Moreover, the highest mineral fertiliser WOM/N-120 increased the content of TOC only in comparison with RS/N-0.

The hot water extractable carbon is well known as a sensitive indicator of changes and challenges in soil management and also of agricultural practices. DOC can contribute significantly to the cycling of soil nutrients. DOC seems to be a major vehicle for leaching many elements from the litter. The primary sources of DOC are considered to be the leaching of substances from fresh litter and the products of plant

residue decomposition (Qualls et al., 1991; Smolander & Kitunen, 2002). The concentrations of DOC in these investigations ranged from 0.04 to 0.045 g kg<sup>-1</sup>. The lowest concentrations of DOC were measured in the treatment without organic manure and also when RS/N-0 was used and were equal to 0.04 g kg<sup>-1</sup>. The highest content of DOC revealed by the forest soil equaled to 0.13 g kg<sup>-1</sup>. The labile nature of dissolved C and the reasonable function of its pool (usually constitutes of 3–6% of TOC in soils) give DOC measurements an early indication of the possibility of organic matter loss (Ghani et al., 2003). At the same time, DOC exhibits a better relationship with mineral fertilisation and N<sub>total</sub> and also with N<sub>mineral</sub> forms than TOC in soil. There was also a negative correlation between the content of different forms of organic carbon and N-NH<sub>4</sub><sup>+</sup>.

A significant source of mineral nitrogen in soil is the degradation of organic substances. Its dynamics are affected by mineralisation, immobilisation, leaching, root exudates, and plant uptake (Murdam et al., 1994; Kanal et al., 1999). Sufficient inorganic nitrogen supply for the crop growth is crucial for organic farming to be economically sustainable. On the other hand, minimisation of inorganic nitrogen leaching is a criteria for any farming system to be considered environmentally sound. A significant conflict occurred between these two objectives, since any supply of inorganic nitrogen (through fertilisers or mineralisation from incorporated organic matter) is only partly available to the growing crop, the rest being prone to leaching (Korsaeth et al., 2002). N<sub>mineral</sub> in soils revealed very low concentrations ranging from 13.4 to 35.1 mg kg<sup>-1</sup> in arable soil. The lowest content of N<sub>mineral</sub> was measured in forest soils and equalled to 10.6 mg kg<sup>-1</sup>. The content of N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup> was almost similar in comparison with the background (WOM/N-0). The contents of N-NH<sub>4</sub><sup>+</sup> were twice higher than the concentrations of N-NO<sub>3</sub><sup>-</sup> in soils fertilised with RS/N-0. Similarly with the soil fertilised with RS/N-0, the content of N-NH<sub>4</sub><sup>+</sup> was two times higher than the concentrations of N-NO<sub>3</sub><sup>-</sup> in the very acidic forest soil. In this investigation, the contents of N-NH<sub>4</sub><sup>+</sup> in the fields ranged from 8.4 to 17.5 mg kg<sup>-1</sup>. Similar dates were expressed by Hannolainen (1970), 25–39 mg kg<sup>-1</sup>.

The total amount of the concentrations of N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup> in soil without organic manure (WOM/N-0) were two times higher than in the treatment where 120 kg N per ha was applied with mineral fertiliser. Organic nitrogen fertiliser in the form of RS/N-120 increased 2.6 times the content of N-NO<sub>3</sub><sup>-</sup> in comparison with RS/N-0 (Table 2).

The arable soil of the experimental plots was from slightly acid to neutral. But the humus horizon of the Tiksoja forest soil revealed very acid properties. The relationship between different agrochemical parameters of soil fertility and experimental factors was moderate. However, the content of mineral forms of nitrogen and N<sub>total</sub> was negatively correlated with the treatments of fertilisation. At the same time, the total and easily mineralisable carbon (TOC and DOC) exhibited a positive correlation with organic manure application. The negative impact of mineral nitrogen fertilisers on the accumulation of N-NH<sub>4</sub><sup>+</sup> in soil was mentioned earlier by Kuldkepp and Suitso (1997).

**Table 1.** Agrochemical parameters of soils taken in spring 2003 (average of 9 samples with standard deviation ).

Parameter	Samples					
	S1	S2	S3	S4	S5	S6
	WOM/N-0	FYM/ N-0	RS/N-0	WOM/N-120	FYM/N-120	RS/N-120
pH <sub>KCl</sub>	6.38±0.11	6.51±0.10	6.92±0.11	6.20±0.19	6.31±0.10	6.73±0.10
P mg kg <sup>-1</sup>	116±14	135±14	131±19	124±11	150±11	131±7
K mg kg <sup>-1</sup>	113±13	196±32	190±36	137±11	219±27	195±23
Ca mg kg <sup>-1</sup>	1181±34	1297±80	1646±150	1191±105	1218±113	1443±147
Mg mg kg <sup>-1</sup>	82±4.1	106±4.5	114±12.0	84±6.1	107±8.0	109±8.4
S mg kg <sup>-1</sup>	13.7±2.0	15.9±1.5	24.7±5.3	16.2±1.0	18.0±1.3	28.3±6.5
Humus %	1.57±0.09	1.89±0.13	1.86±0.13	1.64±0.06	1.98±0.07	1.86±0.04

**Table 2.** Agrochemical parameters of soils taken in autumn 2003.

Parameters	Samples						
	S1	S2	S3	S4	S5	S6	S7*
	WOM/N-0	FYM/N-0	RS/N-0	WOM/N-120	FYM/N-120	RS/N-120	
N <sub>total</sub> %	0.090	0.102	0.090	0.094	0.099	0.098	0.174
N <sub>mineral</sub> mg kg <sup>-1</sup>	35.1	22.2	13.4	16.1	21.3	20.5	10.6
N-NH <sub>4</sub> mg kg <sup>-1</sup>	17.5	10.8	9.1	8.4	9.0	9.4	7.3
N-NO <sub>3</sub> <sup>-</sup> mg kg <sup>-1</sup>	17.6	11.4	4.3	7.7	12.2	11.2	3.3
TOC %	0.93	1.12	0.98	0.82	1.11	1.06	2.37
DOC %	0.04	0.045	0.04	0.04	0.045	0.045	0.13
C/N	10.1	10.7	12.0	10.1	11.6	11.0	13.6
pH <sub>KCl</sub>	6.5	6.6	7.1	6.4	6.5	7.1	4.2
Activity of urease [μmol of urea g <sup>-1</sup> .h <sup>-1</sup> d.m]	0.88	1.13	0.97	0.94	1.47	1.75	5.15
Activity of nitrate reductase [μgN·g <sup>-1</sup> d.m. 24h <sup>-1</sup> ]	0.0732	0.1934	0.2507	0.1255	0.1690	0.2410	0.1805
IAA [mg·kg <sup>-1</sup> d.m.]	25.95	26.96	18.97	20.48	24.97	22.47	39.17
				-21.1%	-7.4%	+18.5%	

\* Sample No 7 was taken from the Tiksoja forest

**Table 3.** Crop yields (Mg ha<sup>-1</sup>) of potato, spring wheat and spring barley in 2002 and 2003 under different kinds of fertilisers.

Fertilisers	Amounts of N kg ha <sup>-1</sup>	Potato		Spring wheat		Spring barley	
		2002	2003	2002	2003	2002	2003
Without organic manure – WOM	0, (S1)	16.71	16.97	0.76	1.85	1.30	1.51
		27.02	26.21	1.84	4.30	2.88	5.33
	120, (S4)	<i>58.1%</i>		<i>137.3%</i>		<i>187.2%</i>	
Farmyard manure – FYM	0, (S2)	24.16	20.25	1.34	2.21	1.33	2.48
		31.43	26.86	3.01	4.53	2.87	5.65
	120, (S5)	<i>31.4%</i>		<i>114.8%</i>		<i>121.8%</i>	
Recultivation substance and composts – RS	0, (S3)	18.59	20.03	1.60	1.96	1.03	2.41
		28.47	26.89	2.93	4.24	3.00	5.79
	120, (S6)	<i>43.7%</i>		<i>99.7%</i>		<i>165.8%</i>	

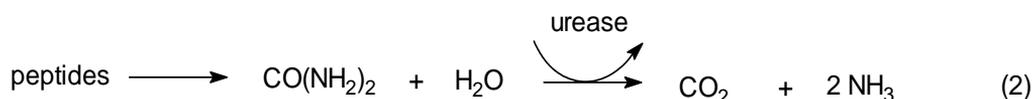
*italics* - mean increase of crop yields from 2002 to 2003 in per cents

The reason of that is supposed to be the enhanced attack of nitrifiers on ammonium. The N-NO<sub>3</sub><sup>-</sup> used to be higher in spring compared with autumn.

The reason of the low level of N<sub>mineral</sub> is probably connected with the late time of sampling in autumn. Therefore, the low temperature and a lack of substrate for microorganisms were the reasons for the low N<sub>mineral</sub>. Niklinska et al. (1999) have shown that the rate of nitrogen mineralisation increases significantly above 15% in autumn. The study showed that DOC represented a negative correlation with mineral forms of nitrogen such as N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup> as well as with TOC and IAA. Although, the highest linear values of correlation were characterised between DOC and IAA (r = -0.90). The correlation between DOC and several agrochemical properties was the following: with pH and Ca content the correlations were positive and strong (r = 0.58), with TOC and N<sub>total</sub> negative (r = -0.48 and r = -0.33, respectively), but with P and K very weak and negative (r = -0.23 and r = -0.02).

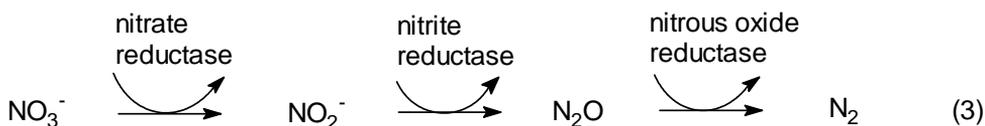
In addition, our investigations showed that the soil treated with organic manure (FYM/N-120) was characterised by high microbial activity represented by the high activity of enzymes. The activity of urease and nitrate reductase in soils fertilised with FYM/N0 and FYM/N-120 and also RS/N-0 and RS/N-120 were higher than in soil treated only with chemical fertilisers. Similar findings presented in our research were observed also in the case of using dairy effluent (Zaman et al., 2002). The synergism of mineral and organic fertilisers always resulted in higher activity of microorganisms in comparison with the manure FYM/N-0 or FYM/120 treatment.

Urease participates in the hydrolytic decomposition of urea. Ammonia produced during this process is strongly absorbed by soil, which makes it safer in the case of larger nitrogen losses, being at the same time easily accessible to plants (2).



The study of urease in the soils revealed a wide range of the activity of this enzyme: from 0.88 to 5.15  $\mu\text{gN}\cdot\text{g}^{-1} \text{ d.m. } 24\text{h}^{-1}$  in the soils of the investigated plots (Table 2). The lowest activity of this enzyme was measured in the soil without any organic fertiliser and the highest in the Tiksoja forest soil. Due to this combination of organic and mineral fertilisers, an increase of the activity of urease was observed. As a result of this application, the highest increase, equal to 79.4%, was measured in the soil by applying composts from RS with mineral fertiliser in a doze of 120 kg N ha<sup>-1</sup> and the smallest (6.8%) using mineral fertiliser 120 kg ha<sup>-1</sup> without organic manure (Table 2).

Denitrification is defined as the “microbial reduction of nitrate or nitrite coupled to electron transport phosphorylation resulting in gaseous N either as molecular N<sub>2</sub> or as an oxide of N” (Martens, 2005). Nitrate reductase is an enzyme involved in the process of denitrification. Nitrogen presence in the structure of this enzyme acts as a terminal acceptor of electrons instead of molecular O<sub>2</sub> by bacteria and is irreversible once NO is formed (Abdelmagid & Tabatabai, 1987). During a low content of oxygen, nitrate ions are reduced to nitrite, and this process is catalysed by nitrate reductase. Further NO<sub>2</sub><sup>-</sup> is reduced to N<sub>2</sub>O by nitrite reductase (Fu & Tabatabai 1989; Ma, 2000). NO is an intermediate substance reacting very lightly with oxygen. The conversion of N<sub>2</sub>O to N<sub>2</sub> is catalysed by nitrous oxide reductase (3). Finally this process leads to the loss of nitrogen in soil mainly in the form of N<sub>2</sub> and N<sub>2</sub>O. The evolution of N<sub>2</sub>O from soil is negative. N<sub>2</sub>O is a greenhouse gas causing significant depletion of the Earth’s stratospheric ozone layer and contributing to the warming of the Earth’s surface (Carnol & Ineson, 1999; Robertson & Klemedtsson, 1996; Zechmeister-Boltenstern et al., 2002). Nitrogen is also removed in the form of NO<sub>3</sub><sup>-</sup> from soil. Nitrates are not absorbed by the sorptive complex and, due to rainfall, they could migrate into the deep layer of soil and also to the ground water. The reduction of nitrates may also transform into ammonium as a result of a contrary reaction to the nitrification process.

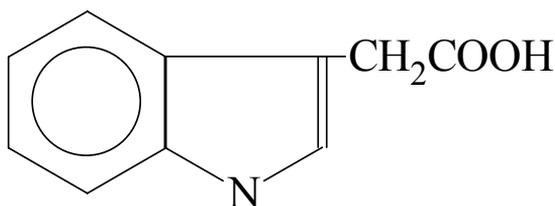


The fertilisers WOM/N-120, FYM/N-120, RS/N-120 had an impact on the changes of activity of nitrate reductase in the soils (Table 2). WOM/N-120 has resulted in a 71.4% increase of the activity of this enzyme, compared with WOM/N-0. The highest increase in the activity of this enzyme in soil with WOM/N-120 suggests the highest loss of nitrogen in the form of volatile compounds such as N<sub>2</sub>O and N<sub>2</sub> from all applied fertilisers. In contrast with WOM/N-120 the different situation was observed

for FYM/N-120 and RS/N-120 (Table 2). These two fertilisers decrease the activity of nitrate reductase by 12.6% and 3.8%, respectively, compared with to FYM/N-0 and RS/N-0, indicating a lower loss of nitrogen than FYM/N-120 from soils.

The high value of the activity of nitrate reductase in forest soil, equal to  $0.1805 \mu\text{gN}\cdot\text{g}^{-1} \text{ d.m. } 24\text{h}^{-1}$ , and the highest content of TOC, equal to 2.37%, and also the highest concentration of  $N_{\text{total}}$  are responsible for the optimum conditions for denitrification. Mazur suggested (1991) that a high content of moisture and organic matter and also neutral and basic pH favour denitrification. The rate of denitrification is also decreased by a high content of oxygen, nitrate,  $\text{H}^+$ , and  $\text{S}^-$  in soil.

Plant growth regulators are considered to be of importance in crop yield and in increasing of soil productivity. IAA seems to perform an important function in nature as a result of its influence on the regulation of plant growth and development. A principal feature of IAA is its ability to affect the growth, development and health of plants. This compound affects root morphology and metabolic changes in the host plant. The physiological impact of this substance is involved in cell elongation, apical dominance, root initiation, parthenocarpy, abscission, callus formation, and the respiration (Tena et al., 1986; Strzelczyk et al., 1992).



### Indole-3-acetic acid

The concentrations of IAA in the cultivated fields ranged from 18.97 to 26.96  $\text{mg}\cdot\text{kg}^{-1} \text{ d.m.}$  The lowest concentration of IAA, equal to 18.97  $\text{mg}\cdot\text{kg}^{-1} \text{ d.m.}$ , was determined in the soils fertilised with RS/N-0 (Table 2). The study revealed that the applying of RS/N-120 increased the highest, by 18.5%, the concentrations of IAA in the soils. Although, the two other fertilisers WOM/N-120 and FYM/N-120 decreased the concentrations of IAA in cultivated fields by 21.1% and 25.0%, respectively. The contents of IAA in the forest soils were higher by more than 30% than in the cultivated fields.

Our results proved evidence that in comparison with crop yields, as a result of applying two different fertiliser dozes, 0 and 120  $\text{N kg ha}^{-1}$ , the mineral fertiliser WOM/N-120 increased crop yields of potato, spring wheat, spring barley much more than organic fertilisers – farmyard manure (FYM/N-120) or recultivation substance and composts (RS/N-120) (Table 3). In this experiment, recultivation substance and composts (RS/N-120) increased the crop yields of potato by 44%, these of spring wheat by 100% and these of spring barley by 166% but farmyard manure (FYM/N-120) by 31%, 115% and 122%, respectively. Such findings agree with the conclusions of Szajdak et al., (2004). They suggested that in long-term investigations on

biologically active substances in soils under crop rotation and continuous cropping of rye, the mineral fertiliser NPK increased the content of bound amino acids, total acidity, phenolic and carboxylic groups in humic acids more than manure.

## CONCLUSIONS

The results obtained in the present study show that the content of different kinds of nitrogen and organic carbon and phytohormone (IAA), the activities of enzymes participating in nitrogen, such as urease and nitrate reductase, and crop yields depend on cultivated plants and the kind of fertilisers. The application of WOM/N-120, irrespective of the plant cultivated, leads to the highest crop yields. WOM/N-120 revealed the highest increase of the activity of nitrate reductase and IAA concentrations and the smallest activity of urease. The highest content of IAA, TOC,  $N_{\text{total}}$ , and the activity of urease measured in the forest soil indicated optimum conditions for denitrification in the soil.

ACKNOWLEDGEMENTS. The authors wish to thank the Agricultural Research Centre (APC) for chemical analysis, and it is also a pleasure to acknowledge the helpful assistance of Mrs. Teresa Stachecka.

## REFERENCES

- Abdelmagid, H.M. & Tabatabai, M.A. 1987. Nitrate reductase activity of soils. *Soil Biology and Biochemistry* **19**, 421–427.
- Albuzio, A. & Ferrari, G. 1989. Modulation of the molecular size of humic substances by organic acids of the root exudates. *Plant and Soil* **113**, 237–241.
- Boguslawski, E. von. 1995. Das Zusammenwirken der mineralischen Düngung mit verschiedenen Formen der organischen Düngung. In *Boden and Düngung*. Tartu, pp. 9–22.
- Carnol, M. & Ineson, P. 1999. Environmental factors controlling  $\text{NO}_3^-$  leaching,  $\text{N}_2\text{O}$  emissions and numbers of  $\text{NH}_4^+$  oxidisers in a coniferous forest soil. *Soil Biology and Biochemistry* **31**, 979–990.
- FAO, ISRIC & ISSS. 1998. *World Reference Base for Soil resources*. World Soil Resources reports 84, Rome.
- Fu, M.H. & Tabatabai, M.A. 1989. Nitrate reductase activity in soils: effects of trace elements. *Soil Biology and Biochemistry* **21**, 943–946.
- Gahni, A., Dexter, M. & Perrot, K.W. 2003. Hot water extractable carbon in soils: a sensitive measurement for determining impacts of fertilization, grazing and cultivation. *Soil Biology and Biochemistry* **35**(9), 1231–1243.
- Hart, S.C., Nason, G., Myrold, D. & Perry, D. 1994. Dynamics of gross nitrogen nitrogen transformation in an old-growth forest: the carbon connection. *Ecology* **75**, 880–891.
- Hannolainen, E. 1970. Content of mobile nitrogen in humus layer of arable lands of Estonian SSR. *EMMTUI Proceedings*, XX, Tallinn, pp. 172–183.
- Kanal, A., Kuldkepp, P., Truu, J. 1999. Microflora activity and chemical properties of Podzoluvisol in field experiment with different manures. *Arch. Acker-Pfl. Boden.* **44**, 507–521.
- Kandeler, E. 1996. Nitrate reductase activity. In *Methods in soil biology* (Schinner F., Öhlinger R., Kandeler E. & Margesin R., eds), pp. 176–179. Springer-Verlag, Berlin Heidelberg.

- Kokk, R. 1995. Distribution and Properties of Soils. In *Estonia. Nature* (Raukas, A. ed.), pp. 430–439. Valgus, Tallinn.
- Korsaeth, A., Henriksen, T.M. & Bakken, L. R. 2002. Temporal changes in mineralization of N during degradation of plant material: implications for the plant N supply and nitrogen losses. *Soil Biology and Biochemistry* **34**, 789–799.
- Kuldkepp, P. 1997. Wirkung und Wechselwirkung unterschiedlicher mineralischer und organischer N- Düngung auf Ertrag und Bodeneigenschaften im IOSDV Tartu (Estland) nach 6 Jahren. *Arch. Acker-Pfl. Boden* **42**, 21–32.
- Kuldkepp, P. & Suitso, A. 1997. The dynamics of nitrate nitrogen content in sandy loam soil during the vegetation period as depending on fertilization. In *Proceedings of NorFA seminar*, pp. 59–63. Jelgava.
- Ladd, J.N. & Butler, J.H.A. 1971. Inhibition by soil humic acids of native and acetylated proteolytic enzymes. *Soil Biology and Biochemistry* **3**, 157–160.
- Ma, R.X. 2000. Effects of allelochemicals on activity of nitrate reductase. *Journal of Environmental Science* **12**, 125–128
- Martens, D.A. 2005. Denitrification. In *Encyclopedia of Soils in the Environment* (Hatfield J.L., Powlson D.S., Crosenzweig D.S, Scow K.M., Singer M.J. and Sparks D.L., eds), pp. 378–382. Academic Press, Amsterdam.
- Mazur, T. 1991. The conversions of nitrogen compounds in soil. In *Nitrogen in agricultural soils* (Mazur T., ed.), pp. 66–98. PWN, Warszawa.
- Murdam, L., Vardjas, N. & Kuldkepp, P. 1994. Efficacy of mineral fertilizers and their danger to environment arise from common field. In *Problems of contemporary ecology* (T. Frey, ed.), pp. 69–74.
- Niklinska, M., Marianski, M. & Laskowski, R. 1999. Effect of temperature on humus respiration rate and nitrogen mineralization: Implication for global; climate change. *Biochemistry* **44**, 239–257.
- Piccolo, A., Nardi, S. & Concheri, G. 1992. Structural characteristics of humic substances as related to nitrate uptake and growth regulation in plant systems. *Soil Biology and Biochemistry* **4**, 373–380.
- Qualls, R.G. & Haines, B.L. 1991. Geochemistry of dissolved organic nutrients in water percolation through a forest ecosystem. *Soil Science Society of America Journal* **55**, 1112–1123.
- Reeuwijk, L.P. van (ed.) 1995. *Procedures for Soil Analysis*. Wageningen: ISRIC, Technical Paper 9.
- Robertson, K. & Klemetsson, L. 1996. Assessment of denitrification in organogenic forest soil by regulating factors. *Plant and Soil* **178**, 49–57.
- Rowel, D.L. 1994. *Soil Science: Methods and Applications*. Prentice Hall, Harlow, 360 pp.
- Schimel, D.S. 1986. Carbon and nitrogen turnover in adjacent grassland and cropland ecosystems. *Biogeochemistry* **2**, 345–357.
- Smolander, A. & Kitunen, V. 2002. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. *Soil Biology and Biochemistry* **34**, 651–660.
- Strzelczyk, E., Pokojaska, A. & Kampert, M. 1992. The effect of pH on production of plant growth regulators by mycorrhizal fungi. *Symbiosis* **13**, 201–215.
- Szajdak, L. & Matuszewska, T. 2000. Reaction of woods in changes of nitrogen in two kinds of soil. *Polish Journal of Soil Science* **33**, 9–17.
- Szajdak, L., Życzyńska-Bałoniak, I., Meysner, T. & Blecharczyk, A. 2004. Bound amino acids in humic acids from arable cropping systems. *Journal of Plant Nutrition and Soil Science* **167**, 562–567.
- Tena, M., Pinilla, J.A. & Magallanes, M. 1986. L-phenylalanine deaminating activity in soil. *Soil Biology and Biochemistry* **18**(3), 321–325.

- Vaughan, D. & Malcolm, I.R. 1979. Effect of humic acids on invertase synthesis of higher plants. *Soil Biology and Biochemistry* **11**, 247–252.
- Vaughan, D. & Malcolm, I.R. 1985. *Influence of humic substances on growth and physiological processes, in soil organic matter and biological activity*. Martinus Nijhoff Publisher, Dordrecht. The Netherlands, pp. 37–76.
- Zaman, M., Cameron, K.C., Di H.J. & Inubushi, K. 2002. Changes in mineral N, microbial biomass and enzyme activities in different soil depths after surface application of dairy shed effluent and chemical fertilizer. *Nutrient Cycling in Agroecosystems* **63**, 275–290.
- Zechmeister-Boltenstern, S., Hahn, M., Meger, S. & Jandl, R. 2002. Nitrous oxide emissions and nitrate leaching in relation to microbial biomass dynamics in a beech forest soil. *Soil Biology and Biochemistry* **34**, 823–832.