Relations between activities and counts of soil microorganisms

E. Popelářová, K. Voříšek, S. Strnadová

Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic

ABSTRACT

Microbial activities and counts of microorganisms were monitored as a part of research projects at 11 localities on arable land of different soil types during the years 2002–2006. The counts of microorganisms (total bacteria count, actinomycetes, micromycetes, *Azotobacter*, oligotrophic and spore-forming bacteria) and microbial activities (respiration, ammonification and nitrification tests) were evaluated using summary statistics, analysis of variance and correlation coefficients. The average counts of microorganisms corresponded with usual counts of microbes in arable soils. There were only some differences among localities in *Azotobacter* counts. Soil respiration is very often used as an indicator of soil microflora activity. Average values of basal respiration were slightly lower (0.45 mg CO_2/h per 100 g dry soil) than usual values, potential respiration with glucose (G) or with ammonium and G (NG) responded to usual values (average G 4.27, NG 9.53 mg CO_2/h per 100 g dry soil). All activities (except actual ammonification) were higher in spring season, but the differences were not significant. There were significant differences in correlation coefficients among the selected criteria; 66% from the total number of correlation coefficients were non-significant, 34% were significant (13% of them was at the significance level 0.05, 10% $P < 0.01$, and 11% $P < 0.001$).

Keywords: respiration; ammonification; nitrification; bacteria; fungi; correlation coefficient; soil

Soil microbiology traditionally deals with the study of microorganisms and their processes in soil. The interaction among organisms and their environments involves soil ecology (Paul 2007).

Soil is a heterogeneous medium of solid, liquid and gaseous phases varying in its properties both across the landscape and in depth (Luo and Zhou 2006). Soil microorganisms significantly contribute to the maintenance of the matter and energy turnover in terrestrial environment. Soil represents one of the most significant places for biogeochemical processes, in which mineralization has a very important role.

Soil respiration is an ecosystem process that releases carbon dioxide (CO_2) from soil root respiration, microbial decomposition of litter and soil organic matter and fauna respiration. Since the global carbon regulates climate change, soil respiration also becomes relevant to climate change, carbon trading and environmental policy. Soil respiration plays a crucial role in regulating atmospheric CO_2 concentration and in climate regulation. The carbon cycle on the global scale involves exchanges of CO_2 among the land biosphere, atmosphere, oceans and the earth crust (Luo and Zhou 2006). Mineralization tests are very often used for testing the soil microbial activity. CO_2 production enables to evaluate both mineralization activity of native soil organic matter and potential activity of soil after addition of nutrients.

Objectives of this study were:

(a) evaluation of microbial activities and counts of microorganisms in different soil types;
(b) differences among tested localities;
(d) differences between spring and autumn seasons;
(e) correlation between tested parameters.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Projects Nos. 4121000005 and 6046070901.

This article was presented at the conference "Practical Solutions for Managing Optimum C and N Content in Agricultural Soils IV", 19–23 June 2007, Prague, Czech Republic.

MATERIAL AND METHODS

Microbial activities and counts of microorganisms were studied during the years 2002–2006. Soil and microbial characteristics were investigated at 11 localities for almost 15 years. All of them were ordinary farm fields with arable land. The localities differ in soil types (from Luvisol, Cambisol to Chernozem), altitude (314–625 m above the sea level) and they are in several climate regions from warm, slightly wet to cold and wet. Selected characteristics of the localities are shown in Table 1.

Soil samples (99) were taken from the topsoil (0–200 mm) at the selected sites twice a year in spring and autumn (March and October). The samples were mixed; stones, plant and animal residues were eliminated and they were sieved (mesh 2 mm). Soil samples were analysed immediately after adjusting.

Cultivation on agar plates was used for evaluation of microorganism counts using different agar media for their identification:

(i) "TOTAL" total bacteria count and "ACT" actinomycetes – Thornton agar;
(ii) "OLIGO" oligotrophic bacteria – Nutrient agar (Oxoid CM3) 1% of recommended dose;
(iii) "SPORE" spore-forming bacteria – Nutrient agar (Oxoid CM3);
(iv) "fungi" micromycetes – Martin agar;
(v) "AZOT" diazotrophic bacterium *Azotobacter* – Ashby agar.

Incubation temperature was 28°C (except micromycetes 20°C). Time of cultivation was 5–7 days. Numbers of colony-forming units were expressed as logarithms per 1 g dry soil.

We have evaluated three microbial activities: respiration (basal and potential respiration with nutrients amendment), ammonification and nitrification (both actual and potential).

Mineralization test (Novák and Apfelthaler 1964) of organic carbon substances (original and after carbon and nitrogen addition) was performed in three variants:

(1) "B" basal respiration – no amendment;
(2) "G" potential respiration with glucose (as carbon source);
(3) "NG" potential respiration with the glucose and ammonium sulphate solution (as carbon and nitrogen sources).

Each of variants contains 50 g soil with 2 ml of water or appropriate nutrition amendment in airproofed Erlenmeyer flasks. The CO_2 production by soil microflora was determined by means of interferometer (Interferometer Carl Zeiss, Jena, Germany) after 20 h incubation at 28°C. Results were expressed in mg CO_2/h per 100 g dry soil.

Ammonification test (mineralization of organic nitrogen substances according to Pokorná-Kozová et al. 1964) was set up in two variants:

(1) "A act" actual ammonification – determination of actual soil N-NH_4^+ content;
(2) "A pot" potential ammonification – ammonium content after peptone amendment.

Ammonification test proceeded at 28°C for 48 h; ammonium was determined using modified Conway distillation method; results were expressed in mg N-NH_4^+ per 100 g dry soil.

Table 1. Basic characteristics of the selected localities

Number	Name of locality	Soil type	Altitude (m)	Clay particles < 2 μm (%)	Fine particles 2–62 μm (%)	Cox (%)
109	Korno	Rendzic Leptosol	381	17.9	25.6	1.73
111	Neumětely	Luvisol	325	26.2	38.6	1.65
125	Čistá u Rakovníka	Cambisol	460	16.3	31.6	1.52
127	Nový Dům	Albeluvisol	430	18.9	28.0	1.43
131	Tursko	Chernozem	314	27.3	34.6	1.87
805	Sloupnice	Albeluvisol	390	15.5	25.5	1.23
806	Mostek 123	Luvisol	370	17.0	27.0	1.43
807	Mostek 140	Luvisol	410	20.0	26.0	1.26
808	Červený Potok	Cambisol	625	22.0	34.3	1.95
809	Červená Voda 1	Cambisol	590	22.0	32.5	1.58
810	Červená Voda 2	Cambisol	565	22.0	37.0	2.10

Nitrification test (soil microorganisms ability to oxidate N-NH_4^+; according to Löbl and Novák 1964) was also set up in two variants:

(1) "N act" actual nitrification – actual content of soil nitrate;

(2) "N pot" potential nitrification after ammonium sulphate addition.

Ion-selective electrode was used for nitrate determination; results were expressed as mg N-NO_3^- per 100 g dry soil.

All results were evaluated using summary statistics, analysis of variance and correlation coefficients.

RESULTS AND DISCUSSION

Bacteria and fungi, the major types of microorganisms found in soil, play an essential role in nutrient transformations. Critter et al. (2002) evaluated both groups of microorganisms in soil samples quantitatively using agar plate counts. He found that amendment with different organic materials affected significantly their quantity. Likewise, the incidence of bacteria in soil samples was evidently dependent on the presence of fresh organic matter rather than the total carbon content in soil (Kubát et al. 1999).

Average counts of microorganisms (Table 2) are expressed as log of CFU per 1 g dry soil and they correspond with usual counts of microbes in arable soils. Average counts of total bacteria and oligotrophic bacteria were similar (6.75 and 6.63 log of CFU per 1 g dry soil, respectively). Counts of actinomycetes were well-balanced (min. 5.29 to max. 5.61 log of CFU per 1 g dry soil). There were no significant differences among tested localities in number of these bacteria.

Average value of spore-forming bacteria was 5.86 log of CFU per 1 g dry soil with significant differences between lower (Cambisol 5.57) and higher (Chernozem 6.08) value. Range for micromycetes among all localities was from 4.65 to 5.04.

There were some differences between localities in the count of *Azotobacter* (0.77–3.02 log of CFU per 1 g dry soil). The lowest level was reported in Luvisol and Cambisol, the highest one in Chernozem (similar as count of spore-forming bacteria).

There were no significant differences between spring and autumn seasons in the counts of microorganisms (except for total bacteria counts that were significantly higher in autumn).

Table 2. Average counts of microorganisms

Number of locality	Average counts of microorganisms (log of CFU per 1 g dry soil)					
	TOTAL	OLIGO	SPORE	ACT	FUNGI	AZOT
109	6.83^{a}	6.70^{a}	6.07^{d}	5.46^{a}	4.86$^{a, b}$	2.53^{d}
111	6.78^{a}	6.80^{a}	6.03$^{c, d}$	5.41^{a}	5.04^{b}	1.61^{c}
125	6.76^{a}	6.67^{a}	6.04^{d}	5.50^{a}	4.77$^{a, b}$	0.77^{a}
127	6.67^{a}	6.65^{a}	5.83$^{b, c}$	5.55^{a}	4.78$^{a, b}$	0.77^{a}
131	6.67^{a}	6.70^{a}	6.08^{d}	5.29^{a}	4.82$^{a, b}$	3.02^{e}
805	6.77^{a}	6.62^{a}	5.77^{b}	5.54^{a}	4.77^{a}	1.13$^{a, b}$
806	6.71^{a}	6.56^{a}	5.82^{b}	5.42^{a}	4.67^{a}	1.30$^{b, c}$
807	6.78^{a}	6.61^{a}	5.81^{b}	5.61^{a}	4.83$^{a, b}$	0.83^{a}
808	6.80^{a}	6.52^{a}	5.57^{a}	5.45^{a}	4.76^{a}	0.87^{a}
809	6.88^{a}	6.63^{a}	5.68$^{a, b}$	5.39^{a}	4.65^{a}	2.52^{d}
810	6.65^{a}	6.50^{a}	5.74$^{a, b}$	5.33^{a}	4.66^{a}	0.94$^{a, b}$
Average values	6.75	6.63	5.86	5.45	4.78	1.48
$P < 0.05$	NS	NS	S	NS	S	S

TOTAL – total bacteria count; OLIGO – oligotrophic bacteria; SPORE – spore-forming bacteria; ACT – actinomycetes; FUNGI – micromycetes; AZOT – diazotrophic bacteria, genus *Azotobacter*

$P < 0.05$ – significance of LSD test (NS – not significant, S – significant); significant differences ($P < 0.05$) in counts of microorganisms are indicated by different letters

Gryndler et al. (2003) evaluated the effect of mineral and organic fertilization on occurrence of soil microorganisms in a field experiment. The colony-forming units of saprotrophic microfungi increased significantly with increasing doses of mineral and organic fertilization; counts of actinomycetes increased in soils fertilized by mineral fertilizers.

Malý et al. (2000) monitored the effect of vegetation composition on various soil microbial properties in abandoned arable land. Microbial numbers and processes were determined in five replicate plots with diverse treatments. The results indicated that differences in plant biomass, plant species diversity and plant species composition had significant effect neither on soil microbial processes (net N mineralization, nitrification and ammonification) nor on the number of colony-forming units of the major microbial groups.

Microbial respiration was determined by measuring either the release of CO_2 or the uptake of O_2. As the atmospheric CO_2 concentration is only 0.036% as compared to 20% for O_2, measurements of CO_2 production are more sensitive than these of O_2 (Kandeler 2007).

The measurement of CO_2 production (Figure 1) was used for investigation of the soil microflora activity in this paper. Basal (control) respiration represented mineralization of native organic substances in soil samples. Average values of basal respiration were slightly lower (0.45 mg CO_2/h per 100 g dry soil) then usual results. Both variants of potential respiration (G, NG) respond to usual values in arable soils (Růžek et al. 2006). Glucose addition increased potential mineralization activity 10 times; average value was 4.25 mg CO_2/h per 100 g dry soil (min.: locality 127, Albeluvisol, 3.11; max.: Cambisol, 5.55 mg CO_2).

Significant correlation ($P < 0.01$) was found only between basal respiration B and potential respiration G ($r = 0.56$). Correlation coefficients are shown in Table 4.

Average values after amendment with nitrogen and carbon (9.50 mg CO_2/h per 100 g dry soil) increased mineralization about 20 times compared to control. Differences between localities were not significant. The lowest rates were found at locality 127 (5.36).

Cookson et al. (2007) used a laboratory incubation of forest, grassland and arable soils at 5 and 25°C. They aimed to clarify the mechanisms of N cycling processes and microbial community composition in relation to dissolved organic carbon (DOC) and N (DON) availability and selected soil properties. Respiration rate was positively correlated with bacterial biomass, DON and DOC/DON ratio.

Average value of actual ammonification (Figure 2) was 15.23 mg N-NH_4^+ per 100 g dry soil, ranging from 12.37 to 18.57 (the lowest one at locality 127; analogous to both potential respiration activities).

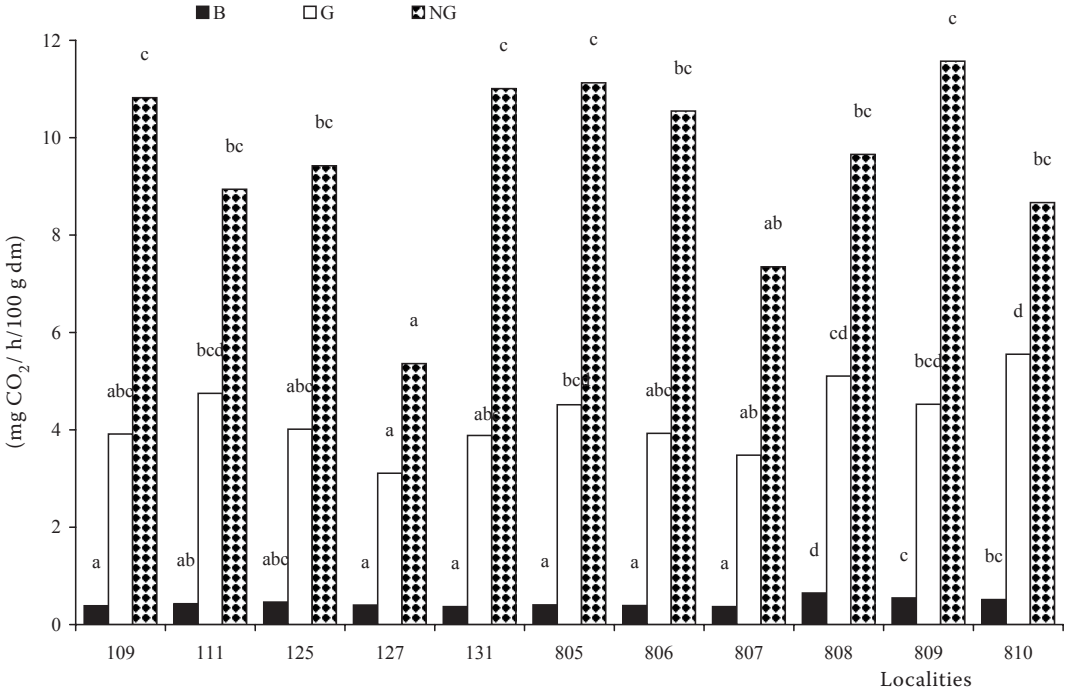

Figure 1. Average values of respiration tests (mg CO_2/h per 100 g dry soil). Explanations in the Table 4; significant differences ($P < 0.05$) are indicated by different letters

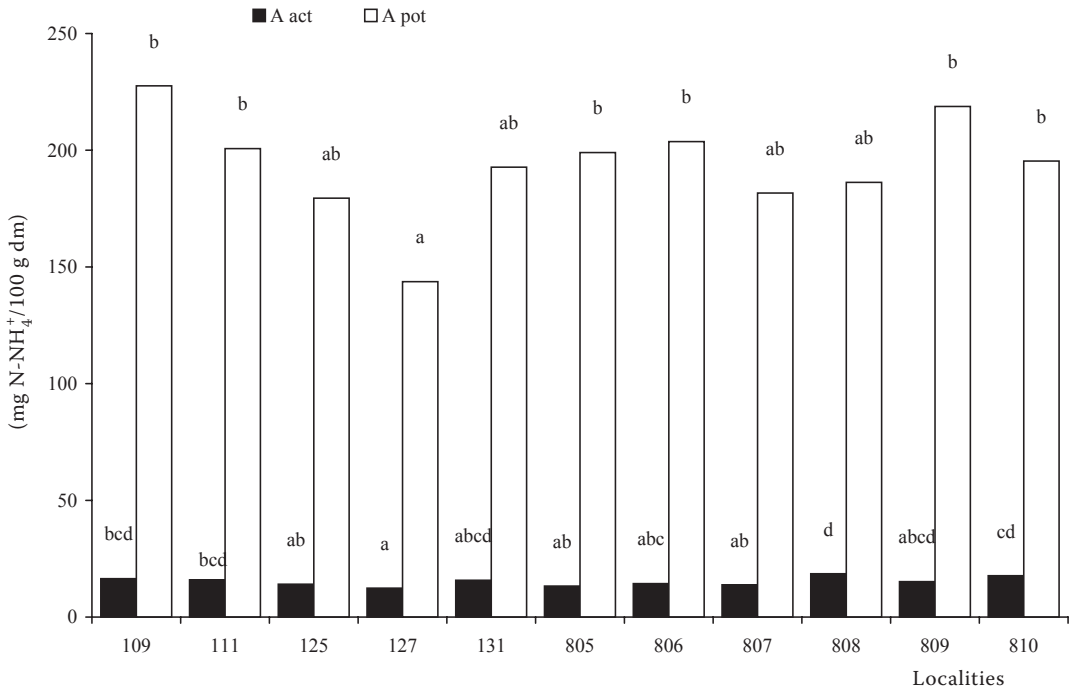

Figure 2. Average values of ammonification tests (mg N-NH_4^+ per 100 g dry soil). Explanations in the Table 4; significant differences ($P < 0.05$) are indicated by different letters

Actual ammonification was significantly higher in autumn.

Potential ammonification activities were more than 10 times higher (193.53 mg N-NH_4^+ per 100 g dry soil) than actual ones. There were no relevant differences among localities. The lowest value of potential ammonification 143.67 mg N-NH_4^+ was found again in locality 127 (Albeluvisol); the highest one in locality 109 (Rendzic Leptosol; 227.58).

Due to the simultaneous carbon and nitrogen mineralization during microbial decomposition of litter and SOM, the rate of nitrogen mineralization often correlates with microbial respiration. Carbon released from microbial decomposition

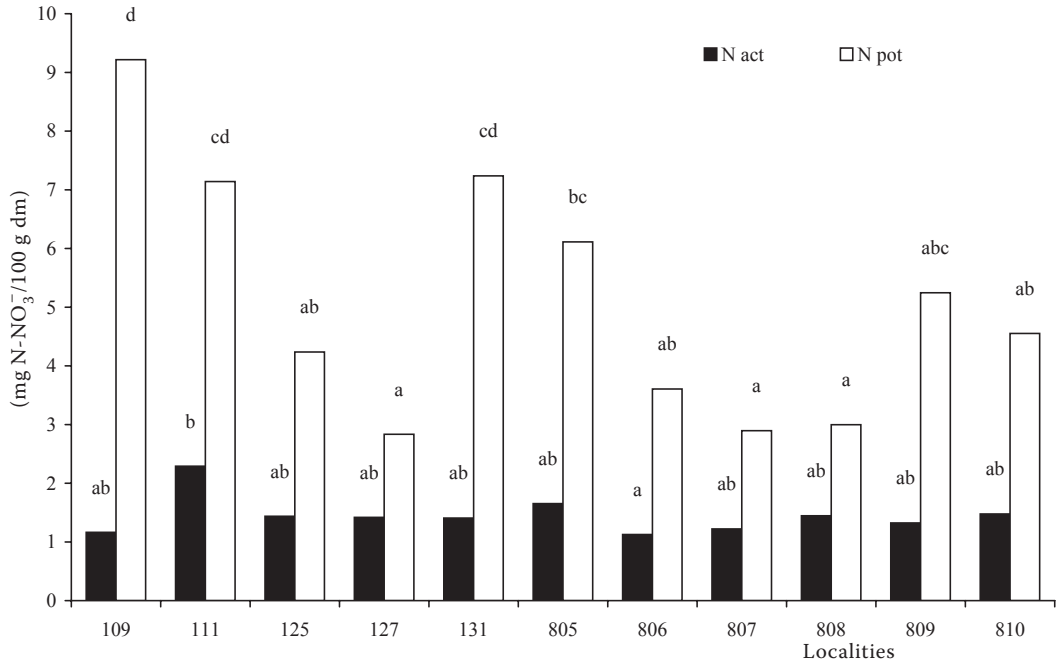

Figure 3. Average values of nitrification tests (mg N-NO_3^- per 100 g dry soil). Explanations in the Table 4; significant differences ($P < 0.05$) are indicated by different letters

Table 3. Correlation coefficients among counts of soil microorganisms

	TOTAL	OLIGO	SPORE	ACT	FUNGI
TOTAL	×				
OLIGO	0.20	×			
SPORE	0.07	0.70***	×		
ACT	0.61**	–0.06	0.06	×	
FUNGI	0.41	0.53**	0.37	0.32	×
AZOT	0.08	0.39	0.39	–0.43*	0.06

TOTAL – total bacteria count; OLIGO – oligotrophic bacteria; SPORE – spore-forming bacteria; ACT – actinomycetes; FUNGI – micromycetes; AZOT – diazotrophic bacteria, genus *Azotobacter*
significant differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

correlated with mineralization nitrogen (Zak et al. 1993).

Similar to our investigations (Table 4), potential nitrification activity was in highly significant correlation ($P < 0.001$) with potential ammonification activity ($r = 0.64$). Correlation of actual ammonification and basal respiration activities ($r = 0.56$) was at the significance level of 0.01. There was also a significant correlation ($P < 0.05$) between potential nitrification and potential respiration of NG activities ($r = 0.51$). It indicates a good status of soil microflora.

Actual nitrification (Figure 3) was the highest (2.29 mg N-NO_3^- per 100 g dry soil) in locality 111 (Luvisol). There were no statistically significant differences among the localities as a result of high variation (SD ± 1.21). Average values were 1.45 mg N-NO_3^- per 100 g dry soil and minimum 1.13 mg N-NO_3^- per 100 g dry soil.

Significant differences in potential nitrification activity were found among the investigated localities. The highest values were in localities 109 and 131 (9.22 Rendzic Leptosol and 7.24 Chernozem, respectively), the lowest ones were at localities 127, 807 and 808 (Albeluvisol 2.83, Luvisol 2.89 and Cambisol 3.00 mg N-NO_3^- per 100 g dry soil, respectively).

The effects of afforestation on potential nitrification, nitrification and ammonification rates were studied at an experimental site in NE Scotland 4.5 years after afforestation of former arable land (Haque et al. 1999). Potential nitrification rates measured in plantation soils were significantly lower than in the unplanted control soil. Nitrification and ammonification rates were also consistently lower, although these differences were only significant in some of the treatments. The results suggest that afforestation of former agricultural

Table 4. Correlation coefficients among soil microflora activities

	B	G	NG	A act	A pot	N act
B	×					
G	0.56**	×				
NG	0.06	0.41	×			
A act	0.56**	0.51*	0.21	×		
A pot	–0.04	0.36	0.76***	0.38	×	
N act	0.07	0.49*	0.33	–0.04	0.13	×
N pot	–0.31	0.24	0.51*	0.21	0.64***	0.24

B – basal respiration – no amendment; G – potential respiration with glucose; NG – potential respiration with glucose and ammonium sulphate; A act – actual ammonification – determination of actual N-NH_4^+ content; A pot – potential ammonification – ammonium content after peptone amendment; N act – actual nitrification; N pot – potential nitrification after ammonium sulphate addition
significant differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

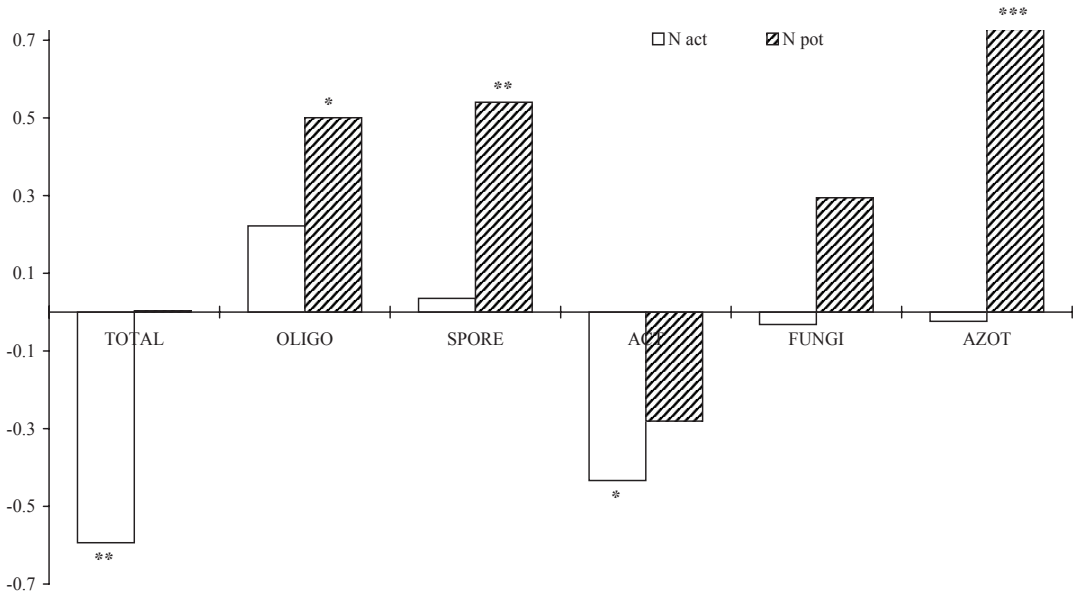

Figure 4. Correlations between nitrification and microorganism counts. For explanations see Tables 2 and 3

soils may cause changes in the soil N cycle soon after planting.

All evaluated activities (except for actual ammonification) were higher in spring season, but differences were not significant; the only significant value was actual nitrification.

Relationship between soil microorganisms counts was mostly insignificant (Table 3). Only correlations of oligotrophic bacteria and micromycetes (0.53) and of total bacteria counts and actinomycetes (0.61) were significant ($P < 0.01$). Also correlation coefficient between oligotrophic and spore-forming bacteria ($r = 0.70$) was highly significant ($P < 0.001$).

Correlation coefficients among soil microflora activities are shown in Table 4. The highest correlation coefficient ($P < 0.001$) was found between potential ammonification and potential respiration NG activities ($r = 0.76$). Correlation of potential respiration G to actual nitrification (0.49) or to actual ammonification activities (0.51) was at the significance level of 0.05.

Correlations among the tested activities and counts of bacteria were mostly insignificant. It might indicate that some other factors (humidity, temperature, pH, aeration, composition of nutrient sources) could be of higher importance for the measured activities than the microorganism counts.

Figure 4 shows correlations between potential nitrification activities and counts of microorganisms. Correlation of potential nitrification to *Azotobacter* (0.73) was highly significant ($P < 0.001$). Significant correlation ($P < 0.01$) was found for potential nitrification and spore-forming bacteria (0.50) and for actual nitrification activity and total bacteria counts (–0.59). Correlations of actual nitrification and actinomycetes (–0.43) and of potential nitrification and oligotrophic bacteria (0.54) were also significant ($P < 0.05$).

In total, 66% of the correlation coefficients were lower than 0.40, i.e. non-significant; 34% were significant; 13% of the latter were in the range from 0.41 to 0.52 (significance level 0.05), 10% were in the range from 0.52 to 0.63 (significance level 0.01), and 11% were higher than 0.64 (significance level 0.001).

Our results characterising the number of microorganisms and their activities in arable soils under standard crop rotation have shown that these biological parameters are very conservative and nearly without significant differences in testing factors (type of soil, season). It is probably related to the ability of soil organisms to compensate small differences in soil management. It has to be stated that the level of microbial activities and the number of microorganisms in tested conditions were very stable.

REFERENCES

Cookson W.R., Osman M., Marschner P., Abaye D.A., Clark I., Murphy D.V., Stockdale E.A., Watson C.A. (2007): Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biol. Biochem., *39*: 744–756.

Critter S.A.M., Freitas S.S., Airoldi C.(2002): Comparison between microorganism counting and a calorimetric method applied to tropical soils. Thermochim. Acta, *394*: 133–144.

Grynder M., Hršelová H., Klír J., Kubát J., Votruba J. (2003): Long-term fertilization affects the abundance of saprotrophic microfungi degrading resistant forms of soil organic matter. Folia Microbiol., *48*: 76–82.

Haque S.M.S., Billett M.F., Grayston S., Ord B.G.(1999): Effects of afforestation on ammonification and nitrification rates in former agricultural soils. Soil Use Manag., *15*: 117–122.

Kandeler E. (2007): Physiological and biochemical methods. In: Paul E.A. (ed.): Soil Microbiology, Ecology and Biochemistry, Vol. 3. Academic Press and Elsevier Inc., Burlington: 53–83.

Kubát J., Nováková J., Mikanová O., Apfelthaler R. (1999): Organic carbon cycle, incidence of microorganisms and respiration activity in long-term field experiment. Rostl. Výr., *45*: 389–395.

Löbl F., Novák B. (1964): Beitrag zur Methodik der Nitrifikationsbestimmung. Zbl. Bakt. II, *118*: 374–378.

Luo Y., Zhou X. (2006): Soil Respiration and the Environment. Academic Press and Elsevier Inc., Burlington.

Malý S., Korthals G.W., Van Dijk C., Van der Putten W.H., De Boer W. (2000): Effect of vegetation manipulation of abandoned arable land on soil microbial properties. Biol. Fertil. Soils, *31*: 121–127.

Novák B., Apfelthaler R. (1964): Contribution to the methods for determination of the respiration activity as an indicator of microbiological processes in soil. Rostl. Výr., *10*: 145–150. (In Czech)

Paul E.A. (2007): Perspective in soil microbiology, ecology and biochemistry. In: Paul E.A. (ed.): Soil Microbiology, Ecology and Biochemistry, Vol. 3. Academic Press and Elsevier Inc., Burlington: 3–24.

Pokorná-Kozová J., Löbl F., Apfelthaler R., Novák B. (1964): Zur Methodik der Fesstellung der Ammonifizeirung in Bodenproben. Zbl. Bakt. II, *118*: 368–373.

Růžek L., Voříšek K., Nováková M., Strnadová S. (2006): Microbiological, chemical and textural parameters of main soil taxonomical units of Czech Republic. Plant Soil Environ., *52*(Special Issue): 29–35.

Zak D.R., Grigal D.F., Ohmann L.F. (1993): Kinetics of microbial respiration and nitrogen mineralization in Great Lakes forests. Soil Sci. Soc. Am. J., *57*: 1100–1106.

Received on December 12, 2007

Corresponding author:

Ing. Eva Popelářová, Ph.D., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Kamýcká 129, 165 21 Praha 6-Suchdol, Česká republika
e-mail: popelarova@af.czu.cz