Effects of organic and mineral fertilisers on biological properties of soil under seminatural grassland

Ľ. Ondrášek, J. Čunderlík

Grassland and Mountain Agriculture Research Institute, Slovak Agricultural Research Centre, Banská Bystrica, Slovak Republic

ABSTRACT

Over 2004–2006, effects of mineral fertilisers (60 N + 30 P + 60 K kg/ha or 120 N + 30 P + 60 K kg/ha) and farmyard manure (12 or 24 t/ha) application on biological and chemical properties of soil were studied in seminatural grassland. The research trial was established at Radvaň site, central Slovakia (altitude 480 m, loamy to sandy-loamy Cambisol). These parameters were investigated during the growing seasons: CO_2 production, intensity of total N mineralisation (TMN), the rate of nitrification (NIT), total C content in microbial biomass (MB-C), C_{ox}, N_t, P, K, Mg and the humic acids to fulvic acids ratio (HA:FA). At the application of mineral fertilisers, the highest N rate significantly increased TMN and NIT, mainly in 2004 and 2005. The manure application showed the strongest effects on the biological parameters. The manure rate of 12 t/ha significantly increased the soil respiration in 2005 and 2006 as well as TMN and NIT throughout the research. The manure application increased also the soil pH, MB-C, N_t, Mg, P and C_{ox}.

Keywords: grassland soil; mineral fertilisers; manure; soil respiration; microbial biomass; nitrogen mineralisation; nitrification

Grassland management practices comprise application of inorganic and organic fertilisers.

Considering the fact that these fertilisers have a great impact on a complex of physical, chemical and especially biological properties of soil, great attention has been paid to this topic (Hatch et al. 2002, Peacock et al. 2002, Parham et al. 2003, Bittman et al. 2005). Another objective of studies is an assessment of fertiliser application from the environmental viewpoints, mainly in relation to possible N losses in a gaseous form or by leaching, as well as quantitative and qualitative changes in soil organic matter as a part of C and N cycling (Casals et al. 2004, Lampe et al. 2004, Mestdagh et al. 2004).

The objective of this paper was to assess research data recorded in a three-year trial. The trial aim was to compare effects of farmyard manure and inorganic fertiliser application on a range of biological and chemical properties of soil under seminatural grassland.

MATERIAL AND METHODS

In 2003, a field trial was established at Radvaň site (near Banská Bystrica in central Slovakia, altitude 480 m; mild climatic region; mean annual temperature 7.0–8.0°C; mean annual rainfall 852 mm; loamy to sandy-loamy Cambisol) on seminatural grassland under three-cut utilisation system.

The trial treatments are given in Table 1. The total annual N rate was applied either once in the spring (treatment 2) or split into two dressings in the spring and to the first cut (treatment 3). Single rates of fertiliser P and K were applied in the spring. Farmyard manure (12 and 24 t/ha, i.e. rates of 60 and 120 kg N/ha as net nutrients, respectively) was applied to treatments 4 and 5 in the autumn of 2003 and 2005.

Throughout the trial, fresh soil was sampled (using a system of mean sample) at 20–100 mm on the dates of the 1st, 2nd and 3rd cuts as well as in the spring and in the autumn. In 2004, the sampling

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.

Table 1. Trial treatments and fertiliser rates

Treatment	Fertiliser rates	Fertiliser type used
1	non-fertilised control	–
2	60 N + 30 P + 60 K (kg/ha)	ammonium nitrate + superphosphate + potassium chloride
3	2 × 60 N + 30 P + 60 K (kg/ha)	ammonium nitrate + superphosphate + potassium chloride
4	12 t/ha	farmyard manure
5	24 t/ha	farmyard manure

dates were 14 April, 4 June, 28 July, 29 September and 26 October. In 2005, the sampling dates were 18 April, 7 June, 25 July, 19 September, and 17 October. In 2006, the sampling dates were 19 April, 26 May, 11 July, 14 September and 3 November.

The soil samples with natural moisture content were passed through a 2 mm sieve and the following parameters were determined:

(a) the absolute soil moisture (gravimetry);
(b) the CO_2 production (gas chromatography after 24 h incubation at 25°C);
(c) the C content of total microbial biomass in soil sampled in the autumn by the fumigation method (ISO 14240-2);
(d) the instantaneous content of NH_4^+-N and NO_3^-- N determined in 1%-extract of K_2SO_4, (spectrophotometry, SKALAR);
(e) the intensity of total mineralisation of nitrogen (TMN) after 14-day aerobic incubation at 25°C;
(f) the rate of nitrification (NIT) after 14-day aerobic incubation at 25°C;
(g) the soil sampled in the year 2006 was dried at laboratory temperature and the following parameters were measured: C_{ox} content (Tjurin); N_t (Kjeldahl); P, K, Ca, Mg (according to the Mehlich III method); pH (*n*KCl) and the humic acids to fulvic acids ratio (HA:FA) by the spectrophotometry method.

Data were analysed by the method of multiple and simple analysis of diffusion with a statistical software package (STRATGRAPHICS). Statistically significant differences between the means were analysed using the LSD test (P = 95.0%). Relationship between some of the research parameters was assessed by the Pearson's type of the coefficient of correlation.

RESULTS AND DISCUSSION

The soil respiration ranks among the oldest and most frequently used parameters used to measure and determine the biological activity of soil. However, the information value, content and interpretation of data recorded by measuring the CO_2 production are largely influenced by the methods applied at the measurements.

In the presented research, soil respiration was measured in soil samples incubated under standard laboratory temperature and moisture conditions. Therefore, the volume of released CO_2 depended only on the amount and availability of organic matter in a soil sample and on the ability of organisms that were present there to utilise the organic substances.

As given in Table 2, the activity of soil respiration was changing in relation to the sampling dates throughout each of the growing seasons. There is a range of influencing factors, such as development stage and activity of soil micro-flora in relation to moisture, temperature, and also the amount of easily decomposable organic matter in soil, dependent on the development of above- and underground plant biomass.

The highest CO_2 production was recorded in the soil samples taken on 14 April, 4 June and 29 September in 2004, on 25 July and 19 September in 2005 as well as on 26 May and 14 September in 2006. The earlier reports (e.g. Tesařová and Gloser 1976) presented that the intensity of respiration in grassland soil is strongly influenced by primary ecological factors, such as temperature and especially the soil moisture content. In our research, a positive relation to the soil moisture content was found only in the samples from 2004 ($r = 0.50^{++}$). In the other years, the intensity of CO_2 output was probably influenced by other factors mentioned earlier, or by their mutual interactions.

The mean data of the treatments showed that the soil respiration activity (Table 2) was influenced – to some extent – by the inorganic and organic fertilisers and their application rates.

At mineral fertilisers treatments, only the highest N rate (treatment 3) showed effects on CO_2 production in 2005. Over the growing season, mean CO_2 production was significantly higher at the fertiliser and manure application treatments than at the control. The earlier detailed research on

Table 2. Soil respiration (mg CO_2/kg/day) over 2004–2006 growing seasons

Treatment	Soil sampling (2004)						Soil sampling (2005)						Soil sampling (2006)				
	14.4.	4.6.	28.7.	29.9.	26.10.	$\bar{x}$	18.4.	7.6.	25.7.	19.9.	17.10.	$\bar{x}$	19.4.	26.5.	11.7.	14.9.	$\bar{x}$
1	121.0	190.6	97.6	109.3	125.0	128.7[a, b]	120.0	96.8	153.3	180.8	99.1	130.0[a]	83.4	221.7	77.6	130.5	128.3[a, b]
2	138.7	125.5	91.0	159.6	80.9	119.1[a]	105.3	128.6	136.6	162.0	113.6	129.2[a]	109.7	173.2	79.9	167.8	132.6[a, b, c]
3	177.8	193.7	91.2	147.4	65.7	135.1[a, b]	177.2	112.1	178.0	170.5	131.6	153.9[b]	82.5	164.9	87.4	152.6	121.8[a]
4	164.5	228.8	94.6	156.7	86.4	146.2[b]	144.1	127.3	161.3	192.4	122.1	149.4[b]	110.7	212.9	122.3	166.4	153.1[c]
5	144.0	283.9	112.8	103.6	91.3	147.1[b]	113.0	146.0	171.7	179.9	119.5	146.0[b]	106.0	189.7	124.8	156.5	144.2[b, c]
$\bar{x}$	149.2[b]	204.5[c]	97.5[a]	135.3[b]	89.8[a]	–	131.9[a]	122.2[a]	160.2[b]	177.1[c]	117.2[a]	–	98.5[a]	192.5[c]	98.4[a]	154.8[b]	–

Mean values not sharing a common letter are significantly different (LSD test, P = 95.0%)

Table 3. Total N mineralisation (mg NH_4^+-N/kg/day) over 2004–2006 growing seasons

Treatment	Soil sampling (2004)						Soil sampling (2005)						Soil sampling (2006)					
	14.4.	4.6.	28.7.	29.9.	26.10.	$\bar{x}$	18.4.	7.6.	25.7.	19.9.	17.10.	$\bar{x}$	19.4.	26.5.	11.7.	14.9.	3.11.	$\bar{x}$
1	0.98	0.84	0.62	0.39	0.39	0.64[a]	1.32	0.24	0.81	1.19	1.41	0.99[a]	1.25	1.93	0.64	1.40	1.23	1.29[a]
2	1.50	0.72	0.72	0.89	0.85	0.94[a, b]	1.13	0.71	0.91	0.73	1.23	0.94[a]	1.89	1.67	0.92	2.54	1.18	1.64[b]
3	1.88	1.09	0.90	0.88	0.81	1.11[b]	3.15	0.88	1.32	0.84	1.55	1.55[b]	1.37	2.64	0.97	1.57	0.86	1.48[a, b]
4	2.07	3.61	0.50	1.63	1.59	1.88[c]	2.27	1.04	1.23	1.09	1.71	1.47[b]	2.13	1.98	1.49	1.58	1.19	1.67[b]
5	1.40	1.51	1.41	0.86	1.29	1.29[b]	1.58	1.06	1.34	1.33	1.48	1.36[b]	1.59	1.58	1.81	1.69	0.80	1.49[a, b]
$\bar{x}$	1.56[b]	1.55[b]	0.83[a]	0.93[a]	0.99[a]	–	1.89[d]	0.79[a]	1.12[b]	1.03[a, b]	1.48[c]	–	1.65[b]	1.96[b]	1.16[a]	1.75[b]	1.05[a]	–

Mean values not sharing a common letter are significantly different (LSD test, P = 95.0%)

the effects of high fertiliser N rates applied during several years on the respiration activity of grassland soil showed a decrease in CO_2 production in the fertilised soil as a result of increased stability of soil organic matter (Ondrášek 1990). However, the most marked impact on the soil respiration was found with manure application. Mainly the low rate of 12 t/ha (treatment 4) resulted in the stimulation effect throughout the trial period, but especially in 2006.

The microbial changes of nitrogen in soil are crucial for plant nutrition. While studying these changes in soil of grassland ecosystem, it is necessary to bear in mind the effects of continuous physiological activity of wide spectrum of plant roots. The plant roots continuously supply the soil micro-flora with small amounts of easily decomposable material through their exudates and dead cell lyses. This material comprises amino acids and other organic acids as well as multi-component organic compounds, such as vitamins, proteins, nucleic acids and their derivatives, the components of cell walls and other compounds. The qualitative composition of all these substances, especially the C:N ratio, is decisive for the course of desamination reactions, that is N release from organic bonds, or for the ammonia nitrogen accumulation in soil. This is similar in other plant (above- and underground litter) and animal (dead soil fauna) materials penetrating into soil and turning into a substrate for the soil microflora. It was generally concluded that the immobilisation of nitrogen predominates over the N mobilisation in grassland soil and this can be explained by the wide C:N ratio in the decomposing organic substances.

The nutritive conditions of soil microflora are influenced, of course, by the N fertiliser or manure application. The N content increases both directly and indirectly in soil through the changed chemical composition of plants and the plant above- and underground litter in which the N content is rising. Consequently, the C:N ratio would narrow and, presumably, due to the mobilisation of ammonia nitrogen in soil through ammonification, microflora would increase.

The significant differences between the total N mineralisation data averaged over the samplings (Table 3) showed marked effects of the ecological factors, similarly to the situation at the soil respiration. The highest mean TMN was recorded in the samples from 14 April and 4 June in 2004, in those taken on 18 April, 25 July and 17 October in 2005, and in those from 19 April, 26 May and 14 September in 2006. The analysis of correlation confirmed the relation to the soil moisture content. The coefficients of correlation were as follows: $r = 0.45^{++}$ in 2004; $r = 0.61^{++}$ in 2005; $r = 0.40^{++}$ in 2006. The total N mineralisation was markedly influenced by grassland fertilisation. The rate of 120 kg N/ha + PK (treatment 3) significantly increased TMN in 2004 and 2005, respectively. However, the increase in TMN at the application of 60 kg N/ha (treatment 2) was significant in comparison with the control only in 2006.

Manure application showed the most notable effects on the total N mineralisation. In 2004, by comparison to the control, the data averaged over the treatments (Table 3) showed threefold higher TMN at 12 t/ha (treatment 4) and twofold higher TMN with 24 t/ha (treatment 5) manure application rates. The reduced stimulation effect of the higher rate could have resulted probably from deteriorated aeration conditions for the ammonification and especially for the nitrification microflora due to the excessive input of organic matter. In the following years, a decrease in stimulation effect continued in spite of the manure application repeated in the autumn 2005. A reason for this situation could have been the reduced rainfall and, consequently,

Table 4. Means of agrochemical analysis of soil samples (x = sampling I.–V.) over 2006 growing season

Treatment	C_{ox} (g/kg)	N_t (g/kg)	HA:FA	P (mg/kg)	K (mg/kg)	Ca (g/kg)	Mg (mg/kg)	pH
1	27.7[a, b]	2.6[a]	0.4[a]	36.5[a]	104.2[a]	1.0[a]	245.7[a]	3.9[a]
2	26.6[a, b]	2.6[a]	0.5[a]	8.9[a]	120.8[a]	1.0[a]	297.2[b]	4.0[a, b]
3	25.7[a]	2.5[a]	0.6[a]	9.5[a]	108.1[a]	1.1[a]	287.7[a, b]	4.2[b]
4	28.9[b]	2.9[a]	0.5[a]	73.1[b]	108.1[a]	1.0[a]	325.7[b]	4.1[b]
5	28.3[b]	3.0[a]	0.6[a]	40.0[a]	109.4[a]	1.2[a]	327.3[b]	4.3[c]

HA:FA – humic acids/fulvic acids ratio

Mean values not sharing a common letter are significantly different (LSD test, P = 95.0%)

the decrease in soil moisture content in 2005 and 2006, respectively. While the total rainfall over the growing season was 502 mm in 2004, it was lower in the 2nd and 3rd harvest years, namely 403 mm and 376 mm, respectively.

A part of microbial changes of N in soil is the nitrification process where ammonia, as a final product of ammonification, is oxidised into nitrates.

In the trial, the course of nitrification was identical with the total N mineralisation. A strong relationship was found between TMN and NIT ($r = 0.90^{++}$) by the analysis of correlation. This indicates that nearly all the nitrogen coming from ammonification was oxidised to the nitrate N, and also that not the low pH (Table 4) but the NH_4^+ content was the limiting factor for the nitrification bacteria.

Grassland soils have very low nitrification ability and their high acidity has been specified as one of the main reasons. The positive relationship between the pH and nitrification activity of grassland soil at a range of altitudes was found in the earlier research (Ondrášek et al. 2004). In the trial presented here, the manure application brought a tendency of decrease in soil acidity (Table 4) bringing also increased nitrification ability of soil.

The determined instantaneous content of mineral N forms in soil is given in Tables 5 and 6. The highest content as well as the greatest variability were found at NH_4^+-N (Table 5). Over all the growing seasons, the highest content was recorded at the samplings on 18 April, 7 June and 17 October of the year 2005. In 2004, the highest values were measured in the samples from 4 June at treatment 3 (13.11 mg NH_4^+-N/kg) and treatment 4 (10.37 mg NH_4^+-N/kg). This data illustrate that the sward was not able to take up the supplied nutrients. Considering the recorded data averaged over the treatments, the values of NH_4^+-N were significantly higher than the control at treatments 2 and 3, especially in 2004 and 2006. Throughout the trial period, the incidence of NO_3^--N in soil (Table 6) was mostly lower than that of ammonia N. The highest content was recorded with the highest mineral fertiliser rate at the second sampling dates (21.34 mg and 15.09 mg NO_3^--N; 4 June in 2004 and 26 May 2006, respectively). Averaged over the growing season, the content of NO_3^--N was significantly higher than the control at treatment 3 in 2004, at treatment 5 in 2005 and at treatment 3 in 2006.

However, it should be noted that – considering the absolute values – the data measured at NH_4^+-N and NO_3^--N were mostly low. This indicated the ability of sward to utilise sufficiently the nutrient rates supplied as fertiliser or manure.

The determination of total microbial biomass is an inseparable part of microbiological analysis of soil. The microorganisms have an irreplaceable role not only at transformation of organic substances but also as a source and reserve of nutrients in soil. Therefore, the quantity and the activity of microbial biomass are important parameters of soil biological activity playing the decisive role in creating and maintaining the soil fertility.

However, the effects of fertiliser and manure application on the total C content in microbial biomass in soil were not unambiguous, but were mostly positive during the research years (Table 7). Only in 2005, the level of MB-C was not influenced by the manure rate of 12 t/ha (treatment 4). In 2006, the MB-C decreased at the highest manure application rate (treatment 5).

It was concluded that the effects of fertiliser and especially manure application on the investigated microbiological parameters of soil were positive. The manure application enriches the soil not only with many organic substances but also with numerous forms of microorganisms enhancing and intensifying the biological activity of soil. This conclusion is in agreement with a range of reports on the impact of organic fertilisers and animal excreta on grassland resulting either from direct application or from gazing (Lovell and Jarvis 1996, Bardgett et al. 1998, Bittman et al. 2005).

Mineral fertiliser and especially manure application result in changes in the agrochemical properties of soil. The data in Table 4 show above all a tendency of changes in soil organic matter. The content of C_{ox} and N_t increased with the manure application.

The increase in C_{ox} was significant in comparison to the highest fertiliser application rate (treatment 3) where the C_{ox} content decreased. Moreover, there was a tendency to improvement in the humic acids to fulvic acids ratio (HA:FA).

A decrease in P content was recorded at treatments 2 and 3, despite the application of phosphorous fertiliser. It may be concluded that P was taken up by sward that was growing more intensively as a result of mineral fertiliser N applied.

As to the other investigated agrochemical properties of soil, the manure application rate of 12 t/ha increased Mg and P content and reduced the soil acidity. The increase in agrochemical parameters of grassland soil resulting from organic fertiliser application was previously reported by other authors (Hsieh et al. 1997, Gondek and Filipek-Mazur 2005).

Table 5. The instantaneous NH_4^+-N content (mg/kg) over 2004–2006 growing seasons

Treatment	Soil sampling (2004)						Soil sampling (2005)						Soil sampling (2006)					
	14.4.	4.6.	28.7.	29.9.	26.10.	$\bar{x}$	18.4.	7.6.	25.7.	19.9.	17.10.	$\bar{x}$	19.4.	26.5.	11.7.	14.9.	3.11.	$\bar{x}$
1	4.50	4.85	1.96	1.86	1.36	2.91[a]	4.43	4.27	2.33	3.13	4.29	3.69[a]	4.15	3.79	1.88	1.44	4.43	3.14[a, b]
2	3.84	6.83	2.91	7.24	1.37	4.44[b, c]	5.32	3.90	2.14	2.09	4.15	3.52[a]	6.33	5.02	2.83	1.62	2.44	3.65[b]
3	4.10	13.11	2.43	2.61	2.02	4.85[c]	4.54	4.76	5.89	2.18	5.59	4.59[b]	6.17	7.35	1.74	1.92	0.93	3.62[b]
4	3.68	10.37	1.32	3.06	1.74	4.03[a, b, c]	5.43	4.02	2.18	2.71	4.61	3.79[a]	5.56	3.51	1.66	1.78	2.13	2.93[a, b]
5	3.26	6.59	2.33	2.33	1.74	3.25[a, b]	4.65	4.12	1.81	2.67	3.66	3.38[a]	4.92	3.18	1.57	2.13	0.93	2.54[a]
$\bar{x}$	3.88[c]	8.35[d]	2.19[a, b]	3.42[b, c]	1.64[a]	–	4.88[b]	4.22[b]	2.87[a]	2.56[a]	4.46[b]	–	5.42[b]	4.57[b]	1.94[a]	1.78[a]	2.17[a]	–

Mean values not sharing a common letter are significantly different (LSD test, P = 95.0%)

Table 6. The instantaneous NO_3^--N content (mg/kg) over 2004–2006 growing seasons

Treatment	Soil sampling (2004)						Soil sampling (2005)						Soil sampling (2006)					
	14.4.	4.6.	28.7.	29.9.	26.10.	$\bar{x}$	18.4.	7.6.	25.7.	19.9.	17.10.	$\bar{x}$	19.4.	26.5.	11.7.	14.9.	3.11.	$\bar{x}$
1	1.36	1.86	0.85	0.52	0.96	1.11[a]	0.46	0.47	1.73	1.58	1.73	1.19[a]	1.15	0.94	0.94	1.93	4.63	1.92[a]
2	2.10	1.83	1.69	1.53	2.91	2.01[a, b]	0.21	0.39	1.68	0.70	2.11	1.02[a]	1.40	1.56	1.12	3.04	6.23	2.67[a, b]
3	3.08	21.34	3.90	1.36	2.67	6.47[c]	0.00	0.77	3.47	0.57	2.69	1.50[a]	1.26	15.09	0.90	2.79	5.57	5.12[b]
4	1.59	3.43	2.60	0.93	6.01	2.91[a, b]	1.57	0.54	1.88	0.57	1.67	1.25[a]	1.37	0.00	1.42	3.64	9.31	3.14[a, b]
5	2.26	5.19	6.13	1.30	7.11	4.40[b, c]	1.47	1.30	4.38	0.90	3.25	2.26[b]	2.34	0.00	1.77	4.58	5.81	2.77[a, b]
$\bar{x}$	2.08[a]	6.73[b]	3.04[a]	1.13[a]	3.93[a, b]	–	0.74[a]	0.69[a]	2.63[b]	0.86[a]	2.29[b]	–	1.50[a]	3.39[a]	1.23[a]	3.20[a]	6.31[b]	–

Mean values not sharing a common letter are significantly different (LSD test, P = 95.0%)

Table 7. The total content of soil microbial biomass (mg C/kg) in the autumn soil samplings in 2004, 2005 and 2006

Year (autumn)	Treatment				
	1	2	3	4	5
2004	1026.9[a]	1093.8[a, b]	1391.6[c]	1355.5[b, c]	1584.8[c]
2005	789.9[a]	889.0[b]	901.3[b]	792.0[a]	1069.4[c]
2006	1335.6[b]	1569.5[d]	1337.8[b, c]	1449.7[c]	1198.5[a]

Differences between treatments in one year only not sharing a common letter are significantly different (LSD test, $P = 95.0\%$)

REFERENCES

Bardgett R.D., Keiller S., Cook R., Gilburn A.S. (1998): Dynamic interactions between soil animals and microorganisms in upland grassland soils amended with sheep dung: A microcosm experiment. Soil Biol. Biochem., *30*: 531–539.

Bittman S., Forge T.A., Kowalenko C.G. (2005): Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biol. Biochem., *37*: 613–623.

Casals P., Garcia-Pausas J., Romanyä J., Camarero L. Sanz M.J., Sebastiä M.T. (2004): Effects of livestock management on carbon stocks and fluxes in grassland ecosystems in the Pyrenees. Grassland Sci. Eur., *9*: 136–138.

Gondek K., Filipek-Mazur B. (2005): The effects of mineral treatment and the amendments by organic and organomineral fertilisers on the crop yield, plant nutrient status and soil properties. Plant Soil Environ., *51*: 34–45.

Hatch D.J., Sprosen M.S., Jarvis S.C., Ledgard S.F. (2002): Use of labelled nitrogen to measure gross and net rates of mineralization and microbial activity in permanent pastures following fertilizer applications at different time intervals. Rapid Commun. Mass Spectrom., *16*: 2172–2178.

Hsieh C.H., Hong C.M., Hong K.Y. (1997): Effect of surface application of cattle manure on soil physical and chemical properties on pangola grass pasture. J. Taiwan Livest. Res., *30*: 395–409.

ISO 14240-2: Soil Quality – Determination of Soil Microbial Biomass – Part 2: Fumigation-Extraction Method. International Organization for Standardization, Geneva.

Lampe C., Dittert K., Wachendorf M., Sattelmacher B., Tuube F. (2004): Nitrous oxide fluxes from grassland soils with different fertiliser regimes. Grassland Sci. Eur., *9*: 334–336.

Lovell R.D., Jarvis S.C. (1996): Effect of cattle dung on soil microbial biomass C and N in a permanent pasture soil. Soil Biol. Biochem., *28*: 291–299.

Mestdagh L., Lootens P., Carlier L. (2004): Variation in organic carbon content in Flemish grassland soils. Grassland Sci. Eur., *9*: 133–135.

Ondrášek Ľ. (1990): Some changes in biological soil characteristics of the grassland ecosystem under different management intensity. Agriculture, *36*: 25–32.

Ondrášek Ľ., Uhliarová E., Valihora B. (2004): Evaluation of biological and chemical soil properties under meadows and pastures in Kremnicke vrchy. J. Mountain Agric. Balkans, *7*: 168–179.

Parham J.A., Deng S.P., Da H.N., Sun H.Y., Raun W.R. (2003): Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. Biol. Fertil. Soils, *38*: 209–215.

Peacock A.D., Mullen M.D., Ringelberg D.B., Tyler D.D., Hedrick D.B., Smith K.A., Beckwith C.P., Chalmers A.G., Jackson D.R. (2002): Nitrate leaching following autumn and winter application of animal manures to grassland. Soil Use Manag., *18*: 428–434.

Tesařová M., Gloser J. (1976): Total CO_2 output from alluvial soils with two types of grassland communities. Pedobiologia, *16*: 364–372.

Received on December 12, 2007

Corresponding author:

RNDr. Ľudovít Ondrášek, CSc., Slovenské centrum poľnohospodárskeho výskumu, Výskumný ústav trávnych porastov a horského poľnohospodárstva, Mládežnícka 36, 974 21 Banská Bystrica, Slovenská republika
phone: + 421 484 132 541, fax: + 421 484 132 544, e-mail: ondrasek@vutphp.sk