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The variation of mobile humic acids and mineral nitrogen in the soil as affected by the use of perennial grasses for green manure

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Abstract. The paper presents the findings of the experiments conducted on a clay loam gleyic *Cambisol* at the Lithuanian Institute of Agriculture's Joniškėlis Research Station and the results of analyses done at LIA's Laboratory of Chemical Research in 2007–2008. The tests were designed to establish the effects of the use of perennial grasses – red clover (*Trifolium pratense* L.), lucerne (*Medicago sativa* L.), their mixtures with festulolium *(*x *Festulolium)*, pure festulolium and their biomass as green manure on the variation and migration of mobile humic acids and mineral nitrogen in the soil of organic farms. Experimental evidence indicated that the content of mobile humic acids depended little on the plant species, however, when mixed management of the aboveground mass of perennial grasses (herbage of the first cut is removed from the field; second and third cut herbage left for green manure) was used, it increased significantly compared with the treatment where herbage had been removed from the field. Before ploughing-in of perennial grasses, the content of $N_{min.}$ in the 0–30 cm soil layer was significantly reduced (by 18.3 and 10.1%) by the cultivation of red clover and lucerne in mixtures with festulolium, compared with pure crops of respective legumes. The averaged data suggest that the highest $N_{min.}$ content in the soil was recorded in the treatments where mixed management of herbage had been used compared with the treatments where herbage had been removed from the field. In late autumn, 2.5 months after herbage ploughing-in, $N_{min.}$ concentration in the –30 cm and 3–60 cm soil layers increased by 8.9–53.9%, 7.8–93.7%, compared with that present in the soil at the end of August. Significantly lower $N_{min.}$ concentration in the 0–30 cm soil layer was found after festulolium cultivation, compared with red clover, and its content was also the lowest in the deeper layer (30–60 cm). When the herbage of perennial grasses had been used as green manure by four–time mulching, N_{min} concentration significantly increased in the topsoil layer (0–30 cm) by 23.1% and tended to increase in the deeper soil layer (30–60 cm) by 11.4%.

Key words: perennial grasses, mobile humic acids, mineral nitrogen, *Cambisol.*

INTRODUCTION

One of the main sources of nitrogen on organic farms is legume plants, especially perennial legumes. However, their use on animal production farms is limited. Much research has been devoted to the technologies of the use of legumes as green manure (Schröder, 2005). Some researchers suggest that one of the problems with using legumes as green manure is rapid mineralization of their above ground mass and nitrogen losses (leaching) during the autumn–winter–early spring period (Tripolskaja, 2005). Seeking to more effectively utilise biological nitrogen fixed by legumes and to prevent environmental pollution, researchers suggest mulching (cutting, chopping and spread) of the aboveground mass of perennial grasses on the soil surface 2–3 times during the growing season (Hatch et al., 2007). A chopped, nitrogen-rich mass of legumes rapidly mineralises and free nitrogen is bound again by intensively growing (re-growing) perennial grasses or is fixed in the soil organic compounds (Olesen et al., 2009). Plants start utilising nitrogen 14 days after the spread of mulch (Schäfer et al., 2001). The objective of the present study is using aboveground mass of perennial grasses for green manure to determine the concentration and migration of $N_{min.}$ and mobile humic acids on heavy loam *Cambisol* under organic farming conditions.

MATERIALS AND METHODS

Experiments were set up in 2007–2008 at the LIA's Joniškėlis Research Station on an *Endocalcari–Endohypogleyic Cambisol*, whose parental rock is limnoglacial clay. According to its texture, the soil is clay loam on silty clay with deeper lying sandy loam (*p2/m2/p1)*. The plough layer's agrochemical characteristics are as follows: pH_{KCl} 6.4, available P_2O_5 and K_2O 135 and 229 mg kg^{-1}, respectively, N_{total} 0.135%, $C_{org.}$ 1.68%. The following experimental design was employed: Factor A: perennial grasses: A_1 red clover (*Trifolium pratense* L.) (B1 control treatment); A_2 mixture of red clover and festulolium; A_3 lucerne (*Medicago sativa* L.); A_4 mixture of lucerne and festulolium; A_5 festulolium (x *Festulolium*). Factor B: management methods of above ground mass of perennial grasses: B_1 all herbage is removed from the field; B_2 herbage of the first cut is removed from the field, herbage of the second and third cuts is left for green manure (mixed method); $B_{3,}$ all herbage is used for green manure (mulching).

In the first experimental year (2007), spring barley (cv. Ula) was undersown with perennial grasses in compliance with the experimental design: red clover (cv. Vyliai, at a rate of 7.5 million seeds ha^{-1}), lucerne (cv. Birutė, at a rate of 7.5 million seeds ha^{-1}), intergeneric hybrid festulolium (cv. Punia, at a seed rate of 6.2 million ha^{-1}) and mixtures of both grasses with festulolium (seed rate ratio of legume to grass, 2:1). In the second and third treatments, every 30–40 days, depending on weather conditions, grass for green manure was cut by a self–propelled mower, equipped with a mulching device, then chopped and evenly spread on the plot. In the second half of August, the plots of all treatments were disked and 2 weeks later were ploughed at the 25 cm depth. Before sowing, the field was cultivated and harrowed at the same time. Winter wheat cv. Tauras was sown at a rate of 220 kg ha^{-1}. The seed was treated by a seed treater Biokal. Soil samples for mineral nitrogen ($N–NO_3$ + $N–NH_4$) determination were taken after the last cut of grasses (on 24-08-2008) and late in the autumn (on 12- 11-2008) from two depths (0–30 and 30–60 cm). Ammonia nitrogen ($N–NH_4$) in the soil was determined spectrometrically with a Carry 50 instrument, nitrate nitrogen ($N–NO_3$) by ionometric technique. Soil samples for determination of the mobile humic acids fraction were taken after the last cut of grasses (on 24-08-2008). The content of these acids was measured after the Tyurin method modified by Ponamariova and Plotnikova. Experimental data were processed by ANOVA using a software package 'Selekcija'.

The experimental period was characterised by the alteration of dry and excessive moisture periods. The longest dry period occurred during the plant growing season in

May and June, and again in September. Excessive moisture in October may have determined nutrient migration into deeper soil layers.

RESULTS AND DISCUSSION

The fraction of mobile humic acids is attributed to active forms of humus and is characterised by a low carbon and elevated hydrogen and nitrogen content. Literature sources suggest that this fraction of soil organic matter is formed of plant residues, root secretion, dead soil fauna and micro-organism populations, and abiotic fraction of soil organic matter (Teit, 1991). This mobile part of soil organic matter differs from the stable part in that it can be used by micro-organisms as a carbon and energy source. During mineralization of soil mobile humic acids the soil is enriched with nutrients necessary for the nutrition of growing plants and for the formation of aboveground mass. Having compared individual plant species, it was found that the highest content of mobile humic acids in the 0–30 cm soil layer occurred when perennial grasses had been grown in pure stands (Table 1).

Table 1. The variation of mobile humic acids ($C_{org.}$% of soil) in the soil as affected by cultivation of perennial grasses and different methods of their management (before ploughing on 24-08-2008).

Perennial grasses (A)	Management methods of perennial grasses (B)						Mean for factor A	
	removal from field		mixed		mulching			
	soil layer cm							
	0–30	30–60	0–30	30–60	0–30	30–60	0–30	30–60
Red clover	0.078	0.021	0.094	0.023	0.081	0.023	0.084	0.022
Red clover and festulolium mixture	0.078	0.028	0.092	0.024	0.080	0.022	0.083	0.025
Lucerne	0.072	0.029	0.098	0.025	0.090	0.026	0.087	0.027
Lucerne and festulolium mixture	0.083	0.019	0.082	0.019	0.081	0.024	0.082	0.021
Festulolium	0.080	0.026	0.097	0.020	0.081	0.024	0.086	0.023
Mean for factor B	0.078	0.025	0.093*	0.022	0.083	0.024	0.084	0.024
LSD_{05} (0–30 cm) A–0.0140; B–0.0110; AB–0.0240; (30–60 cm) A–0.0080; B–0.0060; AB–0.0140								

* −differences significant at $P < 0.05$ level.

As averaged data indicate, the highest content of these acids was identified after lucerne and festulolium grown in monocrops, by 3.6 and 2.4% higher than that after red clover. Cultivation of legume and festulolium together in mixtures tended to reduce the content of mobile humic acids. Significantly higher content of these acids (by 19.2%) was found when a mixed perennial grass management method had been used, compared with the treatment in which herbage had been removed from the field and was by on average 6.4% higher than that in the plot where herbage had been left on the soil surface and mulched four times. This might have been determined by meteorological conditions and a slower microbiological process in the second half of the plant growing season. In a deeper soil layer (30–60 cm) mobile humic acids varied from 0.019 to 0.029% $C_{org.}$ The differences between individual treatments were ambiguous. Lucerne had the most marked effect on the increase in mobile humic acids, irrespective of perennial grass management methods. It determined an increase in mobile humic acids by on average 0.005 percentage points or by 22.7%, compared with red clover.

The differences in N_{min} concentration stood out before ploughing-in of perennial grasses due to organic matter accumulated in the root rhizosphere and mulched plant aboveground mass mineralization intensity. Correlation–regression analysis shows that mineral nitrogen concentration in the 0–30 cm soil layer significantly correlated with the organic carbon content accumulated in this soil layer and was inversely proportional to it (r = – 0.57; $P \le 0.05$). Depending on perennial grass species in the topsoil layer (0–30 cm), the data distributed in the following $N_{min.}$ increasing order: festulolium < legume/ festulolium mixture< legume (Table 2).

Table 2. The variation of mineral nitrogen (mg kg^{-1} soil) in the soil as affected by cultivation of perennial grasses and different methods of their management (before ploughing on 24-08-2008).

Perennial grasses (A)	Management methods of perennial grasses (B)						Mean for factor A	
	removal from field		mixed		mulching			
	soil layer cm							
	0–30	30–60	0–30	30–60	0–30	30–60	0–30	30–60
Red clover	5.01	3.59	5.09	3.83	4.51	3.77	4.87	3.73
Red clover and festulolium mixture	3.64*	3.49	4.40	4.02	3.90	4.30	3.98*	3.94
Lucerne	4.09	3.21	4.82	2.92	4.74	3.88	4.55	3.34
Lucerne and festulolium mixture	3.44*	3.69	4.20	3.32	4.63	3.49	4.09*	3.50
Festulolium	2.99**	2.73	3.89	2.78	3.45*	3.46	3.44**	2.99*
Mean for factor B	3.83	3.34	4.48*	3.37	4.25	3.78	4.19	3.50

LSD_{05} (0–30 cm) A–0.679; B–0.526; AB–1.177; (30–60 cm) A–0.698; B–0.541; AB–1.210

* −differences significant at P<0.05 level, ** − at P < 0.01

The highest $N_{min.}$ content was after legume and their green manure use, which has been confirmed by the data of other authors (Tripolskaja, 2005). After red clover and lucerne, $N_{min.}$ concentration in the soil was by on average 41.6 and 32.3% higher compared with festulolium. Lucerne, compared with red clover, determined lower $N_{min.}$ concentration in the soil (by on average 6.6%). Cultivation of legume in mixtures with festulolium, which has a higher demand for nitrogen, significantly reduced $N_{min.}$ concentration. After red clover and festulolium mixture, $N_{min.}$ declined by 18.3%, after lucerne and festulolium by 10.1%, compared with the respective pure legume stands.

Comparison of management methods of perennial grasses revealed that the highest $N_{min.}$ content in the 0–30 cm soil layer occurred in the treatments where mixed management of perennial grasses had been used or the whole above ground mass had been mulched, which was by 17.0 and 11.0% higher compared with the plot where herbage had been removed. When applying the mixed herbage management method, the difference was significant. Under the interaction of perennial grasses and their management methods, the highest $N_{min.}$ content was identified when the red clover aboveground mass had been removed from the field or had been used under mixed management. However, significantly lower $N_{min.}$ concentration was determined after the red clover and lucerne mixture with festulolium when all the herbage had been removed from the field (27.3 and 31.3%, respectively) and after pure festulolium when herbage had been removed or mulched (by 40.3 and 31.1%, respectively), compared with the control treatment. In the deeper soil layer (30–60 cm), mineral nitrogen concentration was by 16.5% lower than that in the topsoil layer. Having compared individual species of perennial grasses it was found that significantly lower $N_{min.}$ content occurred only after festulolium compared with red clover. The use of herbage as green manure by four–time mulching during the growing season increased mineral nitrogen migration into deeper soil layers and its content tended to increase (13.2%), compared with the treatment where all herbage had been removed from the field. Under the interaction of perennial grasses and their various management methods, the highest $N_{min.}$ content was identified after red clover and festulolium grown in mixtures applying the mixed herbage management method or mulching of whole above ground mass – by 12.0 and 19.8% higher compared with the control treatment. After winter wheat emergence and reaching tillering stage (BBCH 13), late in the autumn, irrespective of the fact that winter wheat had used part of the mineral nitrogen for nutrition, its concentration increased in the topsoil layer (0–30 cm) by on average 2.68 mg kg^{-1} or 64.0% and in the deeper layer (30–60 cm) by on average 1.59 mg kg^{-1} or 45.4%, compared with the respective data before the ploughing-in of perennial grasses (Table 3).

Table 3. The variation of mineral nitrogen (mg kg^{-1} soil) in the soil as affected by cultivation of perennial grasses and different methods of their management (late autumn 12 11 2008).

Perennial grasses (A)	Management methods of perennial grasses (B)						Mean for factor A	
	removal from field		mixed		mulching			
	soil layer cm							
	0–30	30–60	0–30	30–60	0–30	30–60	0–30	30–60
Red clover	8.29	6.39	6.59	4.13*	7.03	5.47	7.30	5.33
Red clover and festulolium mixture	5.32*	3.86*	8.79	5.87	8.34	6.19	7.48	5.31
Lucerne	8.13	5.95	5.21*	4.33*	9.39	5.97	7.58	5.42
Lucerne and festulolium mixture	6.09	5.08	5.76	4.67	7.51	6.76	6.45	5.50
Festulolium	3.52**	3.66*	6.83	4.59	6.31	3.40*	5.55*	3.88*
Mean for factor B	6.27	4.99	6.64	4.72	7.72*	5.56	6.87	5.09

LSD_{05} (0–30 cm) A–1.683; B–1.303; AB–2.914; (30–60 cm) A–2.151; B–1.666; AB–3.726

* −differences significant at P<0.05 level, ** − at $P < 0.01$

With increasing $N_{min.}$ concentration, before ploughing in perennial grasses, $N_{min.}$ increased in late autumn also. Medium strong correlation ($r = 0.53$; $P \leq 0.05$) at 95% significance level was identified between these indicators. The highest $N_{min.}$ content in the topsoil layer occurred after lucerne, red clover and their mixture with festulolium. The lowest $N_{min.}$ content was after festulolium, that is significantly less difference, making up, on average 24.0%, compared with red clover. Mulching of the entire aboveground mass of perennial grasses significantly increased (by on average 23.1%) the $N_{min.}$ concentration in the soil compared with herbage removal from the field.

Late in the autumn (2.5 months after ploughing-in) the effect of perennial grasses and their management methods on soil $N_{min.}$ concentration became more marked. In the 0–30 cm soil layer, in the treatment where aboveground herbage mass had been removed from the field, the highest $N_{min.}$ content was determined after ploughing-in of pure legume. Their cultivation in mixtures with festulolium considerably reduced $N_{min.}$ content (after red clover by 35.8%, after lucerne by 26.5%, compared with respective pure legume stands). However, the $N_{min.}$ content after the pure festulolium crop was several times (2.4) lower compared with the control treatment. Using mixed management of herbage, $N_{min.}$ data were ambiguous. However, when the whole aboveground mass had been used for green manure with four-time mulching, $N_{min.}$ increased in the 0–30 cm soil layer in all treatments (except for red clover), compared with that in the treatment where herbage had been removed from the field. When herbage had been used as green manure (mulched), the highest $N_{min.}$ content accumulated after lucerne. With increasing $N_{min.}$ content in the topsoil layer it also increased in the deeper layer (r = 0.80; $P \leq 0.01$). In this layer (30–60 cm), $N_{min.}$ content varied in a similar pattern to that in the topsoil layer, but the differences were lower. Compared with red clover, the lower (by on average 27.2%) mineral nitrogen accumulation was determined by festulolium. Cultivation of the legume in mixture with festulolium did not have any marked effect on $N_{min.}$ migration into the deeper soil layer. However, when all the herbage had been used as green manure (mulched), $N_{min.}$ content in the deeper soil layer tended to increase (by 11.4%) compared with the treatment where herbage had been removed from the field.

CONCLUSIONS

The content of mobile humic acids was little dependent on plant species, however, when mixed management of aboveground part of perennial grasses was used, it increased significantly. In late autumn, $N_{min.}$ concentration in the 0–30 and 30–60 cm soil layers increased by 8.9–53.9%, 7.8–93.7%, respectively, compared with that during the end of the perennial grass vegetation period (end of August). The lowest $N_{min.}$ contents were recorded in both soil layers after festulolium cultivation. When fresh mass of perennial grasses had been used as green manure and four-time mulching, N_{min} significantly (by 23.1%) increased in the top soil layer (0–30 cm) and showed a trend of increasing in the deeper layer.

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