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NITROGEN AND PHOSPHORUS AVAILABILITY EFFECT ON ACTIVITY OF CELLULOLYTIC MICROORGANISMS IN MEADOWS

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Abstract

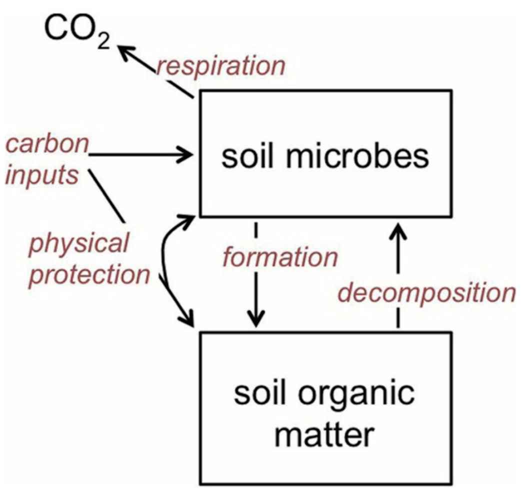
KINTL ANTONÍN, NAWRATH ADAM, ELBL JAKUB, TŮMA IVAN, MUCHOVÁ MARCELA, BRTNICKÝ MARTIN, KYNICKÝ JINDŘICH. 2016. Nitrogen and Phosphorus Availability Effect on Activity of Cellulolytic Microorganisms in Meadows. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 64(4): 1173–1179.

Meadows occupy more than 23 % of agricultural land in the Czech Republic and also represent the largest pool of carbon in soil organic matter. The organic material is an essential component of healthy soil. Decomposition of organic matter is a biological process, affected by high amount of N and P fertilizer applied in the second half of the twentieth century. This work presents the analysis of the effect of available nutrients nitrogen and phosphorus on the activity of cellulolytic microorganisms in permanent grassland of Sanguisorba-Festucetum comutatae association in the soil surface in the Protected Landscape Area of Žďárské Hills. Contents of available nutrients established by the Mehlich III method were measured ex situ. Data referring cellulose decomposition rates were obtained in situ using the standard mesh-bag method. In the field experiment, the highest loss of cellulose was recorded in variants with the addition of nutrients (**PK, 90N + PK** and **180N + PK**) with result 90.38 % on average. The difference was statistically significant, compared with the control variant without added nutrients 27.87 % ($P < 0.05$). The added amount of nitrogen in the variant 90 kg of N + PK per ha had no significant effect on loss of cellulose compared to PK variant, as well as variant 180 kg of N + PK per ha. From the results obtained, it is evident that the highest decomposition of cellulose was observed at variant with added phosphorus, compared to variant with nitrogen. Moreover, the effect of different intensity of grassland utilization was found: the amount of decomposed cellulose was higher in two variants (control and 90N + PK) of two cut system in comparison with the same variant in three cut system.

Keywords: available nutrients, meadows, cellulose decomposition, mesh-bag

INTRODUCTION

Montane and submontane secondary grasslands are integral part of the cultural landscape of the Czech Republic. Historically, they were used for hay production and sheep or cattle grazing. However, the demand for hay has declined and traditional cycles of swing and grazing have lost their economic importance (Uhlířová *et al.*, 2004). A key component for sustaining production in grassland ecosystems is the maintenance of soil organic matter (SOM; organic matter – OM), which can be strongly influenced by management (Conant, 2001). In spite of this, the importance of permanent grasslands increases, irrespectively of direct biomass production. They represent valuable ecosystems supporting unique plant and faunal communities, and together with woods and wetlands form the basis for the ecological stability of the cultural landscape (Uhlířová *et al.*, 2004). Perennial crops can provide multiple ecosystem services essential for sustainable production more effectively than production systems based on annual crops (Jordan *et al.*, 2007). Grasslands can be important sinks of atmospheric CO_2 (Acharya *et al.*, 2012) and they play a major role in the overall carbon cycle fluxes; see the Figure 1: grassland ecosystem is a habitat of microbial communities which utilize large stocks of OM, naturally generated by this ecosystem. Riggs *et al.* (2015) report they predicted N enrichment would have distinct effects on SOM pools, including the pool that is readily available for microbial decomposition, as well as the pools that have been stabilized against microbial decomposition via aggregate occlusion and mineral association.



1: *Theoretical framework for soil organic matter (SOM) dynamics, emphasizing the central role that soil microbes play in both SOM decomposition and formation (Bradford, 2013)*

This land use contains approximately 10 %–30 % of the world's soil carbon reserves (Eswaran *et al.*, 1993). Multi-nutrient fertilization strongly increases plant productivity but may also alter the storage of carbon (C) in soil, which represents the largest terrestrial pool of organic C (Fornara *et al.*, 2013). The cellulolytic activity of various soils as an indicator of agrobiological activity is assessed (Szegi, 1988). Human activities have greatly increased the availability of biologically active forms of nutrients [e.g. nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg)] in many soil ecosystems worldwide (Fornara, 2013). Nutrient concentration is a primary factor influencing organic matter decomposition (Mendelssohn *et al.*, 1999). Soil organic matter is important both from agronomic and environmental perspective because it affects the capacity of soil to sustain crop growth, and it is a source and sink of atmospheric CO_2–C (Lupwayi *et al.*, 1998; Baldock, 2007, Pospíšilová *et al.*, 2011).

Fertilizers are of key importance to sustain modern agriculture (Selibo *et al.*, 2013). Results (Fornara *et al.*, 2013) demonstrate that nutrient fertilization remains an important global change driver of ecosystem functioning, which can strongly affect the long-term sustainability of grassland soil ecosystems (e.g. ability of soils to deliver multiple ecosystem services).

The aim of this paper was to assess and compare the rates of cellulose decomposition in grass stand using four variants of fertilization with different intensity of use.

MATERIALS AND METHODS

Field experiment

The experiment was established on the research plots with the permanent stand of Sanguisorba-Festucetum comutatae association at the cadastre of the village Kameničky in 1992. The village Kameničky is situated in the Protected Landscape Area of Žďárské Hills. The site has a SW exposition and is situated on a slope with the gradient of 3°. Mean annual temperature (1951–2000) is 5.8 °C with mean annual precipitation 758.4 mm. Soil type is acidic Luvic Stagnosol on the gneiss diluvium. The experiment was designed using the method of split compartments in four repetitions. The evaluated factor was fertilization (no fertilization – control, PK fertilization, N90 + PK fertilization, and N180 + PK fertilization). The stands were exploited in two cuts and three cuts systems. The determination of available nutrients was realized according to Mehlich (1984) and Richter *et al.* (1999). This method is described below. The results are presented in the Tab. I. Nitrogen was supplied in the form of ammonium nitrate with limestone (LAV 27 %) at a total dose of 90 kg/ha of N, resp. 180 kg/ha of N. The application of nitrogen (variants 90N + PK and 180N + PK) has been performed in three equal doses since 1993. In the system of three cuts, the nitrogen dose was applied three times (1/3 in the spring, 1/3 after the 1st cut and 1/3 after the 2nd cut), resp. in two terms (1/2 in the spring and 1/2 after the 1st cut). Potassium and phosphorus fertilizers have been applied every year in the spring since 1992; on 30th April in 2012 and 2013. Phosphorus was applied in the form of Hyperkorn (26 %) at the dose of 30 kg/ha P and potassium was applied in the form of potassium salt (DS 60 - 60 % K) at the dose of 60 kg/ha K. In the system of three cuts, the harvest took place in early June, early August and early October. In the system of two cuts, the stands were harvested in the middle of June and in early September. The grass was harvested by the mower model MF-70 equipped with a cutting bar (engagement 1.2 m).

I: *The basic agrochemical parameters of luvic stagnosol at the depth of 0–0.30 m: plant available nutrient content [mg/kg] and soil reaction before fertilization in spring 2013 in individual variants.*

Two cut stands	mg/kg					pH
	P	K	NH_4^+–N	NO_3^-–N	Nmin	
Unfertilized control	37	72	7.95	0.97	8.92	4.47
PK	119	85	9.97	1.14	11.11	4.68
90N + PK	87	66	7.65	1.15	8.80	4.46
180N + PK	107	75	7.98	1.35	9.33	4.45
Three cut stands	**mg/kg**					**pH**
	P	K	NH_4^+–N	NO_3^-–N	Nmin	
Unfertilized control	32	68	7.93	0.98	8.91	4.54
PK	114	70	8.28	1.12	9.40	4.48
90N + PK	123	69	7.80	1.01	8.81	4.48
180N + PK	110	72	7.28	1.08	8.36	4.50

Determination of available nutrients phosphorus, potassium and mineral nitrogen in soil

The contents of mineral nitrogen NH_4^+–N and NO_3^-–N were determined by extraction with 2 M KCl. Extraction was realized in sealed glass containers. From each replication, 20 g of soil were collected on April 30th 2013. This sample was inserted in glass containers and shaken for 60 min with 2 M KCl (Bundy and Meisinger, 1994; Elbl *et al.*, 2014). After shaking, determination of mineral nitrogen NO_3^-–N and NH_4^+–N were realized by distillation-titration method according Peoples *et al.* (1989).

The soil samples, which were collected from individual plots of the field experiment, were analysed using method Richter *et al.* (1999), which was described and used by Maňásek *et al.* (2013): soil was extracted using the Mehlich III extractant. The content of available phosphorus (P) in the extract was determined colorimetrically and the content of available potassium (K) using atomic absorption spectrometry (AAS). The ion-selective electrode (ISE) method was used to determine pH after the extraction in 0.01 M $CaCl_2$. The plant-available nutrient content and soil reaction are listed in the Table I.

Determination of cellulose decomposition rates

Cellulose tests (see the Figure 2) were applied using samples of exactly weighed paper cottonwool in nylon mesh-bags 15 × 10 cm (1 mm of mesh size) according Tesařová (1987) and Hrevušová *et al.* (2012).

2: *Applied mesh-bags on the soil surface (authors)*

The amount of 5 g of dry mass samples was chosen with respect to the length of exposure extending over the whole non-growing and growing season of 364 days (from November 19, 2012 to November 18, 2013). The mesh-bags were exposed on the soil surface. After the exposure, the samples were cleaned and dried at 105 °C in the laboratory, weighed and decrease in dry mass of original sample of cellulose was assessed. The weight of mineral particles fixed to remnants of cellulose was determined by incineration at 550 °C. The cellulose decomposition rate was calculated according to the formula:

$$DR = \ln W_0 - \ln W_1/t_1 - t_0 \text{ [in mg/g·day]}$$

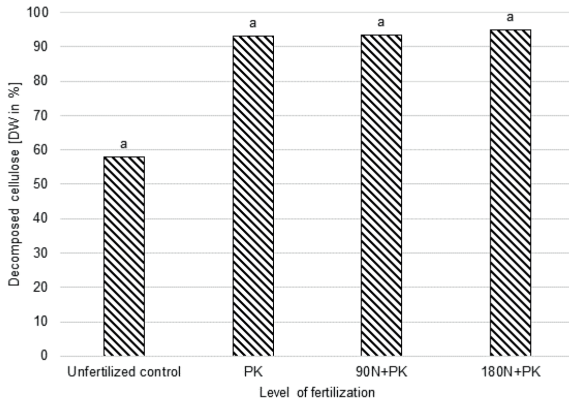
where; W_0 and W_1 is the dry mass of cellulose at the beginning and the end of the exposure, and t_1 - t_0 is the number of days of exposure (Tůma, 1998). Measuring cellulolytic activity in situ is one of the primary methods for determining microbial activity during cellulose decomposition (Semenov *et al.*, 1996).

The potential differences in total yield and decomposition of cellulose were analysed by one-way analysis of variance (ANOVA at the level

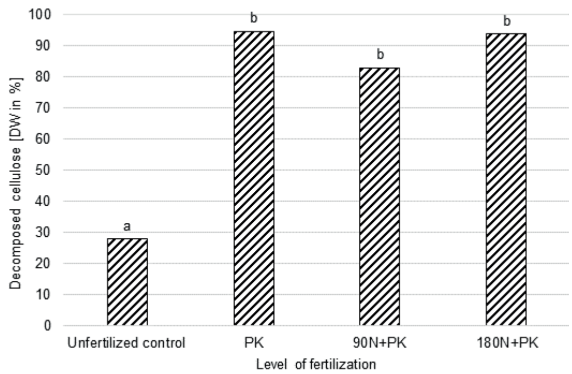
$P < 0.05$) with post-hoc Tukey's HSD test. The results were analysed by Statistica 12 software (Dell Software, Round Rock, USA) and processed graphically in the MS OFFICE 2013 – Microsoft Excel (Microsoft, Redmond, USA).

RESULTS AND DISCUSSION

During the year exposition in the system of two cuts, the rate of cellulose decomposition on the soil surface was higher in the variants with fertilization than in the control variant. This suggests the activity of cellulolytic microorganisms in soil was higher in all variants with added nutrients. Although statistically significant differences in the rates of cellulose decomposition were recorded only in three cut system, between Unfertilized variant (control) and variants with addition fertilizers (PK, 90 + PK, 180N + PK) no difference was found.



3: *The decomposition of cellulose in two cut stand during a year. Comparison of cellulose decomposition in soil surface during 364 days of exposition. The amount of decomposed cellulose is expressed in per-cent of dry mass of the original sample. Different letters denote significant differences at the level P < 0.05.*



4: *The decomposition of cellulose in three cut stand during a year. Comparison of cellulose decpomposition in soil surface during 364 days of exposition. The amount of decomposed cellulose is expressed in per-cent of dry mass of the original sample. Different letters denote significant differences at the level P < 0.05.*

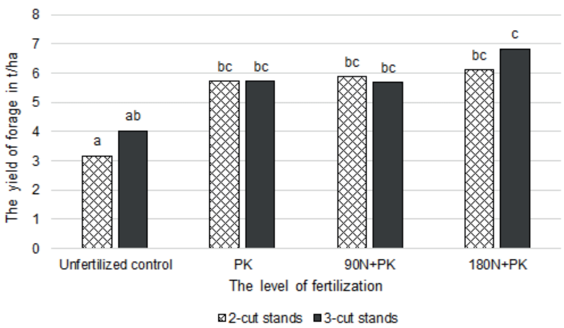
Similar trend in the rate of cellulose decomposition was recorded even in the system of three cuts (the Figure 4 and Table II). Activity of cellulosic microorganisms was verifiably lower in unfertilized variant where weight of exposed cellulose was twice to three times lower (27.87 %) compared to fertilized plots (up to 94.53 % decomposition of the original weight of cellulose). Even with intensive mowing, there has not been observed any demonstrable difference in the degradation of cellulose between variants with different fertilization. It is interesting that after addition of phosphorus and potassium fertilizer, there was significant increase in rate of cellulose decomposition. Anyway, after another addition of nitrogen into fertilized (phosphorus and potassium) plots, there was no further increase in activity of cellulosic microorganisms. Results (Riggs *et al.*, 2015) suggest that addition of N will increase C sequestration by decreasing rate of decomposition of SOM, as well as stabilizing SOM against microbial decomposition in aggregate-occluded pools. The availability of essential nutrients, such as nitrogen (N) and phosphorus (P), can feedback on soil carbon (C) and the soil microbial biomass (Griffiths *et al.*, 2012,). Investigation of the Redfield ratio (106:16:1), which is an atomic carbon (C), nitrogen (N), phosphorus (P) ratio, was found consistent in both plankton and marine waters (Redfield, 1958). Although soil is characterized by high biological diversity, structural complexity and spatial heterogeneity, Cleveland and Liptzin (2007) state that remarkably consistent C : N : P ratios both in total soil pools and in the soil microbial biomass. The availability and amount of essential nutrients e.g. nitrogen (N) and phosphorus (P) can thus affect soil carbon dynamics and microbial biomass (Wang *et al.*, 2010). The presence of these elements significantly affects the activity of the decomposition organisms. Our case shows phosphorus is probably limiting factor for the activity of microorganisms at these permanent grasslands. While its content was low in the fertilized variant, phosphorus content was good at variants with the addition of PK. Limitation caused by phosphorus deficiency is stated e.g. by Šimek (2003). In the micro-site (at the site location of cellulose), ratio of C : P may also significantly increase temporarily because cellulose is a another source of carbon. Decomposition of such mass may be lower at site without the

II: *Cellulose decomposition rates in mg/g/day at the location Kameničky*

	two cut stand	three cut stand
Unfertilized control	0.0051	0.0010
PK	0.0111	0.0103
90N+PK	0.0101	0.0066
180N+PK	0.0106	0.0102

addition of phosphorus. At all variants, there is probably sufficiency of nitrogen which is revealed by the values of total mineral nitrogen in the soil. Even after the supply of nitrogenous fertilizer, the nitrogen was not significantly retained in the soil at the monitored plots. The source of sufficient amount of nitrogen at the site may be partially fallout of nitrogen emissions but it is mainly due to the activity of fixators of atmospheric nitrogen, particularly those living in symbiosis with the roots of *Papilionaceae* plants. Mrkvička and Veselá (2007) state fertilization with phosphorus applied to grassland acts positively on domination of clover plants (for example white clover; family: *Fabaceae*) able to fix atmospheric nitrogen (N2). *Trifolium repens* appeared much more often on the monitored plots fertilized with phosphorus. This corresponds to the highest level of fixed nitrogen in the soil with added phosphorus without added nitrogen fertilizers at variants of two cuts. Obviously, additional input of nutrients may increase the decomposing activity of microorganisms in the soil and thus increase the potential availability of other nutrients from the organic matter contained in the soil but at the same time, it may lead to increased flux rate of CO_2 from the soil.

Fontaine *et al.* (2004) indicates results suggesting that nutrient shortage for soil microbes could decrease C accumulation in soils. Further enhancement of lignin and cellulose decomposition occurred in a medium containing organic nitrogen supplemented with low levels of NO3- (Berg and Matzner, 1997). Grasslands received considerable attention for their C sink capacity (Conant *et al.*, 2001) but they are dynamic and release CO_2 as a response to management practices - cutting and fertilizing (Ammann *et al.*, 2007).



5: *The yield of forage in t/ha (2013). Different letters denote significant differences at the level P < 0.05.*

If we compare the forage yields of the particular variants (the Figure 5), there is a noticeable positive effect of supplied nutrients within both intensities of use. At both intensities of use, there was a significant increase in production just after the addition of P and K fertilizers where the main role was probably played by phosphorus. Phosphorus plays a critical role in energy reactions in the plant. Deficits can influence essentially all energy requiring processes in plant metabolism (Grant, *et al.*, 2001). This conclusion was made also by Verhoeven and Schmitz, (1991); Mrkvička and Veselá, (2007) who add that phosphorus fertilization of grassland acts positively on dominance of clover plants which are able to fix atmospheric oxygen and enrich soil with nitrogen available for plants. Holúbek *et al.* (1997) state phosphorus increases utilization of other nutrients, mainly nitrogen, in grassland and enhances quality and tastiness of forage. Addition of nutrient (N) at two-cut stands did not show any other progress and higher doses of nitrogen fertilizers show to be ineffective not just from the economical point of view but can also present risk for the ecosystem of grassland stand. Stoate *et al.* (2009) state excessive use of fertilizers leads to decrease in biodiversity (number of plant species) and to deterioration of water quality. On the other hand, sufficient supply of nutrients (N) in soil is necessary for achieving adequate crop quality (Lošák *et al.*, 2010). Lucian and Gavril, (2013) recommend using low doses of fertilizers that has no effect on biodiversity and structure of meadow vegetation. At three-cut stands, some increase in production after addition of nitrogen could be observed compared to variant with applied PK fertilizer. The increase in production is not verifiable compared to variant with the addition of phosphorus and potassium. Some positive effect of nitrogen supplies can be seen as three-cut variant cause higher withdrawal of nitrogen from the habitat in form of three times taken aboveground biomass and some addition can balance it. On the other hand, Verhoeven and Schmitz, (1991) state the mowing regime depletes the P pool in soil, while it keeps N inputs and outputs in balance. Importance of nitrogen as a limitation element was proved in long-term experiment held by Královec and Rais, (1990) who reached maximum production using dose of 160 kg/ha N in mesohygrophylous habitat.

CONCLUSION

Fertilization with phosphorus is probably the main factor influencing the increased cleavage of cellulose in monitored grasslands which can result even in greater decomposition activity of micro-organisms able to play a role in increased availability of nutrients from the decomposition of soil organic matter. Further adding of nitrogen fertilizer showed no significant effect on these processes. Similarly, fertilizing with phosphatic fertilizers showed an impact on the increased value of produced grass biomass. However, it was not increased significantly by enriching with the nitrogen fertilization. Intensified decomposition of organic matter can lead to increased release of CO_2 from the soil to the atmosphere. Anyway, such flow may be compensated by retention of carbon in grassland biomass in the variant fertilized with phosphorus. In the final carbon balance, it will depend on the use of harvested and taken biomass perhaps as forage.

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