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CELLULOSE AND CUTISIN DECOMPOSITION IN SOIL OF *ALOPECURETUM* MEADOW

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Abstract

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Plant litter decomposition is a fundamental process to ecosystem functioning regulated by both abiotic and biotic factors. The aim of this study was to determine the decomposition of cellulose and protein (cutisin) substrates on permanent *Alopecuretum* meadow under different methods of management. The treatments were following: 2 × cut, 2 × cut + NPK, 2 × mulch, 1 × cut, 1 × mulch (frequency of mowing per year) and no-treated plots. Cutting or mulching was carried out in October, under the 2 × cut management also in May. In 2007–2009, cellulose and cutisin in mesh bags were placed in the soil and kept from April to October. Total mean ratios of decomposed cellulose and cutisin were 83 % and 40 % of primal substrate weight, respectively. The cellulose decomposition was affected by weather conditions, but not by applied management. The highest mean ratio of decomposed cellulose was found in 2009 (with increased amount of precipitation in May and July), the lowest in 2007. Coefficients of variation within a year and over the years were up to 22 % and 20 %, respectively. The cutisin decomposition was significantly affected by applied management in all three years. Higher rates of decomposition were noted in two times mowed treatments compared to one or not mowed treatments. Significant differences were found between years in 2× cut and 2 × cut + NPK treatments. Coefficients of variation within the year and over the years were both higher by cutisin than by cellulose samples (up to 50 and 42 %, respectively).

permanent grassland, protein decomposition, management, fertilization, mulching, frequency of mowing

Plant litter decomposition is a fundamental process to ecosystem functioning being responsible of carbon, nitrogen and other nutrients cycling (VIRZO DE SANTO *et al.*, 2009). Microbial decomposition is regulated by both abiotic factors (climatic conditions and soil chemical and physical properties) and biotic factors (litter quality and microbial community composition). Size and activity of the soil microbial community are determined by location (ALLISON *et al.*, 2007), soil temperature (KIRSCHBAUM, 1995), moisture (DRENOVSKY *et al.*, 2004), soil pH (HOPKINS *et al.*, 1990), content of soil carbon (UHLÍŘOVÁ *et al.*, 2005), nitrogen (KEELER *et al.*, 2009), phosphorus (VERESOGLOU *et al.*, 2011), and others nutrients such as calcium and manganese (VIRZO DE SANTO *et al.*, 2009), etc. For example, the bacterial community often favors high soil fertility and nutrient availability and the fungi prefer low soil fertility (GRAYSTON *et al.*, 2004).

For a given ecosystem, the litter decomposition is mostly affected by litter quality (LIU *et al.*, 2010). High variability exists between plant species (EISENHAUER and SCHÄDLER, 2011), between individual plants of the same species and between various organs on the same plant (ABIVEN *et al.*, 2005) in depending on differences in litter chemistry. In general, litters with higher N-concentrations decompose faster (LIU *et al.*, 2010). Fast decomposed labile fractions such as cellulose and hemicellulose may be degraded in few months (BERG, 2000). Recalcitrant plant materials are lignin and modified lignin-like humification products (VIRZO DE SANTO *et al.*, 2009). In this way, plant composition of stand can affect soil microorganisms very considerably. Carbon availability for microbe is also determined by plant root exudation and secretion, particularly in sites where carbon is lacking (GRAYSTON *et al.*, 1996). In turn, plants mineral nutrition is often mediated by association with microorganisms, e.g. mycorrhizal fungi and N_2 fixators (LARIMER *et al.*, 2010). Increased diversity of plant derived resources increases the diversity of decomposers and herbivores in soil, which in turn promotes the diversity of other components of the soil food web (EISENHAUER *et al.*, 2011). Higher decomposer diversity often aids in higher ecosystem stability (EISENHAUER and SCHÄDLER, 2011).

Microbial biomass and enzyme activities are also affected by management practices (e.g. grazing, frequency of cutting, mulching, organic and mineral fertilizers application, and grassland restoration). Changes in microbial-community structure after nutrient application, mainly nitrogen, are often discussed. Nitrogen addition can influence decomposition process through the direct influence of inorganic N availability on microbial activity or indirectly through changes in the quality of decomposing litter (LIU *et al.*, 2010). Long-term nitrogen addition experiments have found both positive and negative and neutral effects of added N on decomposition (KEELER *et al.*, 2009) in dependence on other above mentioned factors, mainly on litter quality. The litter decomposition can also be influenced by phosphorus application (VERESOGLOU *et al.*, 2011).

Both fertilization and frequency of cutting determine plant biomass production and, thus, litter inputs in soil and decomposition rate (WANG *et al.*, 2011). Defoliation affects also root morphology, exudation (GUITIAN and BARDGETT, 2000) and plant litter quality. Under mulching, the cut biomass in addition stays on stubble and can change the physical (KVÍTEK *et al.*, 2000) and chemical soil conditions.

As was mentioned above, relations among litter decomposition and sward management are closely related to the botanical composition of sward and locality conditions. The previous experiments with substrate decomposition under different stand management were conducted mainly for mountain meadows (UHLÍŘOVÁ *et al.*, 2005) but humid high yielding meadows were investigated rarely. The objective of this study was to found the amount of decomposed cellulose and protein substrates during a growing period of *Alopecuretum* meadow. We evaluated long term effect of fertilization, not harvesting, different frequencies of cutting and mulching on this value of decomposed organic matter.

MATERIALS AND METHODS

The experiment area is located near Černíkovice village (49°46‘27“N, 14°34‘52“E, 363 m a.s.l.), in Benešov district. Mean annual temperature of the locality is 8.1 °C and mean annual precipitation is 600 mm. The soil at the site is fluvisol/gleysol with a loamy texture, the content of soil organic matter is around 37 g kg^{-1}, and pH (H_2O) 5.7. The experiment plot is a part of fertile meadow with high and fluctuating level of underground water. The meadow belongs to *Alopecuretum* stand type.

The experiment was established in 2001 in six followed treatments: 2 × cut, 2 × cut + NPK, 2 × mulch, 1 × cut, 1 × mulch and no-treated plots. It has block design with four replications. The two times treated plots were cut or mulched in May and October, the once treated plots only in October. The applied NPK fertilizing was added in doses 100 kg N ha^{-1} (as a limestone ammonium nitrate), 40 kg P ha^{-1} (as a triple super phosphate) and 100 kg K ha^{-1} (as a potash chloride) per year.

Cellulose represents a polysaccharide; whereas cutisin is a protein substratum (16–17% N) based on collagen. Cutisin is produced from calcimine by mechanical and chemical treating. The proportions of decomposed cellulose and cutisin were evaluated on eight samples for each treatment.

Filter papers of weight 39.6 g, as a source of cellulose, were fold up in square (125 × 125 mm). Lower weight of the filter paper could meant enhanced danger of whole sample decomposition. Squares of cutisin (100 × 100 mm) weighted 1.5 g. Both filter paper and cutisin were together packed in mesh bags (mesh size 5 mm; bag size around 160 × 160 mm). The bags were vertically put into 10–14 mm deep groove in the sward. The samples were in the soil for six months (from April to October). After samples were taking out, they were carefully cleaned and dried at 105 °C to constant weight. The percentage of decomposed matter was calculated from the decrease of the organic substrate weight.

The data were evaluated by one-way ANOVA tests and Post-hoc HSD tests for unequal n. For variability presentation, the coefficients of variation (CV) were used. CV is defined as the ratio of the standard deviation to the mean value.

RESULTS AND DISCUSSION

Total mean weights of decomposed cellulose and cutisin in six months were 32.9 g (83 % of primal weight) and 0.6 g (40% of primal weight), respectively. However protein substrate cutisin is mechanically and chemically adjusted during production. From this reason, cutisin could be decomposed in different rate than natural protein. These values were used only for comparison of various management among each other in this experiment.

The cellulose decomposition was affected by weather conditions, but not by applied management (Tables I and II). Many studies, e.g. UHLÍŘOVÁ *et al.* (2005) and WANG *et al.* (2011), noted significant effect of applied management to microbial activity. The reason for no-significant effect of management on cellulose decomposition in our experiment

I: *The significance of management on the ratio of decomposed cellulose and cutisin. Results of ANOVA, significant values are faced in bold. Different letters document statistical differences among treatments (α = 0.05).*

	Cellulose	Cutisin						
	P - value	**P - value**	**2 × cut**	**2 × cut + NPK**	**2 × mulch**	**1 × cut**	**1 × mulch**	**No-treated**
2007	0.111	< **0.001**	ab	b	abc	d	acd	cd
2008	0.331	**0.031**	ab	a	ab	ab	ab	b
2009	0.176	**0.001**	ab	a	a	ab	b	ab

II: *Ratio of decomposed cellulose (%), results of ANOVA, significant values are faced in bold. Different letters document statistical differences among years (α = 0.05). CV_w is coefficient of variation within one year. CV_o is coefficient of variation over three years (in columns „mean").*

	2 × cut		2 × cut + NPK		2 × mulch		1 × cut		1 × mulch		No-treated	
	mean	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**
2007	81.8^a	18.1	73.4^a	21.6	86.5^a	17.0	70.0^a	9.2	70.7^a	19.1	79.0^a	14.5
2008	85.0^a	13.1	84.4^{ab}	14.6	81.6^a	16.2	89.2^b	8.4	75.2^{ab}	21.3	84.0^a	12.6
2009	79.8^a	18.5	91.3^b	7.6	94.2^a	6.9	88.0^b	13.5	89.6^b	12.9	89.1^a	12.1
P-val	0.751		**0.026**		0.123		**<0.001**		**0.032**		0.209	
Mean	82.2		83.0		87.5		82.4		78.5		84.0	
CV_o	16.1		16.8		14.3		15.0		19.8		13.4	

III: *Ratio of decomposed cutisin (%), results of ANOVA, significant values are faced in bold. Different letters document statistical differences among years (α = 0.05). CV_w is coefficient of variation within one year. CV_o is coefficient of variation over three years (in columns „mean").*

	2 × cut		2 × cut + NPK		2 × mulch		1 × cut		1 × mulch		No-treated	
	mean	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**	**mean**	**CV_w**
2007	62.3^a	18.8	69.6^a	27.0	52.4^a	42.3	24.3^a	48.7	43.5^a	39.7	29.5^a	43.2
2008	34.7^b	50.0	49.4^{ab}	49.0	49.0^a	40.6	34.1^a	48.8	33.7^a	31.9	23.4^a	42.5
2009	39.3^b	17.4	42.0^b	18.9	42.7^a	22.3	37.6^a	21.0	29.2^a	25.9	33.2^a	22.9
P-val	**0.001**		**0.018**		0.567		0.118		0.089		0.183	
Mean	45.5		53.7		47.8		32.0		35.5		28.7	
CV_o	38.1		39.4		36.5		41.8		37.9		37.2	

could be a longer time of conducted tests because this effect could be evident only in earlier stages of decomposition.

The significant effect of year condition on the cellulose decomposition was described by UHLÍŘOVÁ *et al.* (2005) and HONSOVÁ *et al.* (2007). In our experiment, the highest mean ratio of decomposed cellulose was found in 2009, the lowest in 2007. It could be a consequence of increased amount of precipitation in 2009 (especially in May and July) than in 2007 and 2008. This effect was not stabile across variants of management where 1 × and 2 × cut treatments had highest values in 2008. The lowest values for 2 × cut and 2 × mulch variant were measured in 2009 and 2008, respectively.

The average coefficients of variation within a year (CV_w) in evaluated treatments ranged from 7 to 22%. The obviously lowest CV_ws were in 1 × cut treatment. The CV_ws were in all three years similar. The coefficients of variability over the years (CV_o) were from 13 to 20% (Tab. II).

The ratio of the decomposed cutisin was significantly affected by applied management in all three years (Tab. I). Higher rates of decomposition were noted in two times mowed treatments compared to one or not mowed treatments. Late spring mowing enabled increasing of soil temperature and in this way also the rate of decomposition (KIRSCHBAUM, 1995).

UHLÍŘOVÁ *et al.* (2005) described lower microbial biomass and activity in once a year mulched plots compared to cut plots. In Černíkovice, there were recorded similar values of decomposed cutisin by both treatments (1 × cut and 1 × mulch). The possible explanation may be in a soil temperature. In vegetation seasons the soil temperature under mulch layer keeps lower (KVÍTEK *et al.*, 2000). If mowing of the stands is performed in late autumn, it could have a same effect on soil temperature as mulching of stand.

Adding of mineral nutrients can have positive effect on decomposing activity (KEELER *et al.*, 2009). There were not observed effect of long term NPK fertilizing in Černíkovice experiment. It suggests no strong limitation of decomposition by these nutrients in this locality. However other factors could also play important role, e.g. soil genesis, chemical and biological properties of soil.

Significant differences were found between years in 2 × cut and 2 × cut + NPK treatments. In these treatments, the biomass from the first cut in May was taken away and therefore, the sward can be less resistant to summer drought.

CV_ws of cutisin were highest in 2 × mowed treatments and in 1 × cut treatment in 2008, and in 1 × mulched and not treated places in 2007 (Tab. III). Cutisin samples were more heterogeneous than cellulose samples and ranged from 17 to 50%. Coefficients of variability over years were also higher by cutisin (37–42%) than by cellulose.

CONCLUSION

Cellulose decomposition on *Alopecuretum* meadow in Černíkovice was depended mainly on year to year condition variability. The main factor probably was precipitations that directly influence microbial activity. Proportion of decomposed cutisin was affected not only by year conditions, but also indirectly by management where two cut regimes significantly increased this value in comparison with one cut regime or no harvest plot. It shows, that the soil temperature, which was higher when the stand was mowed in late spring, was important for cutisin decomposition. The cellulose and cutisin decomposition could be also influenced by various chemical properties and by composition of microbial community, which can be the aim of further research.

SUMMARY

Plant litter decomposition is a fundamental process to ecosystem functioning regulated by both abiotic factors and biotic factors. The aim of this study was to determine the decomposition of cellulose and protein (cutisin) substrates during vegetation.

An experiment, established in 2001 on *Alopecuretum* meadow under different methods of management, was used. The following treatments were recorded: 2 × cut, 2 × cut + NPK, 2 × mulch, 1 × cut, 1 × mulch (frequency of mowing per year) and no-treated plots. Cutting or mulching was carried out in October, also in May when mowed twice a year. The applied NPK fertilizing was composed of 100 kg N, 40 kg P and 100 kg K ha^{-1} per year. Over 2007–2009 period, cellulose and cutisin in mesh bags were put in soil for six months (from April to October).

Total mean ratios of decomposed cellulose and cutisin were 83% and 40% of primal weight, respectively. The cellulose decomposition was affected by weather condition, but not by applied management. The highest mean ratio of decomposed cellulose was found in 2009 (with increased amount of precipitation in May and July), the lowest in 2007. This effect was not stabile across variant of management. Coefficients of variation within year were up to 22%, where the lowest was calculated in 1 × cut treatment. Coefficients of variability over years were up to 20%.

The cutisin decomposition was significantly affected by applied management in all three years. Higher rates of decomposition were noted in two times mowed treatments compared to one or not mowed treatments. There were recorded similar values of decomposed cutisin by both one mowed treatments. There was noted no effect of long term NPK fertilizing. Significant differences were found between years in 2 × cut and 2 × cut + NPK treatments. Coefficients of variation within a year were highest in 2 × moved treatments and in 1 × cut treatment in 2008, and in 1 × mulched and not treated places in 2007. Cutisin samples were more heterogeneous than cellulose samples (coefficients of variation were up to 50 %). Coefficients of variation over the years were up to 42%, which was also higher than by cellulose.

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