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What is the effect of long-term mulching and traditional cutting regimes on soil and biomass chemical properties, species richness and herbage production in *Dactylis glomerata* grassland?

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ABSTRACT

Mulching is a type of grassland management in which clippings are crushed into short lengths and left to decompose on the sward surface. This low-cost method has been widely used in the Czech Republic since 1990 to maintain grassland without agricultural utilization. Several studies have examined the effects of mulching on vegetation, but none have focused on nutrients in the soil and herbage. We hypothesized that mulching supports nutrient turnover because of easy decomposition of the mulched biomass. We analysed data from a manipulative experiment in an upland grassland established in 2000 with the following treatments: unmanaged control (U); two cuts per year with biomass removal in June and August (2C); mulching once per year in July (1M); mulching twice per year in June and August (2M); and mulching three times per year in May, July and September (3M). Based on RDA analysis, there were three groups of treatments with a similar response of soil and herbage chemical properties: (i) U; (ii) 2C (iii) 1M, 2M, 3M. After eleven years of the experiment the mulching regimes had not produced any substantial changes in soil and herbage nutrient concentrations in comparison with the unmanaged or/and cut treatments. However, under all mulching treatments there was a tendency for extractable soil P and K concentrations together with P and K concentrations in the herbage to be higher than under treatment 2C and lower than under treatment U. Herbage biomass production in the 2M treatment was significantly higher than in the 2C treatment. Plant species richness was significantly affected by applied treatment (2C > 3M, 2M > 1M, U). Mulching performed two or three times per year can substitute for a conventional cutting regime over eleven years without substantial changes in the soil nutrient properties although a decrease in plant species diversity was observed.

1. Introduction

Most of the grasslands in Central Europe have been created and maintained by traditional agricultural management practices. These include regular defoliation by grazing or by haymaking carried out over many centuries, and their continued existence is dependent on human activities (Hejcman et al., 2013). Political changes in the 1990s led to the breakup of state-run farms in many of the former-communist countries of Central Europe, and one consequence was widespread changes in grassland management (Krahulec et al., 2001; Vassilev et al., 2011). There was a dramatic reduction in the numbers of domestic herbivores that had previously been available for utilization of grassland forage, and in addition many areas of former arable land were converted to grassland. As a result, many grasslands are no longer used for forage production and are now either maintained under extensive management with government subsidies, or have been abandoned (Isselstein et al., 2005; Hejcman et al., 2008, 2010a).

As agriculturally maintained grasslands are often valuable sources of biodiversity, and as the areas they occupy have also been reduced, there is a need to find alternatives to the traditional management practices of haymaking or grazing that are both economically feasible and able to maintain biodiversity. One such practice is mulching, which has been used in the Czech Republic since the 1990s (Fiala, 2007; Gaisler et al., 2013). Mulching is a low-cost method (Dux et al., 2009) based on cutting the sward without herbage removal; instead, the clippings are crushed into small

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pieces that are left on the sward surface to decompose, thereby releasing a large proportion of their mineral nutrient content (Gaisler et al., 2004). This method has also been recommended as an alternative management for the conservation of species-rich grasslands (Kahmen et al., 2002; Moog et al., 2002). In low-production grasslands mulching can substitute for conventional agricultural cutting management without substantial loss of plant species richness and diversity (Mašková et al., 2009; Gaisler et al., 2013), but there is a shortage of information about the effect that mulching has on the more productive meadows which comprise a substantial part of the European landscape.

Different methods of grassland management are known to influence plant species composition and species diversity differently (Ryser et al., 1995; Bakker et al., 2002; Eler et al., 2005; Pavlů et al., 2005; Hejcman et al., 2010c; Pavlů et al., 2011a; Valkó et al., 2012; Gaisler et al., 2013), although less is known about how different management practices, especially mulching, affect soil and herbage nutrient concentrations. Cutting with herbage removal can result in nutrient depletion from the soil, especially when performed over a long period without fertilizer application (Schnitzler and Muller, 1998; Perring et al., 2009; Pavlů et al., 2011b). Nevertheless, the quantities of nutrients removed annually by harvested biomass are very low (Bakker, 1989; and citations therein). In abandoned grasslands, where nutrients are not exported with harvested herbage, nutrient availability in the soil may increase because of the input of nutrients from atmospheric deposition, mineralization of soil organic matter or by weathering of soil minerals (Köhler et al., 2001). However, in contrast to cutting, there is little information about the effects of long-term mulching on soil and herbage chemical properties. It might be expected that management with mulching would support nutrient turnover because of easy decomposition of the mulched biomass. Further, the biomass left on the site might also lead to changes in the physical properties of the soil (Facelli and Pickett, 1991) and then to changes in soil chemical concentrations.

Management regime and soil chemical properties are key factors that affect biomass production in temperate grasslands (Bakker, 1989). It is assumed, as has been shown by Oomes et al. (1996), that because of higher nutrient recycling the biomass production under a mulching regime will be higher than under cutting management with biomass removal. But the results obtained by Mašková et al. (2009) in a long-term management experiment were not so straightforward, and biomass production under mulching was intermediate between cutting and abandonment; therefore, it seems that local conditions and type of vegetation can also play an important role (Römermann et al., 2009).

Some soil and herbage nutrient concentrations are closely linked, so changes in the nutrient status of the soil can be detectable in the herbage. For example, in the experiment of Schaffers (2002) the changes in K concentration caused by management were detectable in the herbage earlier than in the soil. Similar results were shown for P and K by Pavlů et al. (2013).

To investigate whether mulching could be a sustainable alternative management to traditional haymaking we established a long-term experiment on a *Dactylis glomerata* dominated grassland in an upland area (Jizerské hory Mountains, Czech Republic). Over a period of eleven years, different management regimes (mulching, cutting, abandonment) were applied. Unpublished results on changes in botanical composition showed increased cover of forbs and lower cover of grasses under no management in comparison to the cut and mulching treatments, but the cover of forbs and graminoids was similar under all the managed (mulching and cutting) treatments. We hypothesized that mulching supports the turnover of nutrients because of easy decomposition of the mulched biomass and the likelihood of increased nutrient concentrations in the soil, which can consequently affect biomass production, herbage chemical properties and species richness. Within this context, we aimed to answer the following questions: (i) what is the effect of long-term mulching on soil and herbage chemical properties in comparison with cutting and unmanaged treatments? (ii) what are the relations between soil and herbage chemical properties, soil chemical properties and species richness? and (iii) what are the effects of different management regimes on herbage biomass production and species richness?

2. Material and methods

2.1. Study site

The experiment was established in the year 2000 on a site 10 km north-west of Liberec in the Czech Republic (50.851N, 15.037E); altitude 443 m a.s.l). The site is in the Jizerské hory Mountains and has a mean annual temperature of 7.2 °C and the average annual precipitation is 803 mm. The bedrock is a biotic orthogneiss underlying acid Cambisol.

At the start of the experiment, mixed soil samples from the upper 0–10 cm were collected from random points and analysis showed the following chemical properties: pH_{KCl} was 6.3; concentration of organic C (C_{org}) was 27,000 mg kg^{-1}; plant-available concentrations (Mehlich III extraction; Mehlich, 1984) of P, K, and Mg were 28 (satisfactory), 138 (satisfactory), and 290 mg kg^{-1} (very high), respectively. In the year 1990, ten years before establishment of the experiment, the meadow had been drained, limed and reseeded with a high production seed mixture (*D. glomerata*,*Festuca pratensis, Elytrigia repens*, *Poa pratensis*, *Trifolim pratense, Trifolium repens*). The sward was subsequently mown or occasionally grazed by cattle. According to Chytrý (2007), the vegetation of the experimental grassland was classified as an Arrhenatherion alliance. The dominant vascular plant species before the start of the study, in descending order according to their cover, were: *D. glomerata*, *F. pratensis, E. repens*, *P. pratensis*, *Trisetum flavescens*, *Vicia sepium*, *Aegopodium podagraria*, *Galium album* and *Veronica chamaedrys*. The mean cover values of the main grasses, legumes and forbs recorded every year in May before the first management application in all treatments for the years 2007–2011 are given in Table 1. The mean forage yield of the meadow ranged from 3 to 6 t ha^{-1} of dry matter per year.

2.2. Experimental design

The experiment was established in four completely randomized blocks, with rectangular plots (10 m × 5 m) of each of five treatments in each block. The treatments were: (i) unmanaged control (U), (ii) two cuts per year with biomass removal in June and August (2C), (iii) mulching once per year in July (1M), (iv) mulching twice per year in June and August (2M) and (v) mulching three times per year in May, July and September (3M). Mulching was performed using a tractor-driven Uni Maher UM 19 (Ducker, Stadtiohn) machine and cutting was performed using a tractor-driven rotary mower. The mulching machine crushed the plant biomass into pieces 5–10 cm long and spread them into a homogenous layer on the sward surface. Cut biomass (from treatment 2C) was removed immediately following cutting. The height of mulching and cutting was 5 cm above the soil surface and mulching and cutting were performed in the same way as done by local farmers.

2.3. Soil chemical properties

Soil samples were collected in October 2011. Three soil subsamples were randomly collected from the upper 10 cm of the soil

Table 1
Total cover (%) of grasses, legumes and forbs and the cover (%) of the most abundant species in each functional group. Numbers represent mean values for treatments: twice-cutting (2C), unmanaged (U), mulching once per year (1M), mulching twice per year (2M) and mulching three times per year (3M). Numbers represent mean values in each treatment for the years 2007–2011.

Species	Treatment				
	2C	U	1M	2M	3M
Total cover of grasses	**39.0**	**44.0**	**43.4**	**39.6**	**37.3**
Arrhenatherum elatior	0.3	1.3	7.9	0.5	0.2
Dactylis glomerata	11.0	2.5	9.8	11.0	8.5
Elytrigia repens	0.3	8.8	3.8	1.5	1.2
Festuca pratenis	3.3	0.4	3.1	4.0	3.3
Festuca rubra agg.	4.6	21.0	5.4	1.5	2.0
Holcus lanatus	4.3	0.2	0.4	2.5	1.4
Phleum pratense	2.6	1.1	3.7	3.6	2.7
Poa pratensis	3.7	2.7	3.5	4.5	5.5
Trisetum flavescens	4.6	1.0	3.7	5.9	3.5
Total cover of legumes	**13.3**	**2.0**	**8.1**	**10.1**	**11.6**
Trifolium repens	5.5	0.3	0.0	2.4	5.9
Vicia cracca	1.6	0.2	0.7	0.9	0.7
Vicia sepium	4.6	1.2	6.2	6.4	5.0
Total cover of forbs	**40.8**	**44.6**	**41.5**	**42.6**	**41.3**
Aegopodium podagraria	1.0	13.0	14.0	11.0	11.0
Anthriscus sylvestris	0.1	3.1	1.0	1.5	0.3
Cirsium arvense	0.2	3.8	3.8	0.6	1.3
Galium album	15.0	7.5	15.0	8.5	4.5
Plantago lanceolata	2.9	0.1	0.0	0.2	0.3
Rumex acetosa	1.9	0.3	0.8	2.5	1.9
Taraxacum spp.	9.2	0.5	1.6	9.5	9.2
Urtica dioica	0.0	10.0	0.5	0.1	0.0
Veronica chamaedrys	2.7	2.3	1.2	1.6	2.7

profile from each 10 m × 5 m monitored plot. The soil samples were air-dried, biomass residues and roots were removed and the samples were then ground in a mortar to pass a 2 mm sieve. All chemical analyses were performed in an accredited laboratory of the Crop Research Institute in Chomutov. Plant-available P, K, Ca and Mg were extracted by Mehlich III (Mehlich, 1984) reagent (composition: 0.2 M CH_3COOH + 0.25 M NH_4NO_3 + 0.013 M HNO_3 + 0.015 M NH_4F + 0.001 M EDTA, usage: 25 cm^3 reagent per 2.5 cm^3 soil) and then determined by inductively coupled plasma–optical emission spectrometry (ICP–OES). Total N was analysed by the Kjeldahl method and the organic C (C_{org}) by conventional oxidation procedure with chromo-sulphuric acid and colorimetry (AOAC, 1984). The mean of three sub-samples from each monitored plot was used for statistical analyses.

2.4. Herbage biomass production and herbage chemical properties

Dry matter herbage production for the whole vegetation season was assessed in each of the years 2000–2011. It was calculated as the sum of sampled dry matter herbage biomass (harvested in June and August for the 2C and 2M treatments, in July for the 1M treatment, and July and September for the 3M treatment). Dry matter herbage production of the U treatment was determined as the standing biomass at its vegetation peak in July. Dry matter herbage biomass production in all treatments was measured in three sub-plots each of 50 cm × 25 cm within each 10 m × 5 m experimental plot, cut to a stubble height of 3 cm. The sampling sites in the U treatment were collected from different sub-plots in each year in order to avoid any residual effect of herbage collection from the previous year. The harvested herbage was dried in a forced-draught oven at 85 °C until totally desiccated and dry matter biomass production was then determined. The mean of three sub-samples per experimental plot was used for statistical analyses.

Concentrations of N, P, K, Ca and Mg were determined from the herbage samples that had been collected for dry matter production determination at the end of May 2011, and were used for analysis after digestion in *aqua regia* by ICP–OES in the accredited laboratory of the Crop Research Institute in Chomutov.

2.5. Species richness

Data from botanical relevés that were collected in May 2011 before the first management application were used to calculate the number of all plant species and the number of plant species with cover ≥1% per plot.

2.6. Data analysis

One-way ANOVA was used to evaluate the effect of treatment on chemical properties of soil and biomass and on species richness.

Table 2
Mean soil and herbage characteristics and mean number of plant species per plot under the different treatments in 2011. *F*-ratio = *F*-statistics for the test of a particular analysis, *P*-value = corresponding probability value. Numbers represent average of four replicates, ±standard error of the mean (SE). Significant differences ($P < 0.05$) between treatments according to the Tukey's post hoc test are indicated by different letters in the row. Treatment abbreviations (2C, U, 1M, 2M, 3M) are explained in Table 1.

	Treatment							
	Characteristics	*F*-ratio	*P*-value	2C	U	1M	2M	3M
Soil	pH/KCl	0.93	0.472	5.87 ± 0.37	6.56 ± 0.25	6.40 ± 0.25	6.12 ± 0.27	6.08 ± 0.26
	C_{org} mg kg^{-1}	0.45	0.773	28500 ± 1123	30350 ± 755	30050 ± 1405	30875 ± 1983	30075 ± 1021
	N_{tot} mg kg^{-1}	1.54	0.242	2200 ± 95	2440 ± 84	2508 ± 122	2503 ± 150	2580 ± 126
	P mg kg^{-1}	3.88	0.023	17.26 ± 1.32 b	34.14 ± 4.59 a	26.29 ± 4.26 ab	25.40 ± 2.53 ab	20.08 ± 2.65 ab
	K mg kg^{-1}	4.27	0.017	69.50 ± 4.70 b	157.59 ± 13.68 a	141.72 ± 22.01 ab	125.47 ± 25.14 ab	100.44 ± 9.61 ab
	Ca mg kg^{-1}	0.62	0.652	2053 ± 416	2654 ± 301	2464 ± 301	2193 ± 239	2234 ± 144
	Mg mg kg^{-1}	0.60	0.666	282.4 ± 57.2	388.0 ± 37.5	350.5 ± 44.4	345.3 ± 54.6	371.4 ± 61.1
	C:N	3.10	0.047	13.0 ± 0.2 a	12.5 ± 0.5 ab	12.0 ± 0.1 ab	12.3 ± 0.2 ab	11.7 ± 0.3 b
Herbage	N g kg^{-1}	21.08	<0.001	16.52 ± 0.46 bc	22.85 ± 0.64 a	18.58 ± 0.39 b	15.33 ± 0.89 c	17.05 ± 0.67 bc
	P g kg^{-1}	9.72	<0.001	2.46 ± 0.06 c	3.11 ± 0.12 a	2.86 ± 0.05 ab	2.67 ± 0.09 bc	2.64 ± 0.03 bc
	K g kg^{-1}	23.71	<0.001	12.40 ± 0.61 c	30.66 ± 1.61 a	27.29 ± 0.69 ab	25.57 ± 2.08 ab	23.25 ± 1.54 b
	Ca g kg^{-1}	2.29	0.108	7.95 ± 0.67	7.60 ± 0.68	6.13 ± 0.47	6.09 ± 0.58	6.51 ± 0.39
	Mg g kg^{-1}	6.92	0.002	3.35 ± 0.20 a	2.38 ± 0.25 b	1.93 ± 0.17 b	2.09 ± 0.12 b	2.69 ± 0.28 ab
	N:P	2.57	0.081	6.7 ± 0.1	7.4 ± 0.4	6.5 ± 0.2	5.8 ± 0.5	6.5 ± 0.3
	N:K	2.25	0.112	21.2 ± 1.5	30.9 ± 3.3	30.7 ± 2.0	26.0 ± 3.2	26.7 ± 2.9
	K:P	19.37	<0.001	5.1 ± 0.3 b	9.8 ± 0.3 a	9.6 ± 0.3 a	9.6 ± 0.7 a	8.8 ± 0.5 a
Number of all plant species		23.45	<0.001	40.75 ± 0.48 a	25.00 ± 2.04 c	27.50 ± 1.76 c	34.50 ± 0.87 b	35.50 ± 0.65 b
Number of plant species ≥1%		10.16	<0.001	21.25 ± 0.25 a	14.00 ± 1.08 b	15.25 ± 1.44 b	20.25 ± 1.44 a	21.00 ± 0.71 a

A repeated-measures ANOVA was used to evaluate the effect of year, treatment and the year × treatment interaction on herbage biomass production. The use of ANOVA was permitted as all the required assumptions were met. The relationships between soil and herbage chemical properties, herbage production and the C:N ratio in the soil, herbage production and the N:P, N:K, K:P ratios in the herbage, soil chemical properties and species richness were analysed by linear regression analysis. All analyses were performed using Statistica 9.0 (www.statsoft.cz). To reveal the effects of treatment on soil and herbage chemical properties a redundancy analysis (RDA) followed by a Monte Carlo permutation test in CANOCO (ter Braak and Šmilauer, 2002) was used. Each analysis was performed with 999 permutations. Species data were log-transformed ($y' = \log_{10}(y+1)$). The blocks were treated as covariables. Ordination diagrams constructed in the CanoDraw program (ter Braak and Šmilauer, 2002) were used to visualize the results of the analyses.

3. Results

3.1. Soil chemical properties

Significant effects of the different treatments on plant-available concentrations of P and K and on the C:N ratio were recorded (Table 2). The mean concentrations of P and K were lowest under treatment 2C and highest under treatment U, and ranged from 17.26 mg kg^{-1} to 34.14 mg kg^{-1} and from 69.50 mg kg^{-1} to 157.59 mg kg^{-1}, respectively. The C:N ratio ranged from 11.7 (3M) to 13.0 (2C). There was no significant effect of treatment on pH_{KCl}, N_{tot}, Ca, Mg and C_{org} concentrations. The mean concentrations of N_{tot} ranged from 2200 mg kg^{-1} (2C) to 2580 mg kg^{-1} (3M); Ca concentrations ranged from 2053 mg kg^{-1} (2C) to 2654 mg kg^{-1} (U); Mg concentrations ranged from 282.4 mg kg^{-1} (2C) to 388.0 mg kg^{-1} (U); C_{org} concentrations ranged from 28 500 mg kg^{-1} (2C) to 30 875 mg kg^{-1} (2M); pH_{KCl} ranged from 5.87 (2C) to 6.56 (U).

3.2. Herbage chemical properties

There was a significant effect of treatments on the herbage concentrations of N, P, K and Mg and on the K:P ratio (Table 2) but no effect of treatment on the concentration of Ca or the N:P and the N:K ratios. The mean concentrations of N ranged from 15.33 g kg^{-1} (2M) to 22.85 g kg^{-1} (U). The mean concentrations of P ranged from 2.46 g kg^{-1} (2C) to 3.11 g kg^{-1} (U). The lowest concentration of K was 12.40 g kg^{-1} (2C) and the highest was 30.66 g kg^{-1} (U). The mean Ca concentrations ranged from 6.09 g kg^{-1} (2M) to 7.95 g kg^{-1} (2C); the mean Mg concentrations ranged from 1.93 g kg^{-1} (1M) to 3.35 g kg^{-1} (2C) and the mean N:P ratios ranged from 5.8 (2M) to 7.4 (U); the N:K ratios ranged from 21.2 (2C) to 30.9 (U) and the K:P ratios ranged from 5.1 (2C) to 9.8 (U).

3.3. Soil and herbage chemical properties

The RDA showed significant ($P < 0.001$) differences for the first ordination axis and all ordination axes in soil chemical properties and herbage chemical properties. The percentage of explained variability by the first axis and all ordination axes was 48.3 and 56.4, respectively. Soil chemical properties and herbage chemical properties became associated with three treatment groups, according to the management applied: the unmanaged control (U), the treatment cut twice per year (2C) and the mulched treatments (1M, 2M, 3M) (Fig. 1). Higher concentrations of N_{tot} and C_{org} were found in the soil of the mulched treatments (1M, 2M, 3M). In the herbage of the unmanaged control (U) there were higher concentrations of N and P and higher N:P ratio, whereas in the twice-cut treatment (2C) there were higher concentrations of Ca and Mg.

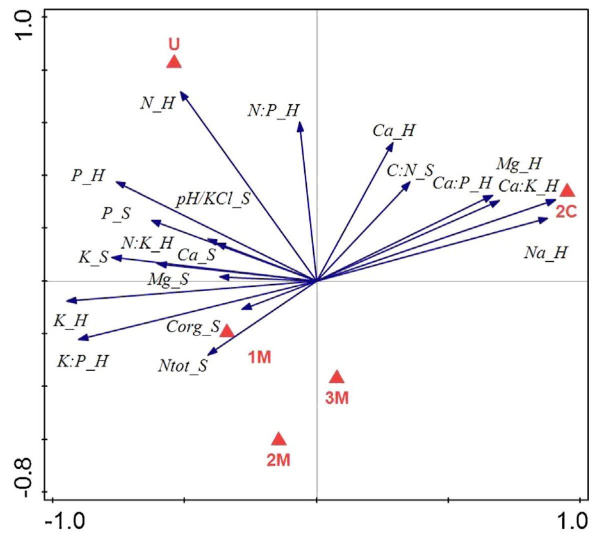

Fig. 1. Ordination diagrams showing the results from redundancy analysis of soil and herbage data. Treatment abbreviations (2C, U, 1M, 2M, 3M) are explained in Table 1.
Abbreviations: pH_{KCl}_S-soil acidity; C_{org}_S-organic carbon; N_{tot}_S-total nitrogen in the soil; P_S, K_S, Mg_S, Ca_S-plant available nutrients; C:N_S-ratio in the soil; N_H, P_H, K_H, Ca_H, Mg_H-nutrient concentration in the herbage; N:P_H, N:K_H, K:P_H-ratios in the herbage.

Positive relationships between P concentration in the herbage and in the soil ($P < 0.001$, $r = 0.66$) and also in the K concentration in the herbage and in the soil ($P < 0.001$, $r = 0.77$) were recorded (Fig. 2a and b). There was no significant relationship between concentrations of N, Ca, and Mg in the herbage and in the soil.

3.4. Species richness

The number of all plant species and the number of plant species with cover values ≥1% were significantly influenced by the treatment (Table 2). The mean total number of plant species recorded ranged from 25.00 (U) to 40.75 (2C) and the mean number of plant species with cover values ≥1% ranged from 14.00 (U) to 21.25 (2C).

A negative relationship was found between P concentration in the soil and species richness ($P = 0.010$, $r = -0.56$) and K concentration in the soil and species richness ($P = 0.001$, $r = -0.67$). No significant relationship between N_{tot}, Ca and Mg concentration in the soil and species richness was found.

3.5. Herbage biomass production

Calculated by repeated-measures ANOVA, a significant effect of year ($P < 0.001$), treatment ($P < 0.001$) and the year × treatment interaction ($P < 0.001$) on herbage biomass production was recorded (Fig. 3). The 2000–2011 mean annual values of herbage biomass production (with inter-annual range in brackets) were as follows: 3M–5.7 t ha^{-1} (4.3–7.2 t ha^{-1}), 2M–5.2 t ha^{-1} (3.5–7.9 t ha^{-1}), 2C–4.5 t ha^{-1} (3.2–6.4 t ha^{-1}), 1M–4.1 t ha^{-1} (2.6–6.1 t ha^{-1}), U–3.6 t ha^{-1} (2.2–6.5 t ha^{-1}). Based on mean annual herbage production and herbage chemical properties in the year 2011 the amounts of nutrients removed per year for the 2C treatment were calculated as 71, 11, 55, 34 and 15 kg ha^{-1} for N, P, K, Ca and Mg, respectively. A negative relationships between the C:N ratio in the soil and herbage biomass production (Fig. 4) was recorded.

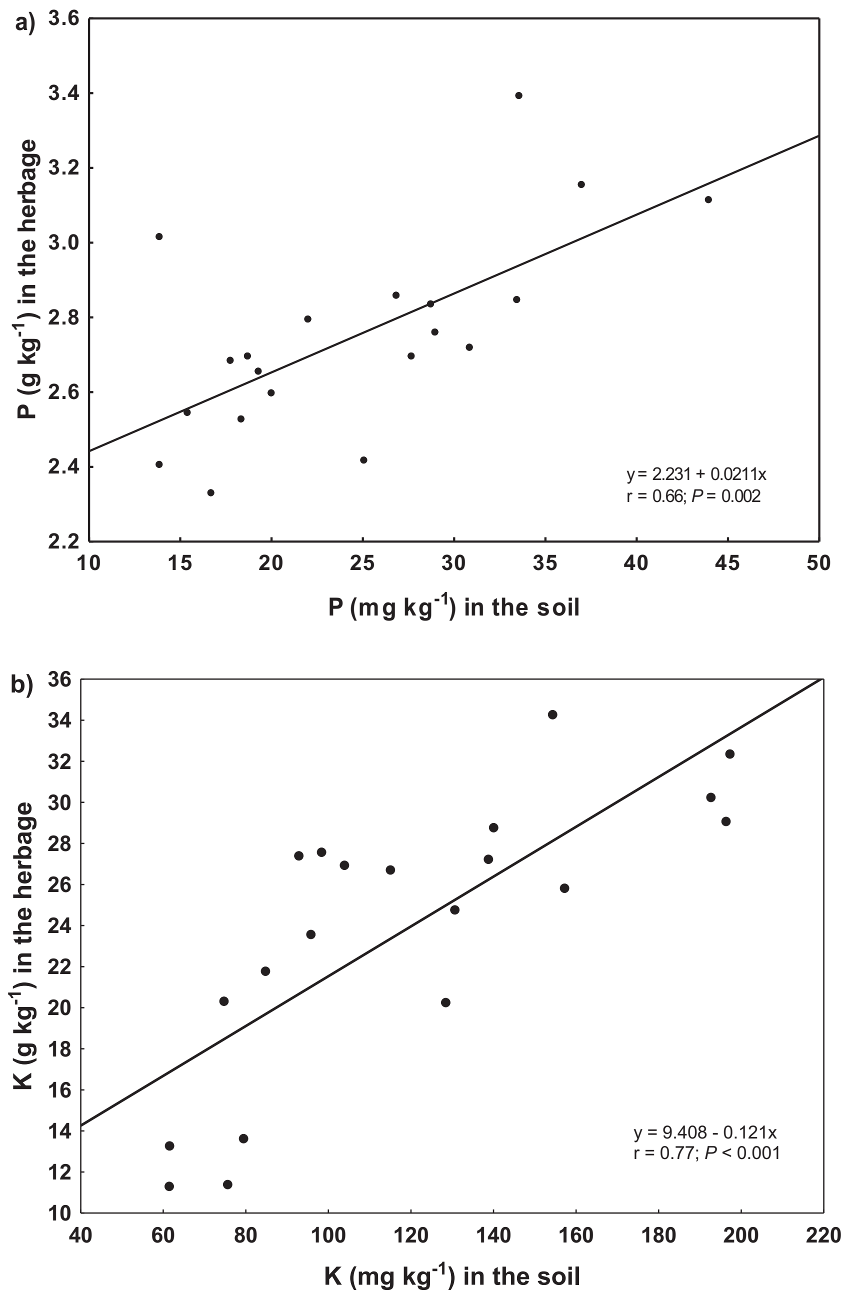

Fig. 2. Relationships between (a) phosphorus and (b) potassium concentration in the herbage and in the soil.

4. Discussion

4.1. Soil chemical properties

An important finding from this study was that P and K were the only nutrients whose plant-available concentrations were affected by the management regime. In our experiment the responses of both plant-available P as well as K concentrations to different management regimes (cutting, mulching, unmanaged) were very similar. It was expected that there would be a significant decrease of plant-available K concentration under cutting treatments with biomass removal, as this response has been reported in several experiments previously (Parr and Way, 1988; Koerselman et al., 1990; Schaffers et al., 1998; Alfaro et al., 2003, 2004; Hejcman et al., 2010a; Pavlů et al., 2013). A recorded decrease in plant-available P concentration under long-term cutting management with biomass removal has, however, been reported in relatively few previous studies (Hejcman et al., 2010c; Pavlů et al., 2011b). The authors of most previous studies found no effect of different management regimes on plant-available P concentrations (Parr and Way, 1988; Schaffers et al., 1998; Köhler et al., 2001; Øien and Moen, 2001; Perring et al., 2009; Pavlů et al., 2013). The significant effect of long-term biomass removal on the decrease in plant-available P concentration is probably connected with the relatively high initial plant available P concentration. In addition, the different management regimes may need to be applied for several decades before there are differences in the soil P status, and many of the experiments that showed no effect of management regime on plant-available P concentration were maintained for only a few years. Although there was no export of P and K by herbage removal under mulching, the soil concentrations of these elements in the mulching treatment were intermediate between those of the

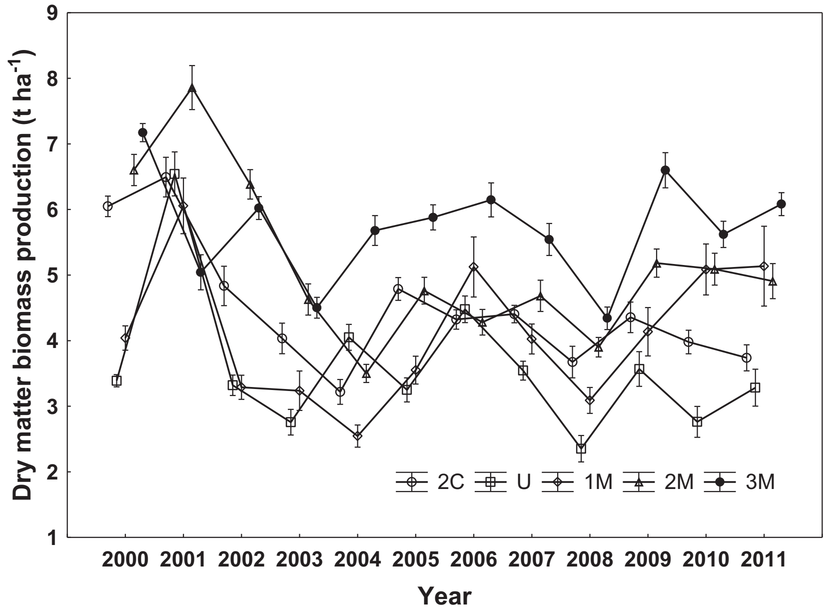

Fig. 3. Dry matter biomass production in investigated treatments in the years 2000–2011. Error bars represent standard error of the mean (SE). Treatment abbreviations (2C, U, 1M, 2M, 3M) are explained in Table 1.

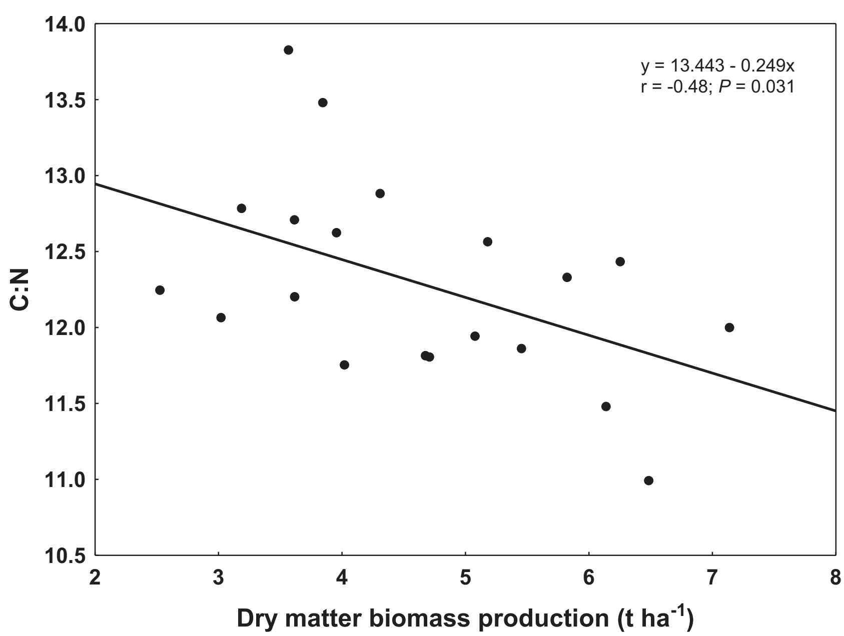

Fig. 4. Relationship between C:N ratio in the soil and dry matter biomass production in all investigated treatments in the year 2011.

unmanaged treatment and the cutting treatment, with a tendency of U > 1M > 2M > 3M > 2C. In contrast to the unmanaged treatment, nutrients from the crushed herbage under the mulching treatments can be easily leached into underground water (Fiala, 2007) and this process can be further exacerbated by the increasing frequency of mulching.

Eleven years of imposition of different management regimes was probably too short a period for any significant differences between treatments in N_{tot} concentration and plant-available concentrations of Ca and Mg. In general, the quantities of nutrients removed annually by harvested herbage is relatively low (Parr and Way, 1988; Pavlů et al., 2011b) and therefore reduction in plant-available P, Ca and Mg concentrations is likely to be a long-term process (Schaffers et al., 1998; Hansson and Fogelfors, 2000; Köhler et al., 2001; Øien and Moen, 2001; Ilmarinen and Mikola, 2009; Perring et al., 2009; Hejcman et al., 2010a; Pavlů et al., 2013). In addition to leaching of P and K, greater amounts of other nutrients including N_{tot}, Ca and Mg could be easily leached from crushed biomass after mulching (Fiala, 2007) than are likely to occur from intact biomass in the unmanaged treatment. Further, the different physical and chemical conditions under the mulch layer, especially soil temperature (Kvítek et al., 2000) as well as light and moisture conditions, could also affect nutrient availability. Therefore, further study of abiotic factors (temperature and moisture) should be the next step for revealing additional effects of mulching on availability of nutrients in the soil.

4.2. Herbage chemical properties

The forage quality of the collected herbage in May was generally quite good, in terms of its value as livestock fodder, in all treatments and it nearly reached the required quality for feeding to dairy cows (Whitehead, 2000). One exception was the high K concentration in herbage from the unmanaged treatment (about 31 g K kg^{-1} dry matter). High K concentrations are common in forage used for livestock nutrition from almost all types of grasslands and animals are able to excrete high proportions of ingested K in their urine. Therefore herbage biomass from the all treatments of the experiment would have been suitable for use as cattle forage in the spring.

Similarly to the results of the soil analyses, P and K concentrations in the herbage were affected by the management regime. This can explain the strong positive correlations between plant-available P and K concentrations and herbage P and K concentrations. However, in studies published by Schaffers (2002) and by Pavlů et al. (2013), K was the only element that showed a strong relationship between plant-available and herbage concentrations. It is likely that a positive relationship between plant-available P and herbage P concentrations may be also apparent in situations where P availability is not limiting for plants, as was recorded in our experiment, because herbage P concentration was above the reported threshold value of 2.1 g kg^{-1} (Liebisch et al., 2013).

Moreover, concentrations of N, P and K in the herbage were not caused solely by low nutrient concentrations in the soil but also by differences in botanical composition. After 11 years of applying different management treatments in this experiment, the cover of forbs was significantly higher and the cover of graminoids was significantly lower in the swards of the unmanaged treatment than in the swards of the cutting and the mulching treatments, and the cover of forbs and graminoids was similar under all managed treatments (Jan Gaisler, unpublished results).

The changes in relative proportions of forbs and grasses also affected the herbage mineral concentrations as forbs generally have higher mineral concentrations than grasses (Pirhofer-Walzl et al., 2011; Liebisch et al., 2013). For example, the highest N concentration in the herbage was revealed in the unmanaged treatments with the highest proportion of forbs, especially the nitrophilous species *Urtica dioica*. Generally, concentrations of N, P and Ca in *U. dioica* are higher than in the herbage from grassland communities (Müllerová et al., 2014); moreover, the herbage production of *U. dioica* is substantially higher than many other plant species so it could have contributed considerably to the higher nutrient concentrations that were recorded in the above-ground biomass of the unmanaged treatment. Nitrogen concentrations in herbage of the cutting and mulching treatments was positively affected by higher proportions of N-rich legumes (especially *T. repens* and *V. sepium*). These legumes are able to transfer biologically fixed N_2 to grasses in amounts that can exceed 100 kg N ha^{-1} (Elgersma and Hassink, 1997; Lüscher et al., 2014). However, the proportion of legumes in the herbage of the all managed treatments was not high enough to reach the same N concentration as that found in herbage of the U treatment. On the other hand, in the twice-cut treatment there was higher concentration of Mg in the herbage, and this was probably due to higher proportions of legumes, which are known for their high Mg concentration (Whitehead, 2000).

Changes in plant species composition connected with changes in herbage nutrient concentration have also been recorded previously. For example, Pavlů et al. (2013) found higher P concentrations in the herbage of abandoned grassland than in herbage of a mown meadow. This was linked to a higher proportion of forbs in the abandoned grassland, although there were no differences in plant-available P concentrations.

Finally, the phenological stage of vegetation was another factor that may have affected herbage nutrient concentration. Thick and high layer of the undecomposed dead biomass in the unmanaged treatment prevented intergrowth of plants. Therefore the vegetation was younger under the unmanaged treatment (Gaisler, personal communication) than under the cutting and the mulched treatments especially at the beginning of vegetation season in May when the samples were taken. This younger vegetation is usually connected with higher herbage nutrient concentration (Duru and Ducrocq, 1997).

4.3. Species richness

Mulching has been proposed as a possible alternative to regular cutting, especially when regular mowing is not feasible for economic or technical reasons or in situations where the absence of defoliation is likely to lead to a significant reduction in species richness Mašková et al. (2009) and Gaisler et al. (2013). On the other hand, experiments showing negative effects of mulching on communities have been reported, leading to dominance of competitive species (Drobnik et al., 2011) and only frequent mulching could be accepted as an alternative management tool for maintenance of grasslands (Römermann et al., 2009). Based on the results of our experiment more frequent mulching could be a possible alternative management to traditional cutting, even though species richness was lower under mulching than under a two-cut management.

The negative relationship between concentrations of P and K in the soil and species richness was revealed in our experiment, and similar responses of species richness to soil P and K concentration have been recorded in other studies on temperate grasslands (Janssens et al., 1998; Hejcman et al., 2010b). However, it is very difficult to identify if the key driver for changes in species richness is the type of management or if it is changes in plant available nutrients caused by the type of management applied.

4.4. Herbage biomass production

The studied grassland was classified as being of high productivity, as the mean herbage biomass production in all treatments during the experiment was above 3 t ha^{-1} per year, the lower limit for high-production grasslands in Central Europe (Hejcman et al., 2010d). Part of the difference in the herbage production between the treatments can be attributed to the different cutting frequencies and times at which herbage samples were taken. This raises the methodological question of how to estimate herbage biomass production when there are different management treatments being compared. Despite these methodological difficulties with herbage biomass production estimation under the different treatments, the highest herbage biomass production occurred in the mulched treatments. It therefore seems likely that recycling of nutrients was responsible for the higher biomass productivity (Doležal et al., 2011). This idea is supported by the C:N ratio in the soil, which was highest (13.0) in the twice-cut management and the lowest (11.7) in the three-times mulched treatment, whereas the mean herbage production was highest (5.7 t ha^{-1}) in the three-times mulched treatment and the lowest (3.6 t ha^{-1}) in the unmanaged treatment. This is consistent with the results of Hejcman et al. (2010b) that lower values of the C:N ratio are linked with higher biomass production in temperate grassland and vice versa. Higher herbage biomass production from the twice-mulched treatment than from the twice-cut treatment was probably supported by higher availability of nutrients in the soil, as both treatments were subjected to the same defoliation frequency and had very similar plant species composition, but they differed in the amounts of nutrient removal. In the unmanaged treatment, litter accumulated on the sward surface, which prevented plants sprouting in the spring, and thus vegetation density and biomass production were reduced.

5. Conclusion

The mulching regimes did not result in substantial changes in nutrient concentrations in either soil or herbage, in comparison with either the unmanaged and cut treatments after eleven years of the experiment. However, under all mulching treatments there was a tendency for increased P and K concentrations in the soil and for concentrations of N, P and K in the herbage to be higher than in the twice-cut treatment but to be lower than in herbage of the unmanaged treatment. The changes in plant-available concentrations of nutrients under the different management regimes occurred relatively slowly and they were detectable in the herbage earlier than in the soil. We can conclude, that in the case of Central European temperate grasslands that are currently not being used for agriculture production, and where the main objective is to maintain ecosystem functions, mulching performed two or three times per year can substitute for a conventional agricultural cutting regime without substantial changes in soil nutrient properties over eleven years, but that possible decreases in plant species diversity need to be taken into account when this procedure is adopted.

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