Effects of cattle slurry application on plant species composition of moderately moist *Arrhenatherion* grassland

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ABSTRACT

Cattle slurry is frequently used fertilizer on grasslands, but little is known about its effect on plant species composition. The aim of this study was therefore to assess effect of different application rates of cattle slurry (S0 – 0, S1 – 60, S2 – 120, S3 – 180, S4 – 240 kg N/ha/year) on the plant species composition of three-cut grassland. The study was performed over 6 years on moderately moist upland *Arrhenatherion* grassland in the Czech Republic dominated by *Alopecurus pratensis*, *Trisetum flavescens*, and *Poa* spp. Species composition recorded in treatments with application of cattle slurry in rate up to 120 kg N/ha/year was similar to the unfertilized control. During first three years, species richness was similar in all treatments and then decreased the most in S4 followed by S3 treatment. Cover of short forbs increased in S0 and decreased with an increase in slurry application rate which supported tall grasses. Application of cattle slurry up to 120 kg N/ha/year can be considered as suitable compromise between maintenance of species rich grasslands and requirements of farmers for sufficient forage production.

Keywords: species richness; water and nutrient availability; functional groups

The plant composition and species richness of permanent grasslands are determined by management practices and site characteristics such as topography, water and nutrient availability, and light conditions (Sebastiá 2004, Tzialla et al. 2006, Wellstein et al. 2007, Araya et al. 2013). Nitrogen (N) is the element most strongly affecting species richness in a grassland sward (Janssens et al. 1998), with its impact dependent on soil phosphorus (P) availability. High soil P, together with N application, allows highly productive species to dominate grasslands (Critchley et al. 2002, Hejcman et al. 2007, Wellstein et al. 2007) and is linked with low species richness (Hejcman et al. 2010). Conversely, high potassium (K) availability is associated with high plant species richness. In general, reducing availability of soil nutrients is expected to increase plant species richness according to 'hump-back' model produced by Grime (2001) which shows species richness peaks at a moderate level of nutrient availability. The reasonable application of fertilizers can even increase plant species diversity in species-poor grassland with low productivity as was reported by Chytrý et al. (2009) in the case of *Arrhenatheretum* meadows on oligotrophic substrates. In the Czech Republic, *Arrhenatherion* meadows are a typical cut grasslands with their conservation made difficult by the scarcity of research on effective management practices (Čámská and Skálová 2012).

In our study, we investigated effect of cattle slurry application on plant species composition of upland *Arrhenatherion* grassland with low availability of P. Cattle slurry is commonly used fertilizer in many countries, but its effect on plant species composition has been little studied (e.g. Liu et al. 2010). The aim of this study was therefore to evaluate the effect of cattle slurry application ranging from 0 to 240 kg N/ha/year on plant species composition of *Arrhenatherion* grassland cut three times per year.

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Table 1. Functional groups of plant species and their abbreviations included in Figure 2

Functional groups	
Tall graminoids	***Alo pr***: *Alopecurus pratensis*, ***Arrhen e***: *Arrhenatherum elatius*, ***Dac glo***: *Dactylis glomerata*, ***Fes pra***: *Festuca pratensis*, ***Hol lan***: *Holcus lanatus*, ***Poa pr***: *Poa pratensis*, ***Poa tr***: *Poa trivialis*, ***Tris fla***: *Trisetum flavescens*
Short graminoids	***Agr cap***: *Agrostis capillaris*, ***Antho od***: *Anthoxanthum odoratum*, ***Luz camp***: *Luzula campestris*
Tall forbs	***Aeg pod***: *Aegopodium podagraria*, ***Anth syl***: *Anthriscus sylvestris*, ***Crep bi***: *Crepis biennis*, ***Ran acre***: *Ranunculus acris*, ***Rum ace***: *Rumex acetosa*, ***Rum obt***: *Rumex obtusifolius*
Short forbs	***Alch vul***: *Alchemilla vulgaris*, ***Cer hol***: *Cerastium holosteioides*, ***Gle hed***: *Glechoma hederacea*, ***Hyp mac***: *Hypericum maculatum*, ***Leont hi***: *Leontodon hispidus*, ***Plan lan***: *Plantago lanceolata*, ***Ran rep***: *Ranunculus repens*, ***Stel gra***: *Stellaria graminea*, ***Tar of***: *Taraxacum officinale*, ***Tri dub***: *Trifolium dubium*, ***Tri pra***: *Trifolium pratense*, ***Tri rep***: *Trifolium repens*, ***Ver arv***: *Veronica arvensis*, ***Ver cham***: *Veronica chamaedrys*

MATERIAL AND METHODS

The study was conducted in 2007–2012 in the moderately moist permanent upland grassland of the alliance *Arrhenatherion* in the central Czech Republic (49°29'31.960''N, 15°16'11.724''E). The soil type was colluvic regosol humic with sandy loams. Available nutrients in the upper 20 cm of soil were determined by Mehlich III extraction at the beginning of the study and categorized according to the levels of Sáňka and Materna (2004) as P = low (14 mg/kg), K = satisfactory (83 mg/kg), Mg = good (156 mg/kg), and Ca = low (1340 mg/kg); pH_{KCl} was 4.2. Altitude was 508 m a.s.l., mean annual precipitation was 660 mm and mean air temperature was 7.0°C. In 2007, five experimental treatments cut three times per year and with cattle slurry applied at various N levels (S0 – 0, S1 – 60, S2 – 120, S3 – 180, S4 – 240 kg N/ha/year) were established in 3 × 5 m plots in a completely randomized block design with three replicates. Slurry was applied three times a year, in early April (S1–S3: 60 kg N, S4: 120 kg N), after second and third cut in late July (S2–S4: 60 kg N) and in late September (S3–S4: 60 kg N). Prior to the start of the study, the site had not been fertilized and had been cut twice per year. The cover of vascular plant species (nomenclature follows Kubát et al. 2002) was estimated visually each year in early June prior to the first experimental manipulation in permanent 1 × 1 m plots established within each 3 × 5 m plot. Hence, the first cover estimation in 2007 was affected by the first slurry application. Vascular plant species richness (number of species per

Table 2. Results of repeated measures ANOVA of species richness, functional groups and dominant plant species, 2007–2012

	Treatment (*df* = 4)		Time (*df* = 5)		Treatment × time (*df* = 30)	
	F-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Species richness	14.06	< 0.001	16.65*	0.002*	1.92*	0.073*
Tall graminoids	31.46	< 0.001	15.06*	0.002*	1.40*	0.226*
Short graminoids	1.24	0.356	2.33*	0.166*	0.87*	0.625*
Tall forbs	2.27	0.134	6.87*	0.018*	0.80*	0.694*
Short forbs	30.49	< 0.001	14.69*	0.003*	3.44*	0.004*
N Ellenberg indicator values	16.61	< 0.001	3.59	0.008	0.68	0.827
Alopecurus pratensis	7.47	0.005	3.46	0.009	0.70	0.806
Poa ssp.	6.61	0.007	19.80*	0.001*	2.28*	0.035*
Trifolium repens	7.57	0.005	14.17*	0.003*	2.13*	0.047*
Trisetum flavescens	3.48	0.050	28.40*	< 0.001*	1.98*	0.065*

df – degrees of freedom; *F*-ratio – derived from *F* statistics; *P*-value – probability value; *results of multiple Wilks test

1 m[2]), cover of functional groups (tall graminoids above mean height ≥ 0.5 m, short graminoids < 0.5m, tall/short forbs, Table 1) and cover of dominant species were calculated. The Ellenberg N indicator value for each relevé was calculated as the mean of indicator values (Ellenberg et al. 1992) weighted with cover of each species.

Redundancy analysis (RDA) followed by the Monte Carlo permutation test with 999 permutations in Canoco 4.5 program (ter Braak and Šmilauer 2002) was used to evaluate the effect of treatments on species composition in particular years. Biplot ordination diagram was used for presentation of the results from 2012. To evaluate effects of slurry application treatments, time and their interaction on species richness and cover of functional groups and dominant species, repeated measures ANOVA was used.

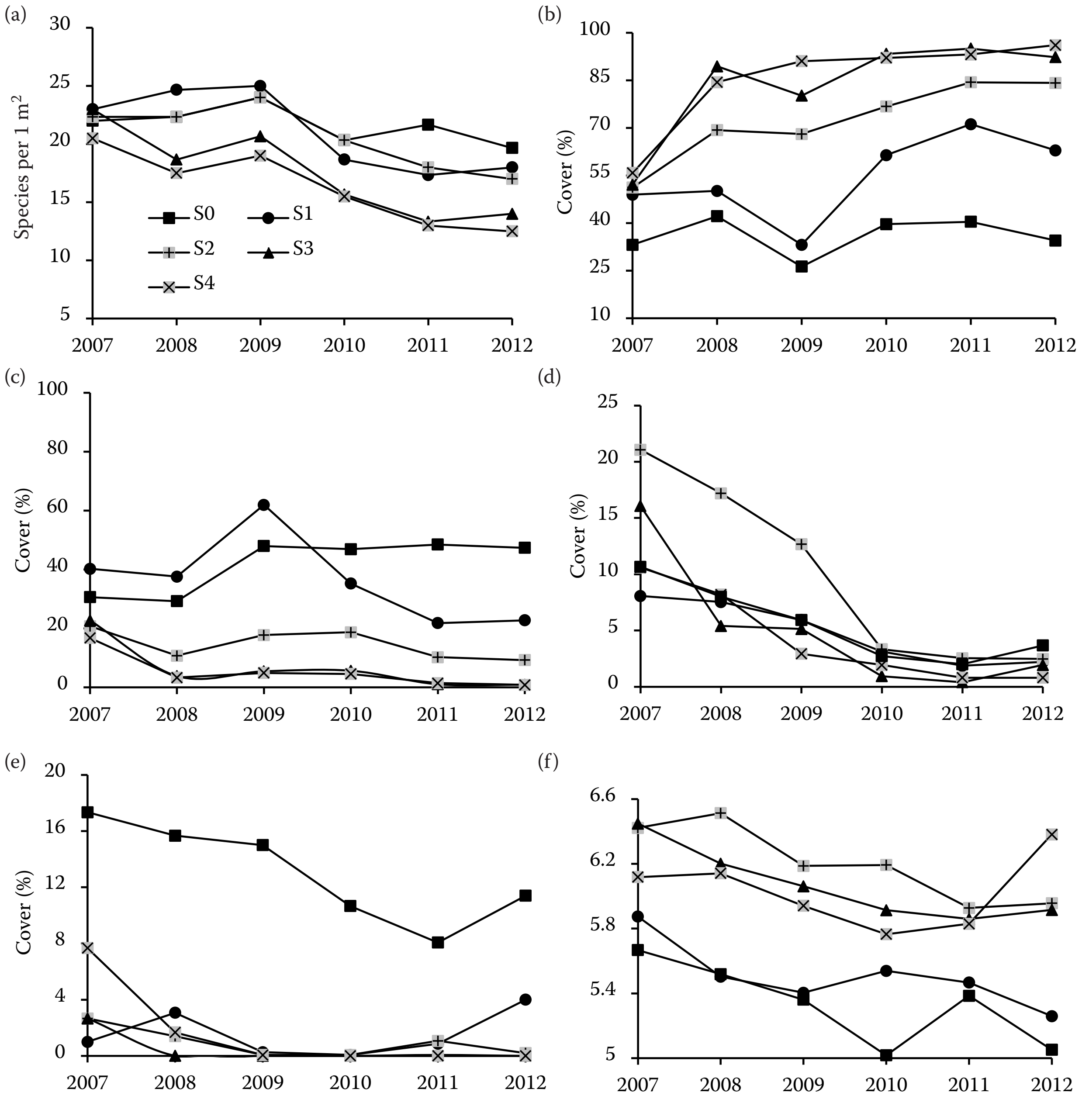

Figure 1. Changes in species richness of vascular plants (a) and mean cover of tall graminoids (b); short forbs (c); tall forbs (d); short graminoids (e), and Ellenberg indicator values for N (f). Treatment abbreviations (S0, S1, S2, S3, and S4) are explained in Table 3

RESULTS

The total number of species in the study site decreased by 3 between 2007 and 2012 to 36. Five forbs rooting in the relevés in 2007 (*Achillea millefolium*, *Campanula patula*, *Cerastium arvense*, *Leucanthemum vulgare*, *Pimpinella saxifraga*) disappeared in 2012, and vice versa, *Veronica serpillifolia* was recorded in S1 and S2 and *Luzula campestris* was recorded in S0, S1 and S2 in 2012.

Species richness was affected by treatment and time significantly (Table 2) and the development of species richness in individual treatments is given in Figure 1a. Species richness decrease was associated with growing competitive advantage of tall grasses (Figure 1b) spreading along with N levels and indicating the significant effect of treatment and time on cover of tall graminoids (Table 2). The lowest species richness was hence observed in S4 with the highest proportion of tall grasses successively suppressing short forbs. Conversely, the highest species richness was found in S0 because of increase in species number and cover of short forbs (Figure 1c). Number of tall forbs decreased along with their cover in all treatments over duration of the study (Figure 1d) explaining significant effect of time (Table 2). The highest species richness was recorded during first three years of the study due to increase of number of short forbs in all treatments. The effect of treatment and time on cover of short graminoids was not significant (Table 2, Figure 1e), but number

Table 3. Results of the effect of treatments on species composition for each year; RDA analysis

Year	Treatment	% expl. var.	*F*-ratio	*P*-value
2007	S0, S1, S2, S3, S4	32.1	1.183	0.239
2008	S0, S1, S2, S3, S4	32.2	1.189	0.236
2009	S0, S1, S2, S3, S4	41.5	1.774	0.013
2010	S0, S1, S2, S3, S4	43.4	1.914	0.034
2011	S0, S1, S2, S3, S4	51.4	2.646	0.004
2012	S0, S1, S2, S3, S4	44.9	2.035	0.011

Applied treatments were three cuts a year with 0, 60, 120, 180, and 240 kg N/ha/year (S0–S4) applied in cattle slurry; % expl. var. – species variability explained by four ordination axes; *F*-ratio – derived from *F* test; *P*-value – related probability value obtained by the Monte Carlo permutation test. Tested hypothesis: Is there any effect of treatment on plant species composition in particular year?

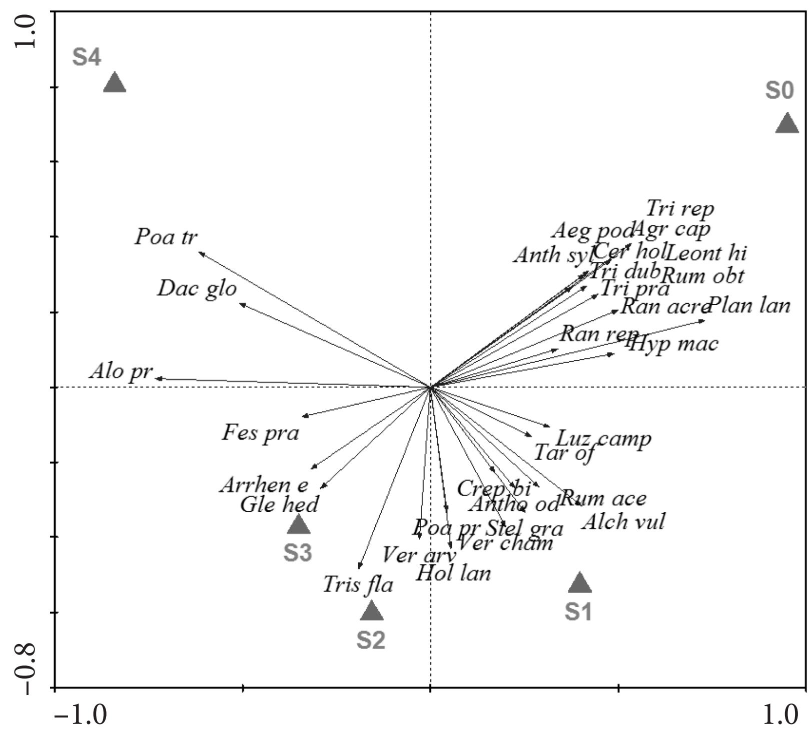

Figure 2. Ordination diagram showing results of the RDA analysis of plant species composition data collected in different treatments in 2012. For species and treatments abbreviations see Tables 1 and 3

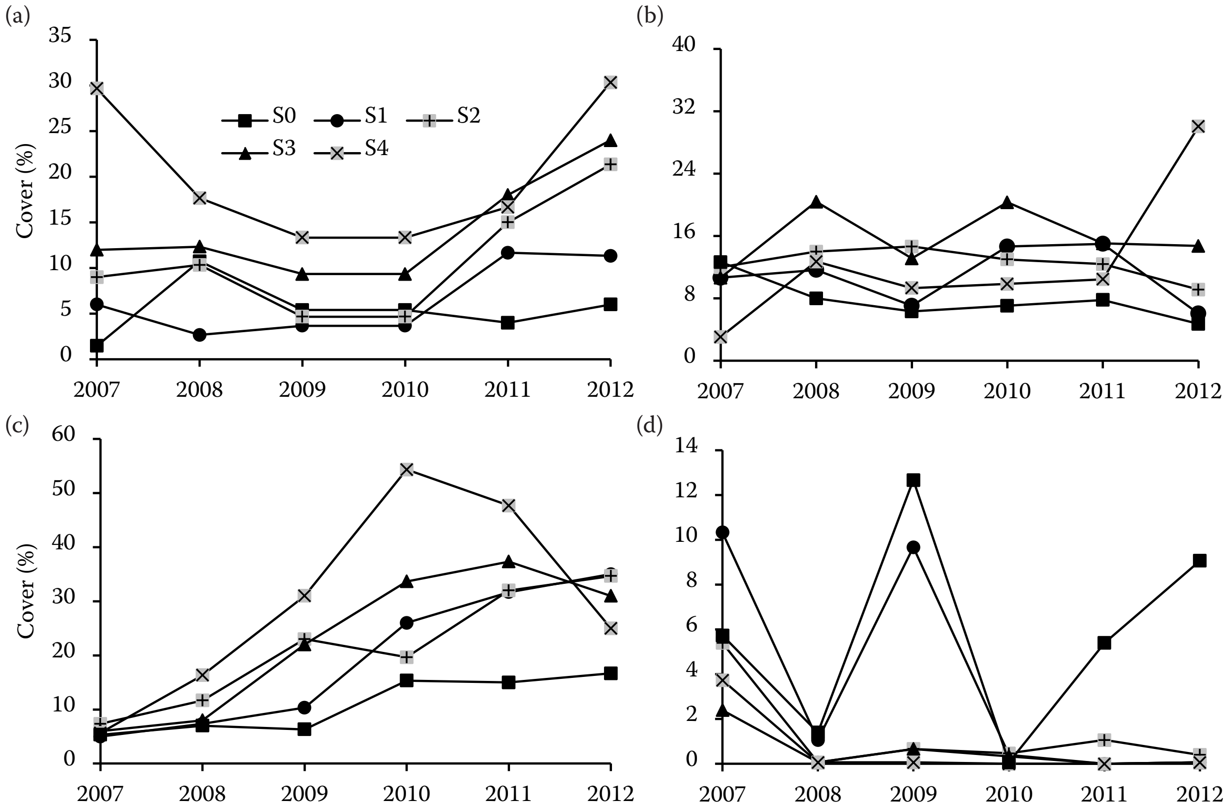

Figure 3. Changes in mean cover (%) of *Alopecurus pratensis* (a); *Poa* ssp. (b); *Trisetum flavescens* (c), and *Trifolium repens* (d). Treatment abbreviations (S0, S1, S2, S3, and S4) are explained in Table 3

of short graminoids increased slightly in S0–S2. Ellenberg indicator values for N was affected by treatment and time significantly with declining trend in all treatments except for S4 (Figure 1f, Table 2).

The effect of treatment on plant species composition was significant since 2009 explaining more than 40% variability of vegetation data (Table 3). If only S0–S2 were tested, the effect of treatment was not significant over all time. Tussock grasses prevailed in all treatments over time particularly in S2–S4 with exceptions of S4 in 2007 and especially in 2012, when rhizomatous grasses spread the most (Figures 2, 3a,b). Owing to spread of rhizomatous grasses, decreasing cover of *Trisetum flavescens* in S4 and S3 was found at the end of the study (Figure 3c). Both lower N levels supported *T. flavescens* until the end of the study. The most supported species by S0 were *Plantago lanceolata*, *Leontodon hispidus*, *Agrostis capillaris* and *Trifolium repens* (Figures 2, 3d). Tall forbs *L. vulgare*, *Anthriscus sylvestris* and *P. saxifraga* were not recorded since 2010, conversely, *Ranunculus acris* and *Rumex acetosa* were observed in all years.

DISCUSSION

All N levels at cattle slurry along with three cuts decreased species richness since 2010. The highest species richness in 2007 was due to previous two cut management enabling species to produce seeds within longer growth period (Buchgraber and Gindl 2004). Likewise, Zechmeister et al. (2003) reported species richness reduction due to more than two cuts and Kramberger and Kaligaric (2008) due to four cuts per year. Relatively constant species richness in all treatments until 2009 was due to keeping equilibrium between tall forbs decrease and short forbs increase. Subsequently, number of tall forbs declined along with decrease in short forbs in S1–S4. Decline of tall forbs was given by their low tolerance to frequent defoliation (Gaisler et al. 2013) because, as strong competitors, they do not tolerate frequent disturbance (Pavlů et al. 2011a). Decline of short forbs was given by an increase of tall grasses because of N addition (Honsová et al. 2007, Kramberger and Kaligaric 2008, Čop et al. 2009, Hejcman et al. 2012). Short forbs in S0 and short graminoids in S0, S1, and S2 treatments were supported by three-cuts per year as a consequence of the disturbance and higher light availability (Zechmeister et al. 2003). In respect of total species richness and number of short forbs, similar trend in S0–S2 was obvious, hence, this type of grassland with low availability of P responded to nutrient inputs without detrimental effect on species richness up to application of 120 kg N/ha/year (Čop et al. 2009, Chytrý et al. 2009). Contrary, Zechmeister et al. (2003) reported meadows with > 90 kg N/ha/year differed in species richness significantly from those with lower levels of fertilizer application.

Significant changes in plant species composition among treatments including cattle and pig slurries application found also Liu et al. (2010). As expected, the cover of tall grasses positively responded to N application, similarly to Pavlů et al. (2011b, 2012), Čámská and Skálová (2012) and Hejcman et al. (2012). *P. trivialis* and *A. pratensis* due to nutrient acquisition by rhizomes (Janeček et al. 2004) favoured after the decrease of tussock grasses such as *Arrhenatherum elatius* or *Holcus lanatus* at the end of our study. *T. flavescens*, as a tussock grass characteristic of cut meadows (Hejcman et al. 2010), was promoted very remarkably by higher N levels from the second to fourth year. *Dactylis glomerata* suffered from three-cut regime, not promoted by N application. *A. capillaris* was the only grass which spread in S0 which is in agreement with Pavlů et al. (2012) who reported it as a low competitive grass negatively responding to fertilizer application. Spread of *P. lanceolata* in unfertilized grassland is in agreement with findings by Hejcman et al. (2010) and by Gaisler et al. (2013). *T. repens* was not nearly found in S2–S4 contrary to Hejcman et al. (2010), but in agreement with Tzialla et al. (2006) favoured by no or low N level (S0, S1). White clover along with *L. hispidus* and *P. lanceolata* were promoted by three-cut regime due to their creeping growth and leaf rosette nearly escaping to defoliation (Gaisler et al. 2013). *R. acris* and *R. acetosa* were maintained in sward due to their support by 60–120 kg N/ha/year (Čámská and Skálová 2012). *Hypericum maculatum* showed prevalence in S0, in contrary to Pavlů et al. (2012) who found it to dominate in unmanaged grasslands.

The main message of this study is that *Arrhenatherion* grassland with low soil P availability fertilized by cattle slurry up to 120 kg N/ha/year showed similar plant composition as unfertilized control. Application of cattle slurry up to 120 kg N/ha/year can be considered as a suitable compromise between maintenance of species rich grasslands and requirements of farmers for sufficient forage production.

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