

## Plant composition, herbage yield, and nitrogen objectives in *Arrhenatherion* grasslands affected by cattle slurry application

RENATA DUFFKOVÁ<sup>1,\*</sup>, JAKUB BROM<sup>2</sup>

<sup>1</sup>Research Institute for Soil and Water Conservation, Prague, Czech Republic

<sup>2</sup>Faculty of Agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

\*Corresponding author: [duffkova.renata@vumop.cz](mailto:duffkova.renata@vumop.cz)

### ABSTRACT

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Cattle slurry is commonly used to fertilize grasslands, so its impact on plant composition and herbage properties is important. Cattle slurry at annual rates of 60 (S1), 120 (S2), 180 (S3), and 240 kg nitrogen (N)/ha (S4) was applied to *Arrhenatherion* grasslands in moderately wet (WS), slopy (SS), and moderately dry (DS) sites cut three times a year over six years, to assess its effects on plant functional types, the Ellenberg N indicator value (Ellenberg N), herbage dry matter (DM) yield, herbage N content and offtake, N nutrition index (NNI), and N use efficiency (NUE). The site-specific changes in an increase in graminoid cover, Ellenberg N, herbage DM yield and N offtake, and NNI along with slurry application rates revealed, while cover of legumes, short forbs, and NUE decreased. In more productive sites (WS and SS), slurry application in the amount of 180 kg N/ha could be suggested as a slurry dose ensuring beneficial agronomic objectives. However, nature conservation requirements via maintaining plant biodiversity were not met. On the contrary, short-term slurry application up to 120 kg N/ha ensured on permeable DS not only sufficient agronomic objectives, but also plant biodiversity conservation requirements.

**Keywords:** herbage production; grasses; plant diversity; agronomic demands; fertilizer

Cattle slurry as a no-cost source of nutrients (especially nitrogen (N)) is commonly applied to grasslands in Europe to increase herbage production and its agronomic value (Douglas and Crawford 1998, Huijsmans et al. 2016). Plant competitive ability, growth, and soil nutrient uptake relative to slurry application are associated with changes not only in herbage yield and herbage N objectives, but also in sward plant diversity (Lemaire 1997).

The most widespread grasslands in Central Europe, traditionally cut two or three times annually with occasional manuring, are those composed of *Arrhenatherion* (Čámská and Skálová 2012). Management changes of these grasslands

including regular intensive N fertilization can result in increased proportion of tall grasses, but decreased species diversity (Vidrih et al. 2009). An increase in herbage dry matter (DM) biomass above 6 t/ha coinciding with N (or N plus phosphorus (P)) application was regarded as the upper limit for species-rich *Arrhenatherion* grasslands in the Netherlands (Oomes 1992). Contrarily, *Arrhenatherion* meadows on oligotrophic substrates need regular nutrient inputs, and are maintained by long-term fertilizer application in the amount of 100 kg N/ha/year (Chytrý et al. 2009).

Despite the abundance of *Arrhenatherion* grasslands, there is little available information on the

effects of repeat organic fertilizer application such as cattle slurry at differing annual rates on plant composition and herbage objectives, which are supposed to be site-specifically modified (Melts et al. 2018). Previous research (Duffková and Libichová 2013, Duffková et al. 2015) evaluated the effect of cattle slurry application on herbage properties and/or plant composition separately in two different *Arrhenatherion* meadows, but without site-specific recommendations related to both agricultural and biodiversity targets. Hence, the aim of this study was to estimate the site-specific impacts followed by repeat cattle slurry application at differing annual rates on plant composition and agricultural demands placed on three types of *Arrhenatherion* grassland. The slurry impact on plant composition was expressed in a simplified way as proportions of plant functional types (PFT) such as graminoids, legumes, and forbs (Duru et al. 2009), and Ellenberg N values (Diekmann 2003). The agronomic demands were derived from herbage DM yield and N objectives (N content and offtake, N nutrition index (NNI), N use efficiency (NUE)). Site-specific cattle slurry rates were derived to meet acceptable agronomic values and/or profits to plant diversity.

## MATERIAL AND METHODS

Three *Arrhenatherion* grassland experimental sites differing in soil, terrain and water characteristics (moderately wet – WS; slopy – SS; moderately dry – DS) were established in the central Czech Republic (WS – 49.4916875N, 15.2702478E; SS – 49.4918303N, 15.2681503E; DS – 49.4914119N, 15.2617989E, Duffková and Macurová 2011). The soil of site WS was Colluvic Regosol Humic with extremely acid sandy loam. Site SS consisted of Haplic Cambisol soil with south-east exposure and 7° incline, with acid loamy sand. The shallow soil of DS site was Cambic Hyperskeletal Leptosol, with acid loamy sand. Sites SS and, especially, DS were drier than WS due to sunny exposure and permeable soil profile, respectively. Mean plant available contents of P and potassium (K) in mg/kg of unfertilized plots extracted from the upper 20 cm soil layer by Mehlich III solution were categorized according to Sánka and Materna (2004) in WS as low (P = 10.2, K = 77), in SS as low (P = 8.4) and satisfactory (K = 108), and in DS as satisfactory (P = 31.5, K = 110), respectively. The altitude of the

sites ranged from 508 m a.s.l. (WS) to 534 m a.s.l. (DS), with a mean annual precipitation of 660 mm and air temperature of 7.0°C. Prior to the study, sites had been cut twice per year with no fertilization.

In April 2007, a factorial experiment was established and replicated three times in a randomised block design of five 15 m<sup>2</sup> treatment plots in each of the three sites. Cattle slurry was applied at 0 (S0); 60 (S1); 120 (S2); 180 (S3), or 240 kg N/ha/year (S4). From 2007–2012, all 45 plots were cut three times annually, in early June, late July, and late September. Slurry was spread manually on the plot surface three times a year. In early April, plots S1–S3 received 60 kg N, and plot S4 received 120 kg N. After the second cutting in late July, plots S2–S4 received 60 kg N; and, after the third cutting in late September, plots S3 and S4 received 60 kg N. The mean DM content of the slurry was 64 g/kg and mean concentration of N in DM was 61 g/kg.

The percent cover of vascular plant species (Kubát et al. 2002) was visually assessed annually prior to the first cut in permanent 1 m × 1 m areas within each experimental plot. Percent cover of PFTs (graminoids, legumes, and tall/short forbs of mean height greater/less than 0.5 m) was calculated. The mean of Ellenberg N values (Ellenberg et al. 1992) weighted for cover of each species was calculated for each relevé.

A representative 1 kg herbage sample was collected from all 45 plots at all cuttings. The herbage DM content and yield were determined for each cut and for the entire season. Herbage N content was determined according to the Kjeldahl method in 2008, 2010, and 2011. The annual herbage N offtake (kg/ha) was calculated as the sum of herbage DM yield multiplied by herbage N content for each cut. To calculate N sufficiency in biomass production, NNI (Lemaire 1997), defined as herbage N content relative to the herbage critical (minimal) N content (N<sub>crit</sub>) needed to produce the maximum amount of dry matter (Hejman et al. 2010), was used. The NNI was calculated as:

$$\text{NNI (\%)} = \frac{\% \text{ N measured}}{\% \text{ N}_{\text{crit}}} \times 100$$

in which % N<sub>crit</sub> = 4.8 (herbage DM yield in t/ha)<sup>-0.32</sup>.

To assess the ability of the investigated grassland to increase herbage yield in response to N applied, NUE was determined as the amount of herbage DM yield in kg produced per 1 kg of N applied in slurry.

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To assess the effects of site, treatment, cutting, and their interactions on PFT, Ellenberg N, herbage DM yield, and N objectives, the repeated measures ANOVA (R package nlme; Pinheiro et al. 2016) was used including random effects of individual plots. Normality of the data was tested by the Shapiro-Wilk test. Data with non-normal distribution were transformed either by logarithmic, root square or rank transformation via the function *rntransform* from R package GenABEL (GenABEL Project Developers 2013). Backward selection based on marginality rules was used for exclusion of non-significant effects from an ANOVA model to acquire a reduced model, which was compared to the previous one using the Akaike Information Criterion. Post-hoc comparison of the differences among tested groups was based on pairwise *t*-tests with *P*-values adjusted by the Holm correction. Relationships among measured variables were assessed by Pearson's correlation coefficient. All statistical analyses were processed using R (Pinheiro et al. 2016).

## RESULTS

The effects of the predictors (site, treatment, cutting, and their interactions) are shown in Table 1. Graminoid cover differed significantly among sites (WS = 69.4%; SS = 58.4%; DS = 36.0%), and also among treatments (S0 = 29.3%; S1 = 46.0%; S2 =

56.8%; S3 = 67.5%; S4 = 73.4%). Slurry application resulted in significantly higher graminoid cover in S1–S4 compared to S0 in all sites (Figure 1a).

Legume cover was higher in DS (13.9%) and SS (11.3%) than in WS, in which cover was minimal (4.3%) and fairly balanced among treatments. Slurry application decreased significantly legume cover with the lowest values in S3 and S4. Legumes in DS were maintained at a good cover across six years followed by 60 kg N/ha and even 120 kg N/ha reaching mean values 23% and 15.4%, respectively (Figure 1b).

Mean cover of short forbs was significantly different among sites and supported the most in DS (Figure 1c). Across sites, slurry application was associated with significantly lower short forb cover in S3–S4. The significant interaction S-T (Table 1) manifested itself in a short forb cover decrease in WS plots applied by 120–240 kg N/ha compared to DS, where short forbs did not decrease its cover significantly by slurry application up to 180 kg N/ha.

The proportion of tall forbs was highest in SS (15.5%), chiefly due to high cover of *Leucanthemum vulgare* in S0 (36.2%, Figure 1d), which is resistant to dry conditions and thrives in N-poor habitats. The spread of this species was attributed to calcium carbonate application up to 10 t/ha in the past (Kvítek 1992).

Based on Ellenberg N values, plot PFT were chiefly those with mild to moderate N demands

Table 1. The effects of predictors (site, treatment, cut) and their interactions on variables expressed by *F*-ratios and *P*-values obtained by repeated measures ANOVA

Data collection	Variable	Site (S)	Treatment (T)	Cut (C)	S-T	S-C	T-C
Annual totals	herbage DM yield (2007–2012)	66.76***	16.55***	–	excluded	–	–
	N use efficiency <sup>++</sup> (2007–2012)	82.93***	54.63***	–	excluded		
	herbage N offtake (2008, 2010, 2011)	47.90***	18.51***	–	excluded	–	–
Before 1 <sup>st</sup> cut (2007–2012)	Ellenberg N <sup>++</sup>	14.15***	10.77**	–	2.31	–	–
	graminoid cover <sup>++</sup>	53.13***	28.19***	–	excluded	–	–
	legume cover <sup>++</sup>	35.77***	30.44***	–	excluded	–	–
	short forb cover <sup>++</sup>	95.37***	4.55*	–	9.37***	–	–
	tall forb cover <sup>++</sup>	7.49**	3.12	–	1.76	–	–
Cuttings (2008, 2010, 2011)	herbage N content <sup>+++</sup>	1.32	excluded	169.11***	excluded	11.85***	excluded
	N nutrition index	17.83***	4.23*	9.28**	excluded	4.42*	excluded

\*\*\**P* < 0.001; \*\**P* < 0.01; \**P* < 0.05; in case of <sup>+</sup>, <sup>++</sup>, <sup>+++</sup> following logarithmic, rank and square root transformations. DM – dry matter

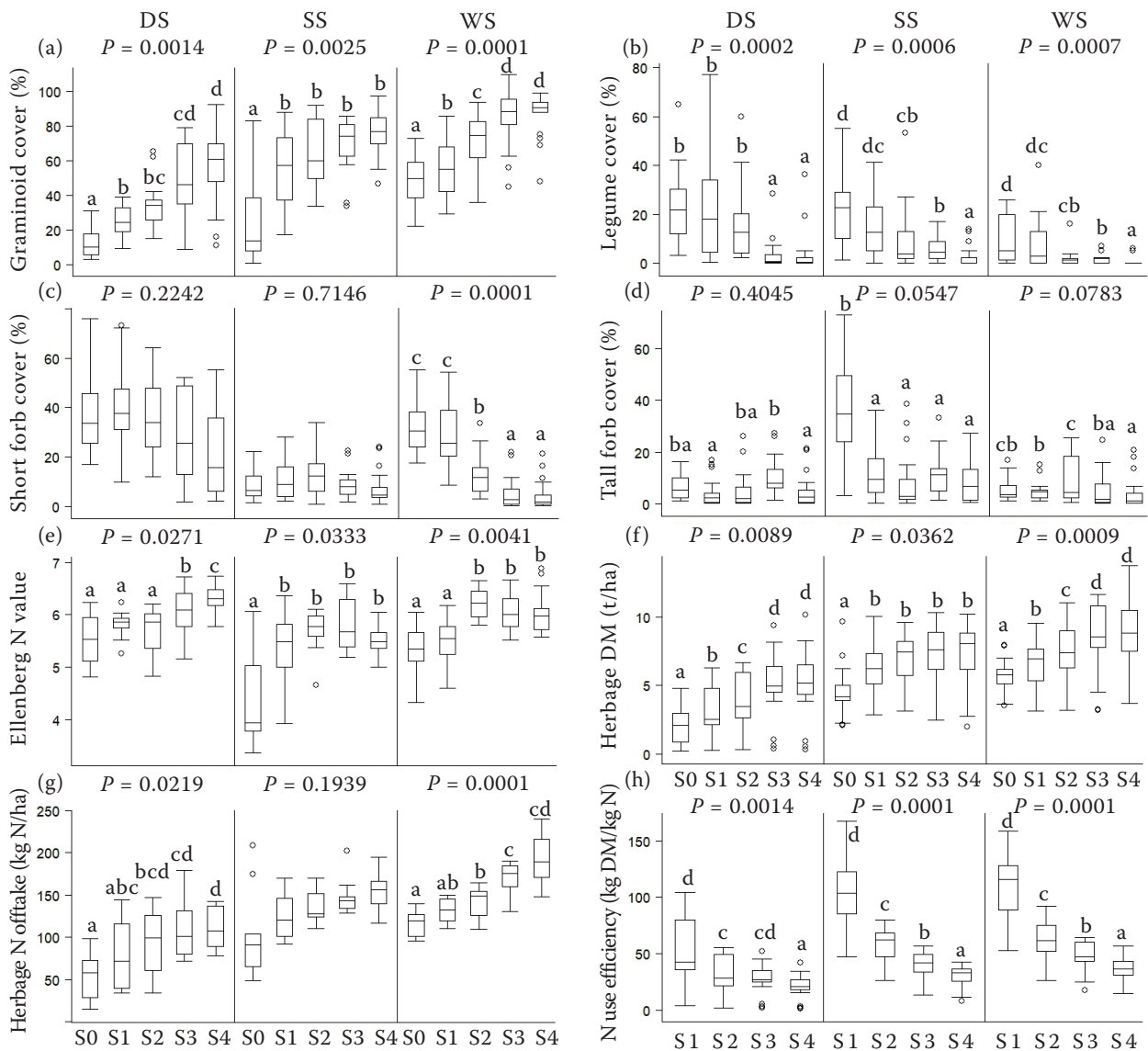


Figure 1. (a) Graminoid cover; (b) legume cover; (c) short forb cover; (d) tall forb cover; (e) Ellenberg N value; (f) herbage dry matter (DM) yield; (g) herbage nitrogen (N) offtake, and (h) N use efficiency in three sites (DS – moderately dry; SS – slopy; WS – moderately wet) as means over 2007–2012 or over 2008, 2010 and 2011 in case of herbage N offtake associated with slurry application treatments S0–S4. P-values obtained from repeated measures ANOVA. Using pairwise *t*-tests, treatments with the same letter were not significantly different. S0 – 0, S1 – 60, S2 – 120, S3 – 180, S4 – 240 kg N/ha

differing in SS significantly from both others (SS = 5.3; WS = 5.8; DS = 5.9). Across sites, the slurry application rate was significantly reflected in Ellenberg N values (S0 = 5.1; S1 = 5.6; S2 = 5.9; S3 = 6.0; S4 = 5.9). Ellenberg N was enhanced significantly by 60, 120, and 180 kg N/ha in SS, WS, and DS, respectively (Figure 1e). In all sites, the increasing proportions of graminoids to the detriment of legumes and short forbs enhanced Ellenberg N values (Figure 2).

The highest mean herbage DM yield was in WS (7.4 t/ha) followed by SS (6.4 t/ha) and DS (3.8 t/ha), in which low soil water availability substantially limited plant growth. In all sites, herbage DM yield in S1–S4 was significantly higher than in S0 (Figure 1f). Herbage DM yield was affected positively by cover of graminoids and negatively by cover of short forbs (all sites), legumes (SS, WS), and tall forbs (SS, WS, Figure 2). Inflection points visible in correlation plots containing herbage DM yield and

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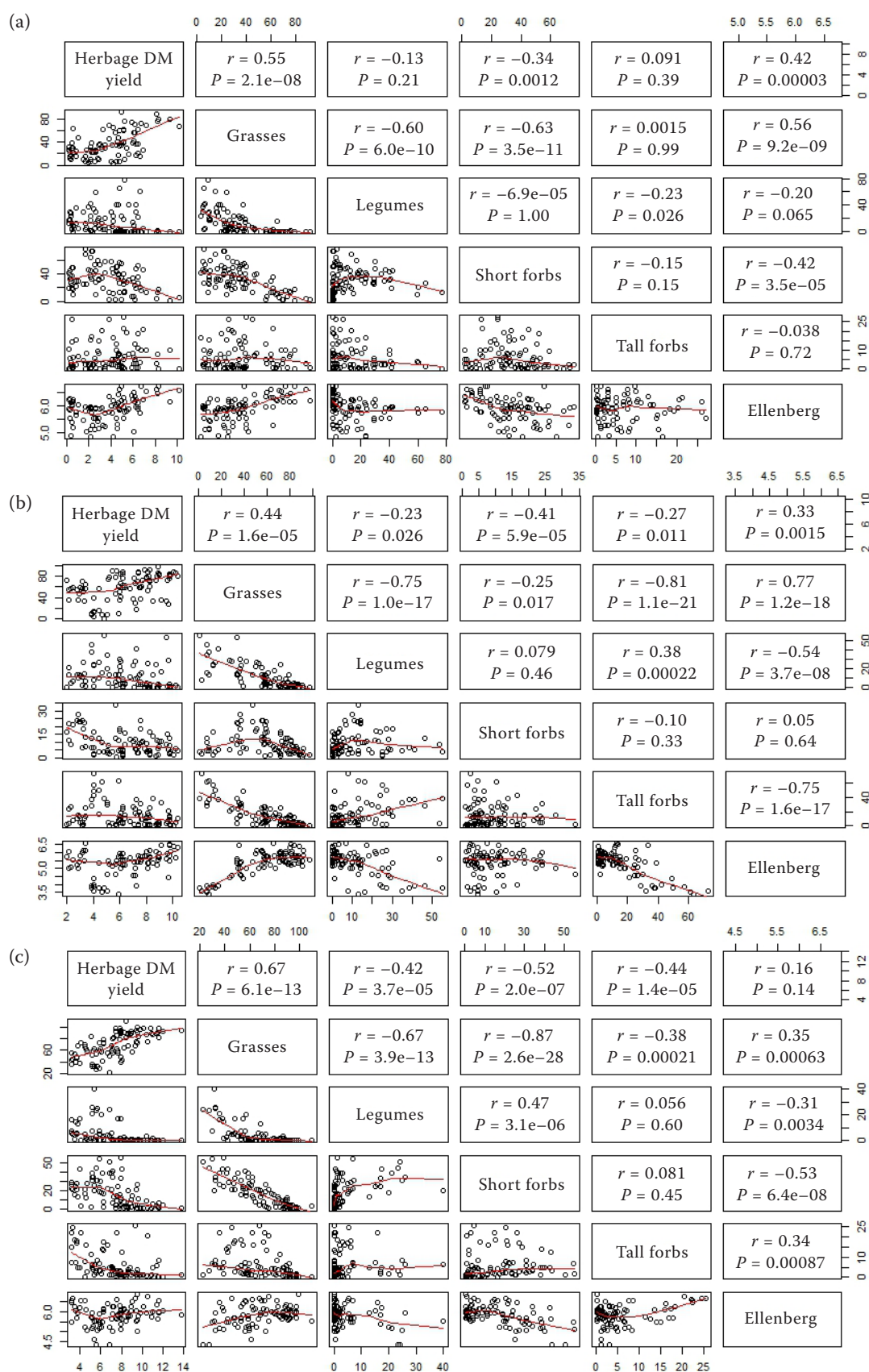


Figure 2. Relationships among herbage dry matter (DM) yield (t/ha), plant functional types (%), and Ellenberg N values for (a) moderately dry; (b) slopy and (c) moderately wet sites.  $r$  – Pearson's correlation coefficient;  $P$  –  $P$ -value



Ellenberg N (Figure 2) indicated an increasing in plant N demands, when herbage DM yield was above 2.5 t/ha in DS and 6 t/ha in SS and WS.

Similar to herbage DM yield, the highest herbage N offtake was in WS (151 kg N/ha) compared to SS (134 kg N/ha) and DS (90 kg N/ha). In DS and WS, S2–S4 treatments enhanced N offtake significantly compared to S0 (Figure 1g).

Nitrogen use efficiency was affected significantly by site with the best results in WS (63.6) followed by SS (57.5) and DS (32.7). Across sites, slurry application decreased NUE significantly along with increasing rates (S1 = 87.8; S2 = 49.8; S3 = 38.0; S4 = 29.4, Figure 1h).

A detailed investigation of plant growth dynamics and herbage N objectives was provided by an analysis of cuttings in 2008, 2010, and 2011. Herbage N content was affected by mowing in accordance with the typical dilution effect, i.e., lower herbage N content at the first cutting in conjunction with the highest aboveground biomass, and vice versa for the third cutting (Tables 1 and 2). No site and slurry application effect on herbage N content was observed (Tables 1 and 2). The herbage N content correlated positively with tall forb cover in WS ( $r = 0.50$ ) and legume cover in SS and DS ( $r = 0.36$  and  $0.44$ , respectively).

All plant communities were affected by N deficiency, which was most pronounced in water-limited DS (NNI = 41.5%) compared to more productive

WS and SS (50.4% and 48.6%, respectively). The significant treatment effect was proven only in WS in S3–S4 plots of the first cut and in S2–S4 plots of the third cut mitigating N deficiency (NNI > 50). Plants harvested in early June showed highest N assimilation, likely due to availability of winter and early spring water supply (NNI = 50.7%), compared to the second and third cutting (42.9% and 47.1%, respectively), which decreased, chiefly in DS (Table 2).

## DISCUSSION

Slurry application lasting six seasons substantially changed plant composition, growth dynamics, and nutritional status in all study sites. Similarly to Douglas and Crawford (1998), the results of the study demonstrated the increase in N availability induced by slurry application, which was accompanied by increased herbage biomass accumulation and N offtake, and also by the spread of more N-demanding and higher yielding grasses, while cover of short forbs and legumes decreased. Positive correlation of herbage DM yield with Ellenberg N indicated effective uptake, not only of soil N, but of other nutrients and factors related to biomass production (Diekmann 2003).

As the mean annual herbage DM yield in S0 in WS and SS was higher than 4 t/ha (5.7 and 4.6 t/ha),

Table 2. Mean herbage nitrogen (N) content and N nutrition index from the first, second, and third (1–3) cuts over 2008, 2010 and 2011 in three sites (WS – moderately wet, SS – slopy; DS – moderately dry)

	Herbage N content (g/kg)									N nutrition index (%)								
	WS			SS			DS			WS			SS			DS		
Cut	1	2	3	1	2 <sup>+++</sup>	3	1	2 <sup>++</sup>	3 <sup>+++</sup>	1 <sup>+</sup>	2	3	1	2	3	1 <sup>+</sup>	2	3
<i>F</i>	2.58	1.99	0.77	0.69	0.58	0.04	1.30	0.19	1.23	3.63	2.52	5.27	0.87	0.41	0.66	1.72	1.63	0.09
<i>P</i>	0.102	0.172	0.566	0.613	0.685	0.996	0.334	0.936	0.358	<b>0.045</b>	0.107	<b>0.015</b>	0.516	0.800	0.635	0.222	0.242	0.985
S0	16.5	17.4	20.2	16.3	18.4	21.8	17.0	16.5	20.3	49.3 <sup>a</sup>	46.5	44.9 <sup>a</sup>	45.7	45.5	43.6	39.7	30.4	41.3
S1	15.3	16.3	19.8	14.5	18.7	21.9	17.7	16.8	18.4	50.2 <sup>a</sup>	44.6	46.4 <sup>ab</sup>	47.5	48.8	46.3	49.8	31.2	40.5
S2	14.3	16.1	20.0	15.3	18.9	22.1	18.6	17.9	19.1	48.2 <sup>a</sup>	44.0	51.2 <sup>bc</sup>	50.7	48.0	52.0	53.6	34.9	38.2
S3	15.5	16.8	20.1	15.3	16.7	21.7	16.7	17.8	19.4	54.9 <sup>b</sup>	46.5	54.1 <sup>c</sup>	53.7	43.5	50.2	53.3	38.8	40.9
S4	17.7	19.3	21.6	14.4	19.7	22.1	15.1	17.4	20.0	62.2 <sup>bc</sup>	54.5	58.7 <sup>c</sup>	51.2	49.9	52.2	50.1	37.3	41.4
Mean	15.9	17.2	20.3	15.2	18.5	21.9	17.0	17.3	19.4	52.9	47.2	51.1	49.8	47.1	48.9	49.3	34.5	40.4

*F*-ratio and *P*-value were obtained by repeated measures ANOVA; in case of <sup>+</sup>, <sup>++</sup>, <sup>+++</sup> following logarithmic, rank and square root transformations. Significant values are in bold. Using pairwise *t*-tests, treatments with the same letter were not significantly different. Abbreviations see Figure 1

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these plots were classified as highly productive (Hrevušová et al. 2015). The high herbage DM yield in WS resulted from its water and nutrient availability and proliferation of graminoids, as opposed to water-limited DS, which produced the lowest herbage biomass.

In more productive sites (SS, WS), S2–S4 treatments decreased short forb and/or legume cover while graminoid cover and Ellenberg N values increased markedly. Hence, reconciling plant diversity and agronomy targets were not encountered (Duffková and Libichová 2013). To support legumes and short forbs in WS more frequent cutting, producing an improved light regime could be useful.

As no increase in the herbage DM yield, herbage N offtake and Ellenberg N revealed in SS and WS plots affected by S4 treatment compared to S3 plots, the amount of 180 kg N/ha could be suggested as a slurry dose ensuring beneficial agronomic objectives. This is in accordance with other authors (Melts et al. 2018) reporting that intensification of herbage production reduces sward plant diversity while improving its agronomic value.

On the contrary, *Arrhenatherion* grassland on permeable DS showed low production (2.2 t/ha) due to limited soil water and nutrient availability. Compared to productive sites, more favourable light conditions in S0–S2 plots in DS resulted in more sparse above-ground biomass allowing maintenance of a satisfactory level of legumes and short forbs accompanied by reduced grass growth without any significant Ellenberg N enhancement. Hence, S1–S2 plots in drier *Arrhenatherion* sustained similar plant diversity as in S0. Similarly, Šmilauerová and Šmilauer (2006) stated short forbs profits based on improving light conditions.

Hence, less productive *Arrhenatherion* grasslands, such as those limited by soil water availability, are suggested to be fertilized by cattle slurry in the amount up to 120 kg N/ha for a short-term period to meet not only sufficient agronomic objectives, but also nature conservation requirements via maintaining plant diversity (Duffková et al. 2015). However, plant species richness would be probably diminished, if suggested management took longer time (Melts et al. 2018).

The amount of available N in slurry was not sufficient to counteract N deficiency in the above-ground biomass, which is commonly reported in grasslands (Liebisch et al. 2013). Bailey et al. (1997) defined severe N deficiency, regardless of

herbage yield, as herbage N content under 20 g/kg. However, in our grasslands, severe herbage N shortage indicated by  $NNI \leq 50$  (Mládková et al. 2015) associated with an extremely low herbage DM yield ( $< 1$  t/ha), and thus high  $N_{crit}$  corresponded to herbage N content of 22.0–26.2 g/kg. A mitigation of N deficiency was connected with higher slurry rates provided sufficient soil water availability (in the first cut and/or in WS).

The optimal range for N in dairy cattle fodder of 19.2–25.6 g/kg (Whitehead 2000) was obtained only at the lowest yielding third cutting. Herbage N deficiency could be mitigated by low-emission application techniques (shallow injection, trailing shoes), according to Huijsmans et al. (2016), who reported more than 90% reduction in  $NH_3$  volatilization compared with broadcasting, and subsequent increase in grassland yields.

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