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ORIGINAL ARTICLE

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Long-term effects of mulching, traditional cutting and no management on plant species composition of improved upland grassland in the Czech Republic

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Abstract

A shortage of available livestock for utilizing grassland biomass in Central Europe is challenging for the management of both semi-natural grasslands and previously intensified (limed, fertilized and reseeded) upland grasslands. An alternative method of grassland management is mulching, in which aboveground biomass is cut, crushed and subsequently spread on the surface. This paper reports on an experiment to compare three different mulching frequencies (one, two and three times per year) with an unmanaged treatment and traditional management of two cuts per year (control) on a previously improved upland meadow. Plant species composition was monitored over 13 years. Traditional management of two cuts with biomass removal was the most suitable method for maintaining plant species richness and diversity, and both were reduced significantly in the once-mulched and especially in the unmanaged treatment. Tall dicotyledonous weeds such as *Urtica dioica*, *Cirsium arvense* and *Aegopodium podagraria* were promoted by the unmanaged treatment and by mulching once a year. Higher frequency of defoliation had positive effects on the spread of short forbs such as *Taraxacum* spp., *Plantago lanceolata* and *Trifolium repens*. After eight years, there were changes in sward structure in the unmanaged and mulched-once-a-year treatments, with increase in the tall/short species ratio. In conclusion, repeated mulching cannot substitute fully for traditional two-cut management in improved upland meadows without decreasing plant species richness and diversity, and changing the sward structure. Although mulching once a year may prevent invasion by shrubs and trees, it also supports the spread of weedy species similar to no management.

KEYWORDS
abandonment, botanical composition, diversity, extensification, functional groups, succession

1 | INTRODUCTION

Permanent grasslands cover substantial parts of the landscape of Central Europe. In the 1990s, political changes in Central and Eastern Europe connected with economic transformation and restructuring led to a reduction in the market for agricultural products, and this in turn resulted in a collapse of agricultural production. In the Czech Republic, the importance of grasslands for fodder production decreased dramatically due to a rapid reduction in cattle and sheep herds, together with an increase in grassland in upland areas on former arable fields (CSO, 2012; Gaisler, Pavlů, Pavlů, & Hejcman, 2013). This resulted in extensification or abandonment of many areas of previously intensively managed grasslands. At the beginning of the 21st century, it was estimated that 30% of the total grassland area in the Czech Republic consisted of non-utilized meadows and pastures (Hrabě & Müller, 2004). In abandoned grasslands, a rapid decrease in the number of vascular plant species, and/or disappearance of rare or endangered plant species has been reported (Krahulec et al., 2001; Mašková, Doležal, Květ, & Zemek, 2009; Pavlů et al., 2005; Weiss & Jeltsch, 2015). If the cultural landscape in Central Europe is to be maintained, and the land kept in agricultural condition while also ensuring the provision of other ecosystem services, it is important that grasslands should be maintained using suitable and economically acceptable methods of grassland management.

The decades of the 1970s and 1980s were the peak period in the Czech Republic for the establishment and state support of improved grasslands. For example, 48,000 ha of meadows and pastures were ploughed and reseeded using productive species and 33,000 ha out of a total of 724,000 ha of grasslands in the Czech Republic were drained in the 1970s (Klesnil, Regal, Štráfelda, Turek, & Velich, 1982). These grasslands were established using highly productive grass-clover seeds mixtures that were species poor and based mainly on the following: *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense*, *Trifolium pratense* and *Trifolium repens*.

To avoid undesirable changes in botanical composition of grasslands that are not currently in agricultural use, mulching has been used since the 1990s in the Czech Republic as a low-cost alternative to grazing or conventional mowing (Fiala, 2007; Gaisler, Pavlů, Pavlů, & Mikulka, 2010; Gaisler et al., 2013). The method involves cutting the aboveground biomass without removal; the clippings being crushed into pieces of several cm lengths and then spread on the grassland surface (Doležal et al., 2011; Metsoja et al., 2012). There are likely to be different responses to mulching management between semi-natural and improved grasslands because of differences in nutrient status, botanical composition and management history. There have been several studies dealing with the effects of mulching management (Bakker, Elzinga, & de Vries, 2002; Duffková, 2008; Kahmen & Poschlod, 2008; Kahmen, Poschlod, & Schreiber, 2002; Mašková et al., 2009; Römermann, Bernhardt-Römermann, Kleyer, & Poschlod, 2009; Tonn & Briemle, 2008); however, few of these were conducted over the long term (Gaisler et al., 2013).

In a previous paper (Gaisler et al., 2013), we reported results from a long-term experiment on the different terms and frequency of mulching and its effect on vegetation of an area of upland semi-natural *Festuca rubra*-dominated, species-rich grassland with low soil nutrient status. Results indicated that mulching performed at least twice a year was able to substitute for cutting management in this type of low-production grassland, without incurring substantial loss of plant species richness and diversity. It was also found that mulching only in September changed the vegetation of the grassland in ways similar to having no management, and therefore, this option was considered suitable only as a means of preventing the establishment of shrubs and trees.

However, there is no information about the long-term effect of this alternative method of management on the vegetation of these formerly improved grasslands. To address this knowledge gap, in 2000 a grassland management experiment was established on a previously improved and managed upland meadow (i.e., sown with a high-production grass-clover seed mixture, limed and fertilized). Analyses performed on this experiment in 2011 revealed that 11 years of different mulching regimes had not caused any substantial changes in soil and herbage nutrient concentrations in comparison with the unmanaged or/and cut treatments (Pavlů, Gaisler, Hejcman, & Pavlů, 2016). Thus, based on these results, we concluded that mulching management performed two or three times per year could substitute for a conventional cutting regime. However, in the paper of Pavlů et al. (2016) detailed evaluation of vegetation development was not taken into account.

Therefore, the aim of the study reported in this paper was to evaluate the effects of different mulching management regimes on vegetation characteristics of a previously improved upland meadow over a 13-year period. In the present paper, we have focused on the following questions: (a) how do different meadow management methods (mulching, cutting and abandonment) affect long-term successional changes in plant species composition, the main plant functional groups and species richness? and (b) At what point in time can the key changes after different management introduction can be detected and are there any sward characteristics that can be used to provide a simple predictor for them?

2 | MATERIAL AND METHODS

2.1 | Study site

The experiment was established in the Jizerské hory Mts. (50°51′N, 15°02′E; 443 m elevation) 10 km north-west of Liberec, in the north of the Czech Republic. The site has a mean annual temperature of 7.2°C and average annual precipitation of 803 mm (Liberec meteorological station). The soil is acid Cambisol overlying orthogneiss. Soil chemical properties in the 0-10 cm soil layer, measured at the beginning of the experiment in 2000, were as follows: pH/KCl 6.3 (pH/H_2O 6.8), organic C content 2.7%; and plant-available (Mehlich III extraction; Mehlich, 1984) P, K and Mg concentrations 28, 138 and 290 mg/kg respectively. Mean soil chemical properties for individual treatments after 11 years of the applied management are given in Table 1 (for details see Pavlů et al., 2016).

In 1990, ten years before establishment of the experiment, the site was agriculturally improved by draining, then limed (500 kg CaO/ha), fertilized (100 kg N/ha, 40 kg P/ha, 80 kg K/ha) and reseeded with a grass-clover mixture suitable for high forage production, comprising *D. glomerata*, *F. pratensis*, *P. pratense*, *T. pratense* and *T. repens*. From 1991 to 2000, the meadow was cut twice each year and occasionally grazed by cattle. The plant community of the study area was classified as *Arrhenatherion* alliance (Chytrý, 2007), and

TABLE 1 Mean soil chemical properties for the different treatments measured in 2011 (Pavlů et al., 2016). Numbers represent mean values for each treatment: two cuts per year (2C), unmanaged (U), mulching once per year (1M), mulching twice per year (2M) and mulching three times per year (3M)

		Treatment				
	Characteristics	2C	U	1M	2M	3M
Soil	pH/KCl	5.87	6.56	6.40	6.12	6.08
	C_{org} mg/kg	28,500	30,350	30,050	30,875	30,075
	N_{tot} mg/kg	2,200	2,440	2,508	2,503	2,580
	P mg/kg	17.26	34.14	26.29	25.40	20.08
	K mg/kg	69.50	157.59	141.72	125.47	100.44
	Ca mg/kg	2,053	2,654	2,464	2,193	2,234
	Mg mg/kg	282.4	388.0	350.5	345.3	371.4
	C:N	13.0	12.5	12.0	12.3	11.7

before the start of the study in 2000, the dominant vascular plant species were *D. glomerata*, *F. pratensis*, *P. pratense*, *Galium album*, and *Veronica chamaedrys*. There were no fertilizer applications to the meadow for at least five years prior to the start and during the period of the experiment. The average yield of forage was about 5 t/ha of dry matter (DM) per year.

2.2 | Experimental design

The experiment was carried out in four completely randomized blocks during the period 2000 to 2013. In each block, there were rectangular (10 m × 5 m) treatment plots, one for each of five treatments. The following treatments were applied: unmanaged (U), two cuts per year (as a control) with removal of cut biomass in June and August (2C), mulching performed 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). A tractor-driven mulching machine (Uni Maher UM 19, Gerhard Dücker GmbH & Co. KG) was used for the mulching treatments: plant biomass was crushed into pieces 5–10 cm long, spread on the sward surface and pressed by the roller. Cutting was carried out using a tractor-driven rotary mower, and cut biomass (in the 2C treatment) was removed immediately following cutting. The residual sward height after mulching and cutting was approximately 5 cm.

2.3 | Plant species composition and functional groups

The coverage of all vascular plant species was determined by visual estimation and recorded directly on a percentage scale. To avoid possible plot-edge effects, only the central area of 8 m × 3 m of each permanent plot was used for the observations. Collection of relevés was carried out annually at the end of May before the first mulching application. Nomenclature of vascular plant species follows Kubát et al. (2002). Weed species in grasslands were considered to be those plant species that (a) significantly reduced forage quality, and/or (b) decreased biomass yield (Mikulka, Pavlů, Skuhrovec, & Koprdová, 2009). A list of weedy species is presented in Supporting Information Table S1.

In accordance with the mean height of vascular plants as listed in the regional flora (Kubát et al., 2002), all plant species recorded in the experiment were a priori categorized into four main groups: short graminoids, tall graminoids, short forbs and tall forbs. The tall/short species ratio was also recorded. Species with a mean height of ≥0.5 m were considered tall, and those below this threshold were considered as short (see Supporting Information Table S1). Shannon diversity (H index) was calculated from the cover data of all vascular plant species in a particular relevé (Begon, Townsend, & Harper, 2005).

The C-S-R functional types (Grime, Hodgson, & Hunt, 1988) were used for evaluating changes in the sward under each different management. Data for determination of C-S-R strategies for each plot were calculated from means of C, S and R values, weighted using the cover of each vascular plant species present in the individual plot, where the sum of all strategies was 1 (100%) (Hunt et al., 2004). Ellenberg nutrient indicator values (EIV) for nutrients, light and soil moisture for each relevé were calculated as the mean of the indicator values (Ellenberg et al., 1992), weighted according to the cover for each vascular plant species.

2.4 | Data analyses

Redundancy analysis (RDA) in the CANOCO 5 program (ter Braak & Šmilauer, 2012) was used in order to evaluate trends in plant species composition. Cover data in RDA were logarithmically transformed [$y = \log(y + 1)$] before analyses in order to down-weight dominant species (Lepš & Šmilauer, 2003). A total of 999 permutations were used in all the performed analyses. Blocks were used as covariables in all analyses. The tested null hypotheses were as follows: A1) the temporal trend in the species composition is independent of the treatment, and A2) there are no directional changes in time in the species composition that are common to all treatments. The data are formed by repeated observations including baseline data. Therefore, the interaction of treatment and year (numeric) were the most important variables. A standard biplot ordination diagram was used to visualize the results of the RDA analysis.

A linear mixed-effects model with fixed effects of treatment, time (factor) and their interaction and random effect of replication was used to evaluate the cover of the most abundant vascular plant species, H index and richness, cover of functional groups, tall/short species ratio and C-S-R strategy. A linear mixed-effects model with fixed effect of treatment and random effect of replication was used to test differences of the cover of the most abundant vascular plant species, species diversity and richness, cover of functional groups, tall/short species ratio and C-S-R strategy in the year 2013. Replication was used as covariate with random effect in all performed analyses. Further, to identify significant differences between individual treatments a post-hoc comparison using Tukey's HSD test was applied. There was no transformation as the data met all requirements of ANOVA. All univariate analyses were performed using Statistica 13.1 (Dell Inc 2016).

3 | RESULTS

3.1 | Species richness and diversity

The species richness of all vascular plants, number of species with a cover value ≥1% and the Shannon index of diversity (H) were all

TABLE 2 Results of linear mixed-effects models for all years (fixed: time, treatment, time×treatment, random: replication) and for 2013 (fixed: treatment, random: replication) for number of plant species, biodiversity, functional groups, CSR strategy, Ellenberg indicator values (EIVs) and dominant plant species

	Effect							
	Time; *df* = 13		Treatment; *df* = 4		Time × treatment; *df* = 52		Treatment in 2013; *df* = 4	
	F-ratio	*p*-value	*F*-ratio	*p*-value	*F*-ratio	*p*-value	*F*-ratio	*p*-value
Number of all plant species	3.20	<0.001	213.50	<0.001	4.00	<0.001	33.24	<0.001
Number of species with cover ≥1%	9.56	<0.001	53.92	<0.001	2.36	<0.001	7.70	0.003
Shannon index of diversity H	6.70	<0.001	120.2	<0.001	3.20	<0.001	42.67	<0.001
Cover of short graminoids	4.88	<0.001	20.97	<0.001	1.75	0.003	0.80	0.546
Cover of tall graminoids	28.01	<0.001	10.70	<0.001	3.97	<0.001	9.99	0.001
Cover of short forbs	14.20	<0.001	231.10	<0.001	3.90	<0.001	70.76	<0.001
Cover of tall forbs	31.00	<0.001	95.67	<0.001	4.12	<0.001	45.09	<0.001
Cover ratio (tall/short species)	21.27	<0.001	94.16	<0.001	9.32	<0.001	24.78	<0.001
C - strategy	17.00	<0.001	342.60	<0.001	5.50	<0.001	73.51	<0.001
S - strategy	18.90	<0.001	303.00	<0.001	5.30	<0.001	69.22	<0.001
R - strategy	14.20	<0.001	359.30	<0.001	5.50	<0.001	75.46	<0.001
EIV for nutrients	7.83	<0.001	33.43	<0.001	2.07	<0.001	21.74	<0.001
EIV for light	11.64	<0.001	54.61	<0.001	2.48	<0.001	5.66	0.009
EIV for moisture	30.80	<0.001	99.87	<0.001	4.61	<0.001	26.24	<0.001
Arrhenatherum elatius	10.10	<0.001	64.22	<0.001	4.92	<0.001	9.22	0.001
Dactylis glomerata	13.10	<0.001	80.09	<0.001	3.87	<0.001	13.02	<0.001
Elytrigia repens	18.39	<0.001	71.78	<0.001	1.27	0.127	10.07	0.001
Festuca rubra	5.27	<0.001	90.02	<0.001	1.48	0.029	46.46	<0.001
Holcus lanatus	8.78	<0.001	40.38	<0.001	2.64	<0.001	9.57	0.001
Aegopodium podagraria	10.34	<0.001	54.47	<0.001	3.38	<0.001	18.65	<0.001
Galium album	19.72	<0.001	38.57	<0.001	1.97	<0.001	5.82	0.008
Taraxacum spp.	25.50	<0.001	234.90	<0.001	7.90	<0.001	107.30	<0.001
Trifolium repens	13.44	<0.001	79.14	<0.001	4.13	<0.001	3.20	0.053
Urtica dioica	10.80	<0.001	171.3	<0.001	8.10	<0.001	18.93	<0.001

Notes. *df*: degrees of freedom; *F*: value derived from *F* statistics in repeated measurements ANOVA; *p*: probability value.

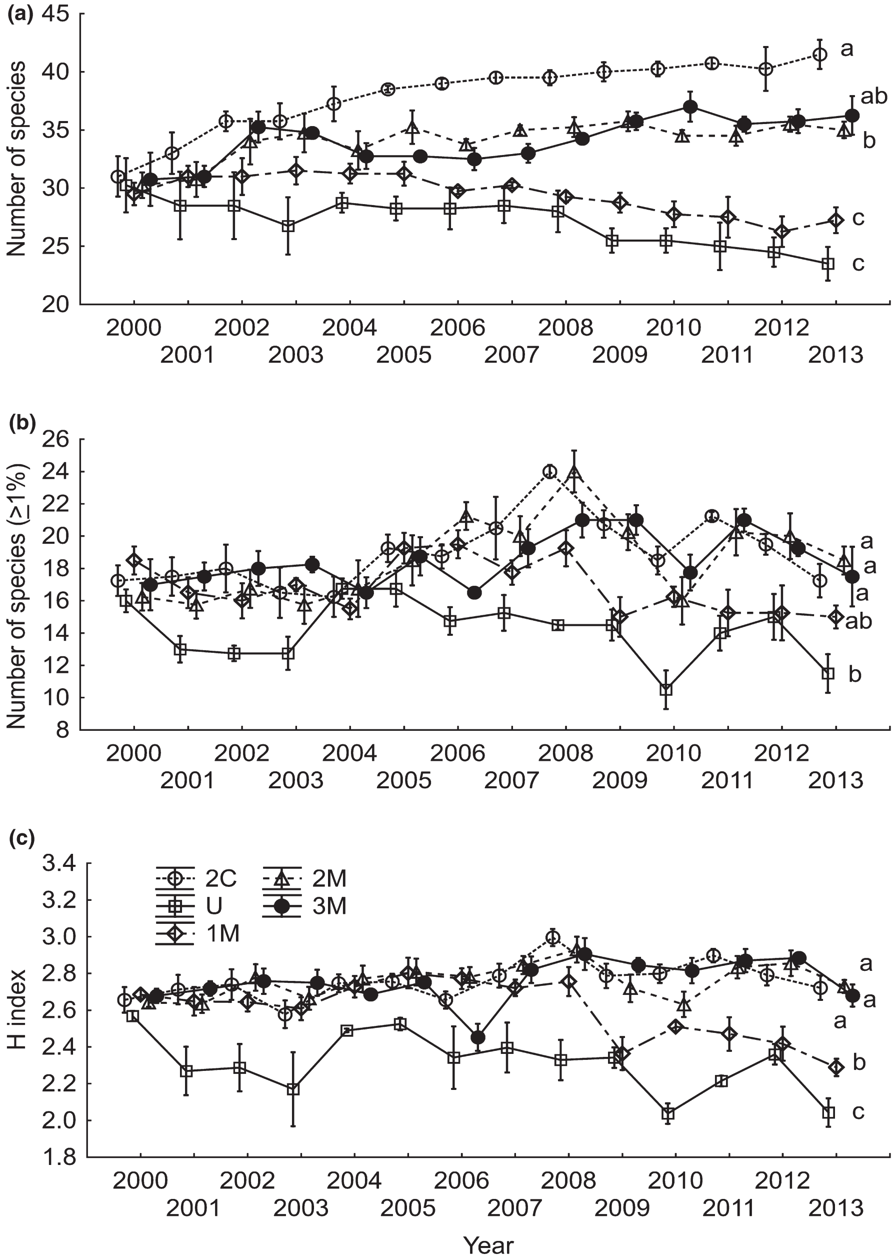

FIGURE 1 Changes in plant species composition of the tested management treatments between 2000 and 2013. (1a) changes in mean number of vascular plant species per 24 m^2, (1b) changes in mean number of vascular plant species that contributed ≥1% cover, (1c) changes in mean Shannon species diversity (H index). Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatments were as follows: unmanaged control (U), two cuts a year with biomass removal in June and August (2C), mulching once a year in July (1M), mulching twice a year in June and August (2M) and mulching three times a year in May, July and September (3M)

significantly affected by time, treatment and by time * treatment interaction (Table 2). Before the start of the experiment, the total number of vascular plant species was about 30 per plot (24 m^2) in all treatments. After 13 years of the different management regimes, the mean numbers of vascular plant species differed significantly according to management intensity in the order 2C>3M, 2M>1M, U. There was a gradual increase in vascular plant species number in the multiple-managed treatments during the course of the experiment, especially in the 2C treatment where biomass was removed immediately after the cut (Figure 1a). In contrast, there was a gradual decrease in the number of vascular plant species in the U and 1M treatments from 2008 onwards. The number of vascular plant species with ≥1% cover did not relate to treatment in the same way as the total species richness related to treatment (Figure 1b). After 13 years of the experiment, the significantly lowest values in the number of vascular plant species were revealed in the U and 1M treatments. Plant species diversity expressed by the Shannon H index was relatively stable in the 2C, 2M and 3M treatments, whereas it decreased in the U and 1M treatments (Figure 1c), where the lowest value was revealed in the final experimental year.

TABLE 3 Results of tested hypotheses to explain differences among the studied management treatments and changes over time independent of treatment, based on Redundancy Analysis. Tested null hypotheses were as follows: A1 – the temporal trend in the species composition is independent of the treatment, and A2 – there are no directional changes in time in the species composition that are common to all treatments. Treatments were unmanaged control (U), two cuts a year with biomass removal in June and August (2C), mulching once a year in July (1M), mulching twice a year in June and August (2M) and mulching three times a year in May, July and September (3M)

Tested hypotheses	Explanatory variables	Covariables	% expl. var. 1st axis, 2nd axis (all axes)	*F*-ratio 1st axis (all axes)	*p*-value 1st axis (all axes)
A1	Year * 2C, Year * U, Year * 1M, Year * 2M, Year * 3M	Year, Plot ID, Block	26.69, 38.06 (43.54)	99.4 (52.6)	0.001 (0.001)
A2	Year * 2C, Year * U, Year * 1M, Year * 2M, Year * 3M, Year	Plot ID, Block	29.17, 36.4 (41.39)	112 (48.2)	0.001 (0.001)

Notes. * indicate interactions of environmental variables; % expl. var.: species, variability explained by one (all) ordination axis (measure of explanatory power of the explanatory variables); *F*-ratio: *F* statistics for the test of particular analysis; *p*-value: probability value obtained by the Monte Carlo permutation test.

3.2 | Plant species composition

There were significant differences among the study treatments and also some remarkable changes over time independent of treatment were detected by the RDA analysis (Table 3). The treatments that had similar plant species composition according to the first ordination axis were sorted into two main groups: U and 1M treatments, and all of the multiple-managed treatments (2C, 2M, 3M) (Figure 2). Although there was a reduction in the cover of the originally sown species *F. pratensis* in all treatments, its presence was supported by management with multiple mulching (2M, 3M) (Figure 2). On the other hand, the cover of the weed grass *E. repens* decreased especially in the multiple-managed treatments, although it increased in the U treatment, where it attained its highest cover value in the final year of the experiment (Figure 3c). At the beginning of the experiment, the occurrence of *Arrhenatherum elatius* was negligible in all treatments, but in subsequent years, it increased under mulching in the 1M treatment (with a cover more than 10%). In this treatment, the final cover was the highest after 13 years of the experiment (Figure 3a). Its cover slightly increased on the unmanaged plots, but there were only a few plants recorded in the 2C and 3M treatments. The unmanaged treatment was also favourable for *F. rubra* agg. Its cover increased from 5% in the year 2000 to 13% in the final year the experiment, although in some years it was more than 25% (Figures 2 and 3d). The cover of *Holcus lanatus* increased in the 2C treatment from 2008 onwards (Figures 2 and 3e). The lowest cover of the originally sown tall-grass species *D. glomerata* was in the U treatment; this was already apparent in the third year (Figures 2 and 3b). The multiple-managed treatments (2C, 2M, 3M) were favourable for short forbs with creeping growth or prostrate rosettes, such as *T. repens* and *Taraxacum* spp., although the prostrate forb *P. lanceolata* was supported by the 2C treatment only (Figures 2 and 4). The positive effects of multiple-management treatments on the cover of *T. repens* disappeared from 2008 onwards, though not for *Taraxacum* spp. The successive increase in the cover of tall weedy forbs as *Urtica dioica*, *C. arvense* and *A. podagraria* was supported by the U and 1M treatments, but these forbs were suppressed in all of the multiple-managed treatments (Figures 2 and 4). The cover of *G. album* was increased in all experimental treatments, and the highest cover was revealed in the 1M treatment (Figure 4b). In the U treatment, woody species occurred 5 years after abandonment. However, they were present only sporadically in one block and therefore not introduced to analyses.

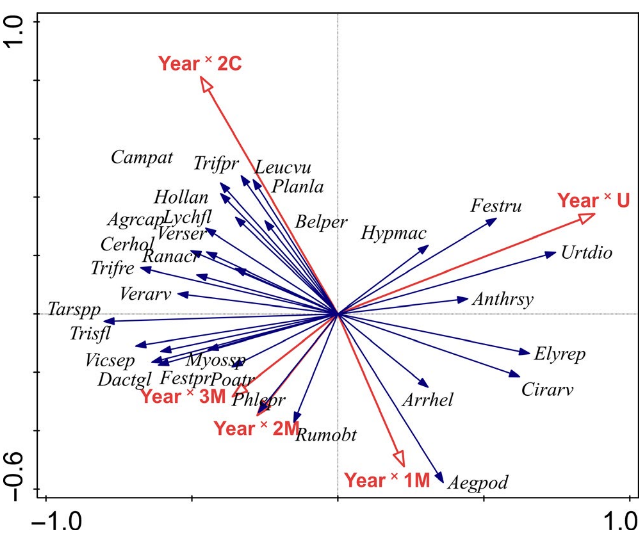

FIGURE 2 Ordination diagrams showing the results of redundancy analysis of plant species composition data (see Table 3 for details). Treatment abbreviations (U, 2C, 1M, 2M and 3M) are explained in Figure 1; * indicate interactions of environmental variables; Species abbreviations: *Aegpod* = *Aegopodium podagraria*, *Agrcap* = *Agrostis capillaris*, *Antsyl* = *Anthriscus sylvestris*, *Arrela* = *Arrhenatherum elatius*, *Belper* = *Bellis perennis*, *Campat* = *Campanula patula*, *Cerhol* = *Cerastium holosteoides*, *Cirarv* = *Cirsium arvense*, *Dactgl* = *Dactylis glomerata*, *Elyrep* = *Elytrigia repens*, *Fespra* = *Festuca pratensis*, *Fesrub* = *Festuca rubra*, *Hollan* = *Holcus lanatus*, *Hypmac* = *Hypericum maculatum*, *Leucvu* = *Leucanthemum vulgare*, *Lychfl* = *Lychnis flos-cuculi*, *Myospp.* = *Myosotis* spp., *Phlepr* = *Phleum pratense*, *Plalan* = *Plantago lanceolata*, *Poatr* = *Poa trivialis*, *Ranacr* = *Ranunculus acris*, *Rumobt* = *Rumex obtusifolius*, *Tarspp* = *Taraxacum* spp., *Trifpr* = *Trifolium pratense*, *Trifre* = *Trifolium repens*, *Trisfl* = *Trisetum flavescens*, *Urtdio* = *Urtica dioica*, *Verarv* = *Veronica arvensis*, *Verser* = *Veronica serpyllifolia*, *Vicsep* = *Vicia sepium* [Colour figure can be viewed at wileyonlinelibrary.com]

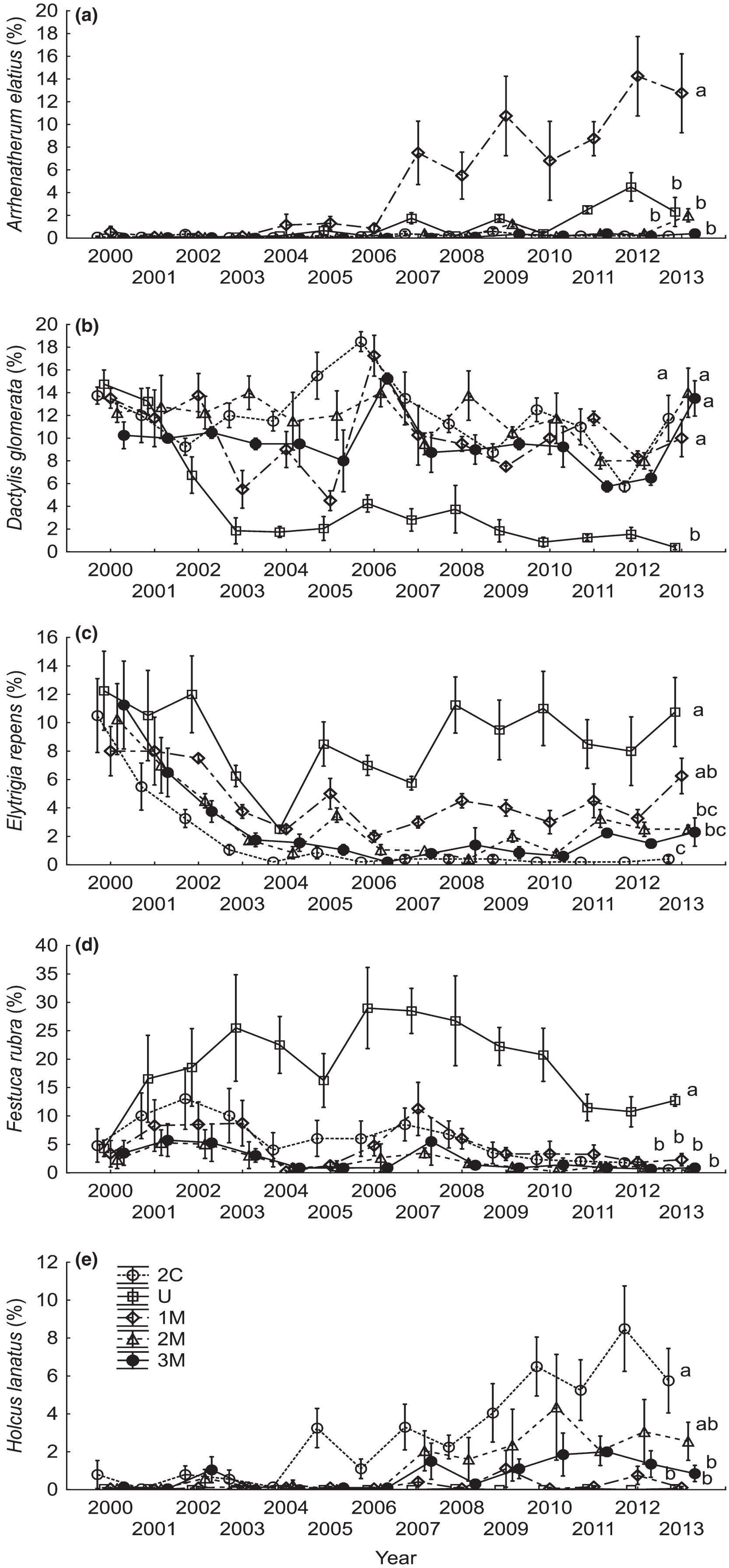

FIGURE 3 Changes in mean cover values of the dominant grass species – (a) *Arrhenatherum elatius*, (b) *Dactylis glomerata*, (c) *Elytrigia repens*, (d) *Festuca rubra* and (e) *Holcus lanatus* in the investigated treatments between 2000-2013. Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatment abbreviations (U, 2C, 1M, 2M and 3M) are explained in Figure 1

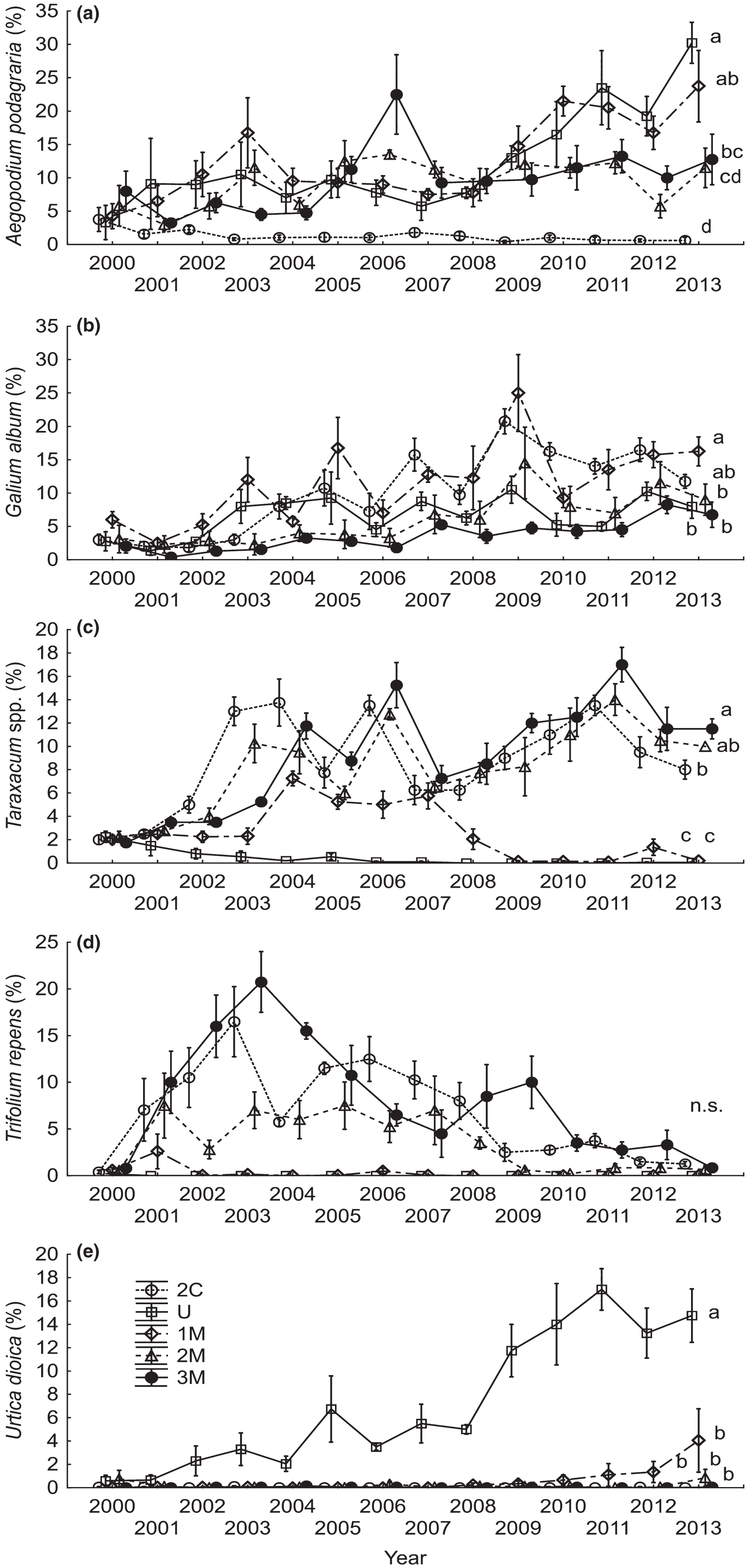

FIGURE 4 Changes in mean cover values of the dominant forb species – (a) *Aegopodium podagraria*, (b) *Gallium album*, (c) *Taraxacum* spp., (d) *Trifolium repens* and (e) *Urtica dioica* in investigated treatments between 2000-2013. Error bars represent standard error of the mean. Significant differences ($p < 0.05$) according to the Tukey post-hoc test are indicated by different letters in the year 2013. Treatment abbreviations (U, 2C, 1M, 2M and 3M) are as explained in Figure 1

3.3 | Functional groups, C-S-R strategies and EIV

The cover of short and tall graminoids, as well as short and tall forbs and the tall/short species ratio, were affected significantly by time, treatment and by time * treatment interaction (Table 2). The initial cover of tall graminoids varied from 52% to 62% at the start of the experiment, and it decreased regardless of treatment during the first four years. Subsequently, there were fluctuations but without any apparent stable trend for the particular treatments. However, the lowest cover of tall graminoids was recorded in the U treatment after 13 years of the experiment (Supporting Information Figure S2a). At the start of the experiment, the cover of short graminoids was very low (maximum of 0.5%). In later years, there was a tendency for an increase in short graminoids in the multiple-managed treatments (2C, 2M, 3M), whereas their proportion remained close to zero in the U and 1M treatments (Supporting Information Figure S2b). The initial cover of tall forbs varied between 13 and 22%, and we observed a progressive increase in the U and 1M treatments. There was a relatively stable cover of tall forbs under all the multiple-managed treatments (2C, 2M, 3M), the lowest proportion being recorded in the final year of the experiment (Supporting Information Figure S2c). On the other hand, the proportion of short forbs decreased in the U and 1M treatments, whereas it increased in the multiple-managed treatments (2C, 2M, 3M) (Supporting Information Figure S2d). The tall/short species ratio was < 6 in all of the multiple-management treatments (2C, 2M, 3M) over the duration of the experiment. After 2008, there was a steep increase in the tall/short species ratio; the highest value of the ratio was in treatment 1M after 13 years of the experiment (Supporting Information Figure S2e).

Time and treatment, as well as their interactions, significantly affected proportions of species as classified by C-S-R functional strategies (competitors, stress tolerators and ruderals) (Table 2). Species characterized by C strategy predominated in all treatments, with proportions higher than 70%, and the C strategy was the one that characterized many of the plant species in the U and 1M treatments (>85%). The lower proportion of C strategy was revealed in both of the multiple mulching treatments (2M, 3M) and the lowest in the 2C treatment. Conversely, U and 1M treatments were least favourable for plant species with S and R strategies. The proportion of S and R strategies was similar and inverse to the C strategy (Supporting Information Figure S3) through the course of the experiment, with a differentiation between treatments in the eighth year of the experiment. The highest value was in the 2C treatment and the lowest the U treatment in after 13 years of the experiment.

The EIV for nutrients, light and moisture were significantly affected by time, treatment and by time * treatment interaction (Table 2). After 13 years of the management imposed, EIV for nutrients were significantly lowest in the 2C treatment, and significantly highest in all of the mulched (1M, 2M, 3M) treatments (Supporting Information Figure S4a). In the final year of the experiment, the EIV for light was highest in the 2C treatment and lowest in the U treatment, while values were similar in all of the mulched treatments (Supporting Information Figure S4b). On the other hand, there were opposite tendencies recorded for EIV of soil moisture, differentiation between treatments from the year 2008 and the highest value was in the U treatment and the lowest in the 2C treatment after 13 years of the experiment (Supporting Information Figure S4c).

4 | DISCUSSION

4.1 | Species richness and diversity

Similar to the findings reported from our previous mulching experiment, which was conducted in a *F. rubra* semi-natural meadow (Gaisler et al., 2013), in all managed treatments, there were only small differences in the number of species that had cover values higher than or equal to 1%. This supports the view that, within the range of treatments implemented, the general plant species composition is relatively stable and remains similar under different management regimes. However, plant species with cover values of less than 1% contributed approximately half of the species richness, and therefore, these species exerted a greater effect on species fluctuation than more abundant plant species (Figure 1). The total number of vascular plant species at the beginning of the experiment (about 30 per 24 m^2) was in the range commonly found in improved upland grasslands in the Czech Republic (Pavlů, Gaisler, Pavlů, Hejcman, & Ludvíková, 2012). Moreover, similar numbers of species have also been recorded for semi-natural grasslands (Pavlů, Pavlů, Gaisler, Hejcman, & Mikulka, 2011). However, there is usually a higher proportion of weedy species (e.g. *A. podagraria*, *C. arvense*, *E. repens*, *U. dioica*) recorded in improved grasslands (Mikulka et al., 2009) as was also revealed in our experiment.

A positive effect of cutting or multiple mulching, in comparison with abandonment, on plant species richness is a common finding and has been reported from several manipulative experiments concerning grassland management (e.g. Doležal et al., 2011; Mašková et al., 2009; Nadolna, 2009). Similar to species richness, the H index was lowest in the unmanaged treatment at the time immediately after abandonment and also for the once-mulched treatments after eight years from the beginning of the study (Figure 1c). This is consistent with the results obtained in a long-term mulching study conducted in *Arrhenatherion* grasslands in Central Germany. Although that study was not conducted on improved grassland, the sward consisted mainly of grasses of agricultural value (*A. elatius and Alopecurus pratensis*) and was managed by two cuts and fertilized (Laser, 2002). In order to avoid the decrease in plant species diversity that occurs in the absence of agricultural utilization, mulching when performed at least twice a year can be recommended as a suitable alternative method of grassland management and be appropriate for different types of grasslands (Gaisler et al., 2013; Laser, 2002; Römermann et al., 2009).

4.2 | Plant species composition and functional group

Based on RDA analyses, both tested null hypotheses (A1– the temporal trend in the species composition is independent of the treatment, and A2–there are no directional changes in time in the species composition that are common to all treatments) were refused. The analyses revealed that the temporal trends in the species composition are dependent on the treatments, and directional changes in time in the species composition are common to all treatments. These expected results are common to long-term manipulative experiments concerning different managements (Gaisler et al., 2013; Pavlů, Pavlů, et al., 2011; Pavlů et al., 2012). The management treatments that involved frequent defoliation enabled the spread of several short forbs that have prostrate, rosette or creeping growth habit (*T. repens*, *Taraxacum* spp., *P. lanceolata*), and this has been a common result from grassland management experiments throughout Europe (e.g. Belsky, 1992; Pavlů, Schellberg, & Hejcman, 2011; Pavlů et al., 2012; Supek et al., 2017). However, not all short forbs behaved in a similar way. For example, the rapid increase in the shade-intolerant (Grime et al., 1988) *T. repens* from its initial negligible cover was followed by successional decrease under repeated defoliation managements. High variation in the cover of *T. repens* between years have also been reported in other long-term manipulative experiments (Gaisler et al., 2013; Pavlů et al., 2012) and are probably associated with nitrogen-driven grass dynamics (Herben et al., 2017). On the other hand, the cover of *Taraxacum* spp. was maintained over the years by frequent defoliation, with relatively low seasonal fluctuations (Figure 4c); a similar result was also revealed in a long-term study conducted in the same region (Supek et al., 2017). Similar to the results obtained in the previous experiment on low soil-nutrient status *Festuca rubra*-dominated, species-rich grassland (Gaisler et al., 2013) our findings reported in the present study support the results of Wahlman and Milberg (2002) that *P. lanceolata* is increased under management with two cuts per year, the traditional cutting frequency in Central European temperate grasslands (Ellenberg, 2009).

Although *G. album* is known to be a species that succeeds in unmanaged or infrequently managed grasslands (Gaisler et al., 2013; Pavlů, Hejcman, Pavlů, & Gaisler, 2007; Stránská, 2004), its cover in all of the managed treatments in our experiment was equal to or higher than in the U treatment (Figure 4b). This is probably connected with the greater competitive ability of *U. dioica*, *A. podagraria*, *F. rubra* and *E. repens* than *G. album* in the U treatment, especially if there is an adequate level of plant available nutrients in the soil (Pavlů et al., 2016). Further treatments with repeated application of management operations during each season (whether as cutting or mulching) significantly reduced the cover of these tall weedy forbs, which are known to be sensitive to frequent defoliation (Pavlů et al., 2007; Přikrylová, 2006). Furthermore, nutrient concentrations in the soil in these managed treatments, especially in the 2C treatment, were low relative to the elevated nutrient demand of these species. The survival and spread of short graminoids (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Luzula* sp.) are usually associated with a management of repeated defoliation, particularly in nutrient-poor acid soils (Gaisler et al., 2013; Pavlů et al., 2007). In the present experiment, therefore, in which both soil pH and plant-available nutrients were relatively high, their cover was low regardless of the treatment imposed. The group of tall graminoids comprised several dominant grasses (*A. elatius*, *H. lanatus*, *F. rubra*, *E. repens* and *D. glomerata*) and these showed different responses to the management applied (Supporting Information Figure S2a). The sown grass *D. glomerata* was supported by all of the managed treatments, whereas for *E. repens* unmanaged or infrequently managed treatments were the most favourable. *Arrhenatherum elatius* was the grass with the highest cover in the once-mulched treatment, whereas for *H. lanatus* it was two cuts per year that appeared to be the most favourable management (Figure 3a,e). Although *H. lanatus* usually prefers fertile soils (Grime et al., 1988), its highest occurrence in our experiment was in the two-cut treatment combined with the lowest soil nutrient level. It seems likely that its preference for unshaded habitats (Grime et al., 1988) in mown meadows (Hejcman, Schellberg, & Pavlů, 2010) is more crucial for this species than the soil nutrient status. The increased cover of *D. glomerata* in all of the managed treatments (Figure 3d) was probably a result of previous reseeding with the grass-clover mixture which included seed of this grass, as well as the subsequent and ongoing defoliation management. A gradual decrease in the amounts of *D. glomerata* in the unmanaged treatment could be connected to the low ability of this early growing tussock grass to sprout through thick layers of plant litter on the sward surface, particularly in early spring. Further, *D. glomerata* could have been suppressed by more productive species over the duration of the vegetation period (e.g. *A. podagraria*, *U. dioica* and *E. repens*). At the beginning of the experiment, *F. rubra* covered about 5% in all treatments and its development in subsequent years was supported by the unmanaged treatment only (Figure 3), similar to the findings reported in a previous study conducted in the same region (Pavlů et al., 2012). However, because *F. rubra* has high plasticity, it can be found in temperate grasslands under a wide range of management regimes (Gaisler et al., 2013; Grime et al., 1988; Krahulec et al., 2001; Pavlů et al., 2007; Pavlů, Pavlů, et al., 2011) and nutrient levels (Hejcman et al., 2014; Honsová et al., 2007; Kidd, Manning, Simkin, Peacock, & Stockdale, 2017).

Results on the tall/short species ratio revealed that the greatest changes occurred in unmanaged and in once-mulched plots after eight years of the treatment introduction (Supporting Information Figure S2e). This shows the high resilience of Central European temperate grasslands to contrasting types of defoliation management and that shifts to different sward structures can be recognized only after several years, as was also revealed in the Oldřichov Grazing Experiment (Pavlů et al., 2007).

Plant species that share C strategy components in the C-S-R plant strategy theory of Grime et al. (1988) were the most thriving group in the experimental grassland of this study. This applied to all the experimental treatments but the proportion of plant species that share C strategy components increased with decreasing level of management intensity (Supporting Information Figure 3). This confirms previous results (Pavlů, Pavlů, & Gaisler, 2010) that a higher percentage of C strategy species occurs in unmanaged grasslands than in grassland cut annually. The proportions of S and R strategists during the experiment were inverse to the proportion of C strategists, and cutting with removal of biomass was the management that was most favourable for plant species with S and R strategies. This also confirmed results from the previous mulching study conducted in *F. rubra* grassland (Gaisler et al., 2013). Similarly to the tall/short species ratio, the C-S-R signatures differed most notably between treatments after eight years of the study. In comparison with the tall/short species ratio, the C-S-R signature for C, S and R strategy could even reflect differences between particular multiple-managed treatments.

EIV for nutrients reflected soil nutrient depletion in the two-cut treatment in this experiment (Pavlů et al., 2016). However, the results of EIV for nutrients for the other treatments were less closely related to the results of the laboratory analyses of soil nutrients, as they were originally related to biomass productivity of the sites (Schaffers & Sýkora, 2000). The EIV for light and moisture showed clear distinctions between unmanaged and two-cut grassland, but they could not discriminate between the different mulching-frequency treatments. Overall, it appears that EIV for nutrients, light and moisture (Ellenberg et al., 1992) may provide good indirect predictors of ecological site conditions over the long term under different management practices.

Our previous study concerning mulching in *F. rubra*-dominated grasslands with low nutrient status (Gaisler et al., 2013) showed that multiple mulching over a period of 12 years affected most plant species similar to that of cutting twice a year. Therefore, mulching performed at least twice a year may be recommended as an alternative management to traditional two-cut management, without any detrimental effects on plant species richness. Similar results have also been described for different low-productive grasslands in many European regions (Laser, 2002; Moog, Poschlod, Kahmen, & Schreiber, 2002; Römermann et al., 2009).

Despite the very small differences between treatments in plant-available nutrients revealed in the previous study on this experiment (Pavlů et al., 2016), the detailed vegetation analyses reported in this paper showed that the long-term mulching management can reduce species richness and increase the proportion of weedy species. Therefore it is not appropriate as a management option for previously intensified grasslands where the soil pH and nutrient status is adequate for agricultural utilization.

5 | CONCLUSIONS

The unique value of this study is that it focuses on the effects of mulching versus other treatments on previously improved agricultural grassland, and particularly so given the relatively long period of the investigation. The main finding was that mulching, even if applied on multiple occasions in each growing season, cannot fully substitute for the traditional two-cut management in upland improved meadow without losses of plant species richness and diversity. The key changes in sward structure occurred after eight years, in the unmanaged and mulched-once-a-year treatments, and were associated with an increase in the tall/short species ratio and the C-S-R signature component for the C strategy. The tall/short species ratio could provide a convenient simple predictor for detecting structural changes in the sward, as affected by management intensity over time, which is easier to apply than the more detailed measures of changes in cover of individual plants, functional groups, species, or in the number of plant species. Mulching performed once a year in improved upland meadows encourages the spread of weedy species and the decline in species richness in a similar way to that of no management. Therefore, mulching may be considered useful only as a means of preventing the encroachment or invasion of improved upland grassland by shrubs and trees.

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