Colchicum autumnale - Control strategies and their impact on vegetation composition of species-rich grasslands

Colchicum autumnale – Strategien zur Kontrolle und ihr Effekt auf die Vegetationszusammensetzung eines artenreichen Grünlands

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Abstract

The meadow saffron *Colchicum autumnale* occurs on agricultural land predominantly in extensively managed grassland, often underlying nature preservation regulations. Due to its high toxicity if fresh or conserved (hay and silage), there is a need of control measures to ensure the future management and sward utilization of sites with occurrence of *C. autumnale*. Until now it is unclear, to what extent common management recommendations affect the vegetation composition of species-rich grassland.

In this study, the effect of different management measures (late hay cut with or without rolling, early hay cut, late mulching in May, early mulching in April, herbicide application with or without reseeding) on the number of *C. autumnale* and the vegetation composition of a moderately species-rich *Dauco-Arrhenatheretum elatioris* (31 ± 4 species per m², mean ± standard deviation) was examined since 2006. The number of *C. autumnale* was first significantly reduced three years after the start of the experiment in the early and late mulching treatments; in the next three experimental years treatment differences in *C. autumnale* reduction did not increase significantly. With respect to vegetation composition, herbicide application had the overriding effect, as it decreased the plant species number and proportions of forbs significantly. The late hay cut preserved the original plant diversity, no negative effect of rolling or the early hay cut was observed. Early mulching resulted in an increase in *Dactylis glomerata* and *Trisetum flavescens* and in the decrease of *Crepis biennis*, *Vicia sepium*, *Tragopogon pratense* and *Trifolium pratense*; it had no negative effect on the total proportion of high nature value (HNV) species. Late mulching resulted in a significantly lower yield proportion of high nature value species in 2012 and less similar in vegetation composition compared to the late hay cut treatment than early mulching; therefore it seems not to be a suitable control measure of *C. autumnale* in plant species-rich grassland on the long-term.

Keywords: *Colchicum autumnale*, extensive grassland, grassland management, plant diversity, vegetation composition

Zusammenfassung

Die Herbstzeitlose *Colchicum autumnale* kommt auf landwirtschaftlichen Flächen vorrangig im Extensivgrünland vor, das häufig Naturschutzauflagen unterliegt. Aufgrund der hohen Giftigkeit, ob frisch oder konserviert (Heu und Silage), werden Regulationsmaßnahmen benötigt, um die zukünftige Bewirtschaftung betroffener Flächen und die Verwendung der Aufwüchse sicher zu stellen. Bisher ist unklar, welches Ausmaß übliche Bewirtschaftungsempfehlungen auf die Vegetationszusammensetzung von artenreichem Grünland haben.

In dieser Studie wurde seit 2006 der Effekt unterschiedlicher Bewirtschaftungsmaßnahmen (später Heuschnitt mit und ohne Walzen, früher Heuschnitt, spätes Mulchen im Mai, frühes Mulchen im April, Herbizidanwendung mit und ohne Nachsaat) auf die Anzahl an *C. autumnale* und die Vegetationszusammensetzung einem mäßig artenreichen *Dauco-Arrhenatheretum elatioris* (31 ± 4 Arten je m², Mittelwert ± Standardabweichung) untersucht. Die Anzahl an *C. autumnale* war erstmalig nach drei Versuchsjahren bei frühem und spätem Mulchen signifikant reduziert; in den nächsten drei Versuchsjahren verstärkten sich die Unterschiede zwischen den Behandlungen nicht signifikant. Hinsichtlich der Vegetationszusammensetzung hatte die Herbizidbehandlung den stärksten Effekt, da sie zu signifikanter Abnahme der Pflanzenartenzahl und des Ertragsanteils der Kräuter führte. Der späte Heuschnitt bewahrte die ursprüngliche Pflanzenartenvielfalt, ebenso wurde kein negativer Effekt durch Walzen oder früheren Heuschnitt im Juni beobachtet. Frühes Mulchen führte zur Zunahme von *Dactylis glomerata* und *Trisetum flavescens* und der Abnahme von *Crepis biennis*, *Vicia sepium*, *Tragopogon pratense* und *Trifolium pratense*; es hatte keinen negativen Effekt auf den Gesamtertragsanteil an high nature value (HNV)-Arten. Spätes Mulchen führte 2012 zu einem signifikant niedrigeren Ertragsanteil an

HNV-Arten und geringerer Ähnlichkeit in der Vegetationszusammensetzung zum späten Heuschnitt als frühes Mulchen; es scheint daher längerfristig nicht die passende Maßnahme zur Regulation von *C. autumnale* in artenreichem Grünland zu sein.

Stichwörter: *Colchicum autumnale*, extensives Grünland, Grünlandmanagement, Pflanzenartenvielfalt, Vegetationszusammensetzung

Introduction

Colchicum autumnale is a plant with high toxicity and a wide distribution in South Germany on extensively managed grassland. Due to its relatively broad ecological amplitude *C. autumnale* is listed by OBERDORFER *et al.* (1990) as character species for the entire class Molinio-Arrhenatheretea in southern Germany. Extensively managed grassland that was formerly used in an intensive way has an increased risk that *C. autumnale* appears because a late first utilization permits the plant the development of leaves and seeds. The increase in *C. autumnale* and other toxic plants often follows the initiation of nature protection programs prescribing late first cuts in order to assure the maintenance of plant species-richness. In general, intensive grassland management with an early first cut in May and high fertilizer application weakens *C. autumnale* (DIERCKS and JUNKER, 1959; JUNG *et al.*, 2011; STÄHLIN, 1969; ELSÄSSER *et al.*, 2009; JUNG *et al.*, 2011). Therefore, *C. autumnale* hardly ever occurs in grassland used for ensiling.

For the conservation of species-rich meadows, the continuation of an extensive management and furthermore a reasonable utilization of the sward are preconditions. However, hay of meadows containing *C. autumnale* cannot be fed to livestock, as its toxicity is preserved during drying (and ensiling). The lethal dosage (LD50) for cattle lays around one mg Colchicin per kg live weight (KÜHNERT, 1991 in CliniPharm / CliniTox, 2013). During ripening of the plants the alkaloid content increases; the highest toxicity is found in seed capsules that are harvested together with the leaves of the late first cut. Whereas grazing animals are usually able to avoid toxic plants on the pasture, it comes to acute poisoning of animals fed on contaminated hay from time to time. To meet both goals of nature protection and animal nutrition, an effective control measure of *C. autumnale* maintaining biodiversity in species-rich grassland is needed. While it is known that a cut in spring can effectively control *C. autumnale* (ELSÄSSER *et al.*, 2009; JUNG *et al.*, 2010), as the maximal exhaustion of stored nutrients and starch in the belowground bulbs is in early spring (FRANKOVÁ *et al.*, 2004; JUNG *et al.*, 2012), and *C. autumnale* was reported to appear rarely in grazed grassland (JUNG *et al.*, 2011), the effect of these measures on vegetation composition of species-rich grassland is unclear. In this study, we therefore compared the following management measures recommended to date: different mulching (April, May) and cutting (beginning of June, July) regimes, a treatment with rolling simulating intensive trampling by grazing animals and herbicide application with or without subsequent reseeding. We addressed the question, which management measures effectively reduce the number of *C. autumnale* but do not result in a change in vegetation composition.

Material and Methods

The experimental site is a plant species-rich (31 ± 4 species per m², mean ± standard deviation, sd, in 2006) permanent grassland with high proportions of *Colchicum autumnale* (578 ± 226 plants per 25 m², mean ± standard deviation, sd, across all plots in 2006), belonging to the plant sociological society *Dauco-Arrhenatheretum elatioris* (POTT, 1992). The site is used as meadow with two cuts per year (late hay cut in the midst of July, second cut around eight weeks later), every two years it is fertilized with around 5 t ha^{-1} of horse manure. The grassland is managed according to requirements of the agri-environmental measure 'extensive management of plant species-rich grassland' (precondition: abundance of at least four plant species from a list of 28 typical species) of the MEKA (Marktentlastungs- und Kulturlandschaftsausgleich, Baden-Württemberg) program. The site is located in Balingen (South Germany), in the low mountain range Swabian Alb. The average annual precipitation for the Swabian Alb foreland is 825 mm, the temperature 7.8 °C

(LANDWIRTSCHAFTSAMT ZOLLERNALBKREIS, 2008). The soil type is a pseudogley (on Opalinus clay); the texture is loamy clay.

In the experiment, management measures were varied (Tab. 1) in order to investigate their effect on the abundance of *C. autumnale* and the vegetation composition. In treatments HER and HER+RS, a herbicide against dicots was applied yearly (between 30^{th} of April and 21^{st} of May); in 2006 Aaherba (2 L ha^{-1}; active components dimethylamine salts [MCPA and 2.4-D]) was used, since 2007 Simplex (2 L ha^{-1}; active components 4-amino-3,5-dichloro-6-fluoro-2-pyridyloxyacetic acid [fluroxypyr] and aminopyralid). From 2006 to 2008, a reseeding in treatment HER+RS took place after herbicide application with a seed mixture (25 kg ha^{-1}) suitable for intensively managed meadows and pastures with fresh soils (in seed weight proportions: 48% *Lolium perenne*, 24% *Phleum pratense*, 4% *Poa pratensis*, 3% *Trifolium repens*). Reseeding took place after the first cut in 2006 or 2008 or after the second cut in 2007. In 2009 no reseeding was necessary as herbicide application did not increase the proportion of gaps and a high amount of seeds was spread through haying (visual observation).

Vegetation analyses were made in 2006 (except for treatments M_E and C_L+R as these had just been carried out at the time of vegetation analysis), 2009 and 2012: plant species and their proportion on dry matter yield (KLAPP and STÄHLIN, 1936) were recorded in April/May (before the first cut) in 25 m^2 circular in the middle of each 150 m^2 (15 x 10 m) plot. Additionally, *C. autumnale* individuals were counted yearly on a 25 m^2 square in the middle of each plot.

Tab. 1 Management measures to control *C. autumnale* with dates (time period across experimental years). The first hay cut on the mulching treatments was at the same time as for the late hay cut treatment.

Tab. 1 *Bewirtschaftungsmaßnahmen zur Kontrolle von C. autumnale mit Angabe des Zeitpunkts (Zeitspanne über die Versuchsjahre). Der erste Heuschnitt der Mulchvarianten erfolgte zur gleichen Zeit wie für den späten Heuschnitt.*

		Treatment
1	C_L	late hay cut (common management; 07/06 – 02/08) as control
2	C_L+R	with rolling (27/04 – 17/05)
3	C_E	early hay cut (26/05 – 03/06)
4	HER	and herbicide treatment
5	HER+RS	and herbicide treatment with reseeding (2006 to 2009)
6	M_L	late mulching (without herbage removal; 11/05 – 21/05: flowering of *Taraxacum officinale*)
7	M_E	early mulching (without herbage removal; 17/04 – 04/05: ~10 cm height of *C. autumnale*)

The treatments were replicated three times in blocks, they were assigned randomly. Univariate analyses were performed with the statistics program R (R Development Core Team 2012, version 2.15.0). The effect of management measures on plant species numbers and yield proportion of grasses, forbs and legumes was determined by an ANOVA using a mixed model with fixed factor treatment and random factor block. The relative change in the number of *C. autumnale* plants was analysed with a mixed model ANCOVA, using the number of *C. autumnale* plants in 2006 as covariable. The assumptions of normality and homogeneity of variance for the response variables were tested visually with normal quantile-quantile (QQ) or residuals vs. fitted values plots (ZUUR *et al.*, 2009). If the assumptions were not met, the response variable was transformed. The Tukey test was used to determine treatment differences post-hoc. Indicator species analyses were performed following the specifications made in BORCARD *et al.* (2011).

Multivariate vegetation analyses were performed with Canoco for Windows (Version 4.5). The vegetation composition of the data set was relatively homogeneous (indicated by a gradient length of 1.667 found in a detrended correspondence analysis); therefore redundancy analyses (RDAs) and principal component analyses (PCAs) were performed. Partial RDAs and PCAs (blocks were used as covariables) were performed and species data log-transformed, apart from that the default settings were used. Monte Carlo permutation tests for testing significant effects of management measures in RDAs were performed with 199 permutations, significant species reactions in response to single management measures were determined by t value biplots (LEPŠ and ŠMILAUER, 2003). Repeated measure analyses based on RDAs were performed according to LEPŠ and ŠMILAUER (2003).

Results

Change in *C. autumnale* numbers

In all treatments the number of *C. autumnale* tended to be lower in 2007 and the consecutive years compared to 2006, the first experimental year (Fig. 1). Only early and late mulching resulted in a significant decrease in *C. autumnale* numbers compared to the other treatments since 2009 ($P = 0.005$, $P < 0.001$, $P < 0.001$ and $P = 0.004$ from 2009 to 2012, respectively). After 2009, no further significant differences between treatments in *C. autumnale* occurred in one of the treatments (determined by an ANCOVA with number of *C. autumnale* in 2009 as covariable). In 2012, the early hay cut resulted in a similarly large – though not significant - reduction in *C. autumnale* as the mulching treatments. The effectiveness of some of the management measures increased with the onset of the experiment, resulting in a year effect ($P < 0.001$), treatment effect ($P < 0.001$) and a treatment x year interaction ($P = 0.026$).

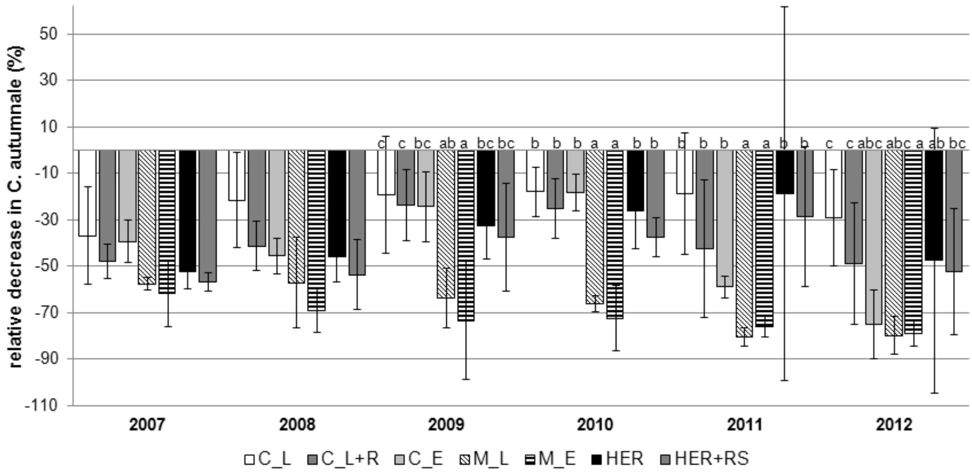

Fig. 1 Change in number of *C. autumnale* compared to 2006 within treatments. Significant differences between treatments are indicated by different letters (P ≤ 0.01, respectively; determined by ANOVAs with the number of C. autumnale in 2006 as covariable).

***Abb. 1** Veränderung der Anzahl an* C. autumnale *im Vergleich zu 2006 innerhalb der Varianten über die Versuchslaufzeit. Signifikante Unterschiede zwischen den Varianten innerhalb eines Versuchsjahres (jeweils P ≤ 0.01; ermittelt über ANCOVAs mit der Kovariable* C. autumnale*-Anzahl in 2006) sind durch unterschiedliche Buchstaben angezeigt.*

Management effects on vegetation composition

There was no difference in vegetation composition between the treatments examined at the start of the experiment in 2006. In 2009 and 2012, after three or six years of different management practices, vegetation composition differed significantly between treatments ($P = 0.005$ in 2009 and 2012, respectively; Monte Carlo permutation test). Both herbicide treatments and early mulching explained a significant part of the variance in the vegetation composition in both years.

In 2009 and 2012, the herbicide application dominated the effect on vegetation composition: herbicide application built the first PCA-axis in both years, i.e. herbicide treatments were highly negatively correlated, while the late hay cut was positively correlated to that axis (data not shown). The plant species number and yield proportion of forbs was considerably reduced through the herbicide application (Tab. 2). The proportion of legumes was larger in treatments without herbicide application and in the early hay cut compared to the late hay cut with rolling in 2009.

When the herbicide treatments were not considered, early mulching (-0.70), the early hay cut (0.50) and late mulching (0.34) were highly correlated to the first PCA-axis that explained 19.5% of the remaining variance in vegetation composition (data not shown). The late hay cut was highly correlated (-0.53) to the third axis, that explained 12.2% of the variance in vegetation composition. In Table 3 plant species whose abundance was significantly affected by at least one of the management measures are presented. According to their highest correlation with treatments, they were assigned to groups representing the effect of the date of cutting (no effect of rolling was observed). The abundance of *Rhinanthus alectorolophus* was higher in the late hay cut compared to the other treatments in 2012; it was identified as indicator species of this treatment, i.e. occurred in higher proportions and steadiness in this treatment. In the early mulching plots, *Cardamine pratensis* (also significant in t-value biplot analysis) was indicator species in 2009, but then decreased significantly until 2012 (by -1.6%). *Festuca rubra* was indicator species of the early mulching treatment in 2012, the absence of *Galium album* was an indicator of the herbicide treatment without reseeding.

The number and yield proportion of high nature value (HNV) species (mainly forbs) was considerably lower in herbicide-treated plots (Fig. 2). Within non-herbicide treated plots the HNV species proportion was comparable with exception of the late mulching treatment that had a significant lower HNV yield proportion than the late hay cut in 2012.

There was a directional temporal change in the vegetation composition between 2009 and 2012. A significant development of the vegetation composition in time was found for the early mulching (P = 0.010, determined by manual selection based on the C2 analysis: see LEPŠ and ŠMILAUER, 2003). From 2009 to 2012, there was a significant decrease in the abundance of *Crepis biennis* (-3.9 vs. +0.3%), *Trifolium pratense* (-4.2 vs. -1.2%), *Tragopogon pratense* (-1.2 vs. -0.5%) and *Vicia sepium* (-1.6 vs. +0.3%), while the grasses *Dactylis glomerata* (+8.0 vs. + 2.4%) and *Trisetum flavescens* (+3.3 vs. -0.9%; mean difference between 2009 and 2012 in early mulching vs. the other treatments, respectively) increased (significant correlations were determined by t value biplot based on the C2 analysis). *T. pratense* also decreased significantly in the late mulching (-4.9%) and early hay cut treatment (-3.0%).

Tab. 2 Plant species number and yield proportions of grasses, forbs and legumes (mean ± sd) of the treatments (in 2006, there was no vegetation analysis in M_E and C_L+R: na). Management effects were determined by ANOVAs; differences between treatments are indicated by different letters.

Tab. 2 *Pflanzenartenzahl und Ertragsanteile von Gräsern, Kräutern und Leguminosen (mean ± sd) der Behandlungen (in 2006 wurde keine Vegetationsanalyse in M_E und C_L+R durchgeführt: na). Bewirtschaftungseffekte wurden mit ANOVAs ermittelt; Unterschiede zwischen Behandlungen sind durch unterschiedliche Buchstaben gekennzeichnet.*

	C_L	**C_L+R**	**C_E**	**M_L**	**M_E**	**HER**	**HER+RS**	**P-Value Treatment**
	Plant species number							
2006	30 ± 5 a	na	32 ± 4 a	31 ± 6 a	na	31 ± 2 a	33 ± 5 a	0.888
2009	36 ± 6	34 ± 6 ab	37 ± 5 b	37 ± 4 ab	28 ± 5 ab	25 ± 4 ab	25 ± 3 a	0.027
2012	39 ± 3 b	33 ± 5 ab	37 ± 6 b	29 ± 7 ab	34 ± 3 ab	25 ± 2 a	26 ± 5 a	0.014
	Grass, %							
2006	41 ± 6 a	na	43 ± 8 a	43 ± 3 a	na	39 ± 3 a	41 ± 4 a	0.485
2009	43 ± 10 a	65 ± 22	43 ± 15 a	48 ± 16 a	55 ± 9 ab	87 ± 6 c	81 ± 10	0.005
2012	44 ± 3 a	53 ± 8 a	49 ± 5 a	58 ± 23	58 ± 3 ab	84 ± 8 c	77 ± 12	0.002
				Forbs, %				
2006	54 ± 6 a	na	52 ± 7 a	52 ± 3 a	na	57 ± 3 a	55 ± 5 a	0.570
2009	53 ± 10	32 ± 19 ab	43 ± 10	46 ± 13 b	36 ± 5 ab	13 ± 6 a	19 ± 10	0.003
2012	52 ± 3 c	44 ± 9 c	46 ± 7 c	38 ± 18	40 ± 5 c	15 ± 7 a	20 ± 10	0.002
	Legumes, %							
2006	5 ± 1 a	na	5 ± 1 a	5 ± 0 a	na	5 ± 1 a	4 ± 2 a	0.627
2009	4 ± 2 bc	3 ± 3 b	14 ± 7 c	8 ± 2 bc	9 ± 5 bc	0 ± 0 a	0 ± 0 a	<0.001
2012	3 ± 1 a	3 ± 1 a	5 ± 2 a	4 ± 5 a	2 ± 3 a	1 ± 1 a	3 ± 2 a	0.348

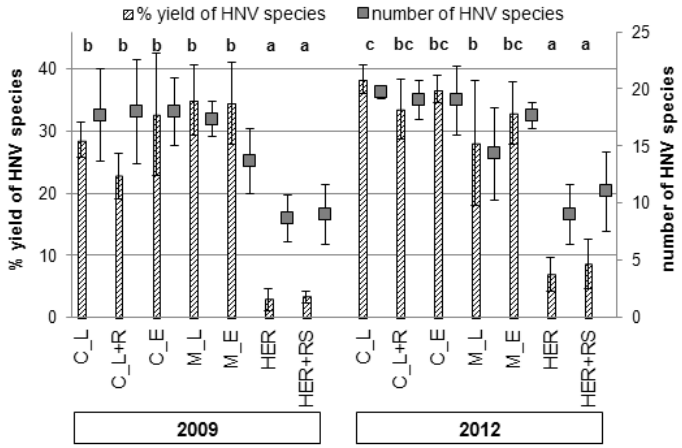

Fig. 2 Yield proportion and number of high nature value (HNV) species in plots of different management measure. Significant differences in the yield proportion of HNV species between treatments within 2009 or 2012 are indicated by different letters (P ≤ 0.001).

Abb. 2 *Ertragsanteil und Anzahl an high nature value (HNV)-Arten in den unterschiedlich bewirtschafteten Flächen. Signifikante Unterschiede im Ertragsanteil der HNV-Arten zwischen den Behandlungen innerhalb 2009 bzw. 2012 sind durch unterschiedliche Buchstaben (P ≤ 0.001) gekennzeichnet.*

Tab. 3 Mowing (M) and treading tolerance (T; ELLENBERG et al., 1992) and mean yield proportion of selected species (mean yield proportion ≥ 0.5%) whose abundance was significantly positively (+) or negatively (-) influenced by one or more not herbicide-treated treatments (values in brackets, respectively; $P < 0.05$; determined by t value biplots; such correlations of these species were not found in 2006). For the herbicide treatments no statistical test was possible, as the variance in species abundance within these treatments was negligible.

***Tab. 3** Mahd- (M) und Tritttoleranz (T; ELLENBERG et al., 1992) sowie mittlerer Ertragsanteil ausgewählter Arten (≥ 0.5% mittlerer Ertragsanteil), deren Vorkommen signifikant positiv (+) oder negativ (-), (Angabe jeweils in Klammern) durch eine oder mehrere der nicht Herbizid-behandelten Varianten beeinflusst war (P < 0.05; ermittelt über t value biplots; solche Art-Behandlungs-Korrelationen wurden nicht in 2006 gefunden). Für die Herbizid-Behandlungen war aufgrund geringfügiger Varianz im Vorkommen der Arten kein statistischer Test möglich.*

		M	T	CUTl	CUT$_l$+M	CUT$_e$	MUL$_l$	MUL$_e$	HER	HER+S
				Early cutting effect						
Vicia sepium	2009	6	2	1.4	1.3	1.7	1.1	2.3 (+)	0.1	0.2
	2012			2.0	1.7	1.7	1.1	0.7	0.7	0.5
Alopecurus pratensis	2009	7	4	5.0	6.7	0.4	2.3	7.7	2.3	9.4
	2012			5.3	8.7	1.7	4.7	10.7	3.0	8.3
Dactylis glomerata	2009	8	6	8.0	10.0	9.0	9.0	4.3 (-)	13.3	10.0
	2012			10.3	12.3	11.0	15.0	12.3	13.3	11.7
Holcus lanatus	2009	6	4	0.7	1.1	0.7	0.5	0.0 (-)	2.3	2.1
	2012			1.7	1.3	2.4	1.0	0.4	3.3	2.7
Anthoxanthum	2009	7	5	0.7	0.2	0.4	0.0	0.0 (-)	1.4	1.1
	2012			1.3	1.4	0.7	0.4	2.3	3.0	2.3
				'Medium' cutting effect						
Crepis biennis	2009	6	2	0.7	0.7	4.7	1.0	4.7 (+)	0.0	0.0
	2012			1.7	1.1	4.0	1.1	0.7	0.0	0.7
Taraxacum officinale	2009	8	7	3.3	6.4	5.0	2.7	2.7	0.2	0.2
	2012			2.1	1.7	3.7	2.3	3.3	0.1	1.0
Plantago lanceolata	2009	7	6	1.4	0.7	2.7	1.7	2.0	0.1	0.2
	2012			2.1	1.7	3.7	1.7	5.3	1.1	1.7
				Late cutting effect						
Rhinanthus alectorolophus	2009	4	2	0.1	0.0	0.1	0.0	0.0	0.0	0.0
	2012			2.3	1.3 (+)	0.4	0.0	1.0 (+)	0.0	0.1

Discussion

Change in *C. autumnale* numbers

In all treatments, at least a slight decrease in *C. autumnale* was observed. Herbicides reduced – in accordance with the producer information – only affect dicot species, but did not affect the monocot *C. autumnale*. The effectiveness of herbicide application depends on its selectivity; therefore the suitability of herbicides in reducing *C. autumnale* may vary (WINTER *et al.*, 2011). Trampling intensive grazing in spring (GEHRING and THYSSEN, 2004; ROTH and KOLLAS, 2009) or rolling are recommended (BRIEMLE, 2006) as control measures in order to disrupt the substance exchange between above- and belowground parts of *C. autumnale*. In the study, the expectation of a significant reduction of *C. autumnale* due to its low trampling tolerance (value 3: ELLENBERG *et al.*, 1992) was not confirmed; it was observed that only few *C. autumnale* stems were broken.

Mulching in April or May were the only management measures that significantly reduced the number of *C. autumnale*; this positive effect was first observed after three experimental years. From March to May the leaves of *C. autumnale* are intensively growing and simultaneously the belowground reserves in the mother corm are depleted (FRANKOVÁ *et al.*, 2004). The nutrient and starch content in the corm is lowest in April, but then increases again until June or July (JUNG *et al.*, 2012). Accordingly, the late hay cut, occurring during that time, had no effect on *C. autumnale* and moreover was too late to foreclose the ripening and further spread of the seeds. The early hay cut – on the other hand – carried out when the restoring of energy reserves into the belowground (daughter) corm was just in progress (FRANKOVÁ *et al.*, 2004) but had not ceased yet (JUNG *et al.*, 2012) - clearly decreased *C. autumnale* - though not significantly - after six experimental years. Interestingly, the differences between treatments found in 2009 did not increase significantly in the following years. Our findings are in accordance with JUNG *et al.* (2010) and WINTER *et al.* (2011), who found a reduced number of *C. autumnale* in all early cutting regimes, especially in the April and May cut regime.

Management effects on vegetation composition

The application of the not selective, dicot-specific herbicides had an overriding effect on vegetation composition; it resulted in grass-dominated species-poor swards with significantly lower number and yield proportion of HNV species. Accordingly the abundance of several frequently occurring forb species like *Geranium pratense* and *Trifolium pratense* were around zero. Above others the newly arisen gaps were taken over by two of the most abundant grasses: *Lolium perenne* and *Trisetum flavescens*. As the seed formation at the cutting date of the herbicide treatments was sufficient for closing gaps (personal observation: Jörg Schmid), reseeding – that had not affected vegetation composition in 2009 – was stopped in 2009.

Species-rich grassland typically comprises large proportions of plant species with late (and thus slower) development (NITSCHE and NITSCHE, 1994). Advanced cutting dates therefore result in decreased plant species-richness and changes in vegetation composition (KIRKHAM and TALLOWIN, 1995). The cutting date – amongst other factors – determines if plant species come to flower and seed spread, as some species are rather in their vegetative growth after the first cut (DIERSCHKE *et al.*, 2002). Furthermore the storage of reserves is affected by the cutting date. If the sward is repeatedly cut before sufficient reserves are stored, a plant species' regrowth and maintenance is at risk (VOIGTLÄNDER *et al.*, 1987). Apart from that, in mulching treatments the mulch material might – depending on the height of the mulch layer - decay slowly and thus suffocate subjacent plants especially on relatively moist sites. Thus, the late hay cut as the common management on the site, preserved the initially high plant species-richness and moreover facilitated the annual *Rhinanthus alectorolophus* that was identified as indicator species of this treatment the most. Rolling did not affect vegetation composition significantly.

Early mulching – by contrast – had significant effects on vegetation composition in 2009 and 2012 and resulted in a significant change in vegetation composition during these three years. A significantly lower proportion of the HNV species *Anthoxanthum odoratum*, a species 'susceptible to variations in cutting date' (KIRKHAM and TALLOWIN, 1995), was found in 2009, but not in 2012. This inconsistency might be related to 'cyclic changes' (VAN DEN BERGH, 1979 in OOMES and MOOI, 1981) related to naturally unsteady environmental conditions, resulting in changing resource conditions for plant species (DAVIS *et al.*, 2000). On the other hand, there was a general increase in several grass species at the expense of some larger growing dicot species typical for extensively used meadows like *Crepis biennis* (-3.9%), *Vicia sepium* (-1.6%), *Trifolium pratense* (-4.2%), and *Tragopogon pratense* (-1.2%): *Trisetum flavescens*, occurring on extensively used meadows, and *Dactylis glomerata* that tolerates frequent utilization, increased significantly from 2009 to 2012. Likewise, the abundance of the rapidly developing grass *Alopecurus pratensis* (OOMES and MOOI, 1981), suitable for frequent (VOIGTLÄNDER *et al.*, 1987) and early cuts, was positively correlated to early mulching in 2012 and *Festuca rubra*, that reached higher yield proportions in the early mulching compared to the other treatments in 2009 and 2012 was identified as indicator species in 2012. Higher abundance of *F. rubra* in plots with advanced utilization compared to a late hay cut was also found by SMITH *et al.* (2002). As the mean interval between mulching and the following late hay cut was with around seven or ten weeks (late and early mulching treatment, respectively) relatively long, these effects are unlikely to be due to mowing in short intervals (DIERSCHKE *et al.*, 2002). The change in forb and grass proportions was therefore likely due to a higher sensitivity of the forbs against the almost complete removal of their photosynthetic organs (VOIGTLÄNDER *et al.*, 1987) at an early stage of regrowth. It is, however, startling that mulching in April affected a larger number of forb species compared to mulching in May, as the growth of plant species will be more advanced then. *Cardamine pratensis*, indicator species of the early mulching treatment in 2009, decreased significantly from 2009 to 2012; the growth of this early developing species was obviously disturbed by early mulching. Similar, but fewer effects as for the early mulching treatment were found for other treatments with advanced cutting date: *D. glomerata* increased significantly in the late mulching treatment (+6%) and *Taraxacum officinale* and *Plantago lanceolata*, whose regrowth ability is hardly affected by a cut as they grow close to the ground, also profited from better light conditions in some of the treatments with advanced cutting date. *T. pratense*, a species common in extensively used grassland, in contrast decreased in the late mulching (-4.9%) and early hay cut treatment (-3.0%).

As a survey on how plant species of high value in plant species-rich grassland are affected by the treatments, the number and yield proportion of high nature value plant species (PAN *et al.*, 2011) were examined. In 2012, the proportion of HNV species was significantly lower in the late mulching treatment compared to the late hay cut. The Bray-Curtis dissimilarity (TER BRAAK and ŠMILAUER, 2002), that measures the dissimilarity in vegetation composition between treatments by considering both the plant species present and their yield proportions is in accordance with this result: the early mulching treatment was more similar to the late hay cut than the mulching treatment (0.34 ± 0.04 vs. 0.37 ± 0.04, mean ± sd, respectively), in spite of the above mentioned significant effects of the early mulching treatment on vegetation composition. In May, the storage reserves are more depleted than in April. Therefore, regular cuts in May, typically aiming at ensiling the sward, results in species-impoverished swards on the long-term (e.g. JEFFERSON, 2005). Otherwise, a late second cut allowing seed spread following an early first cut might – with respect to the maintenance of plant species-richness - be an alternative to a late first hay cut (PLANTUREUX *et al.*, 2005).

To conclude, both early and late mulching effectively reduced the number of *C. autumnale*, but simultaneously affected the vegetation composition – though the effects found were not always consistent in 2009 and 2012. Early mulching changed the proportion of several grass and dicot species more than other treatments with advanced cutting date, while late mulching resulted in a decreased proportion of HNV species in 2012. Further observations are needed to verify the suitability of the mulching treatments in preserving plant species-richness while controlling *C. autumnale*, as both might have long-term effects on the sward composition and its structure.

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