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What is the effect of 19 years of restoration managements on soil and vegetation on formerly improved upland grassland?

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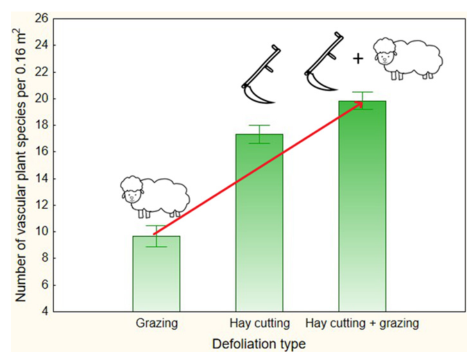
HIGHLIGHTS

- Extensive grazing is often a recommended tool for conservation management.
- Defoliation type was the key driver influenced plant species diversity.
- Grazing only managements supported grasses at the expense of forbs.
- Cutting and combinations of cutting and grazing increase plant species diversity.
- Long-term continuous extensive sheep grazing can be deleterious to plant diversity.

GRAPHICAL ABSTRACT



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ABSTRACT

Finding the best management strategies to restore grassland diversity and achieve a compromise between agricultural use and biodiversity protection is a global challenge. This paper reports novel data relating to the impacts of 19 years of restoration managements predicted to increase botanical diversity within reseeded upland temperate grassland common in less favoured areas in Europe. The treatments imposed were: continuous sheep grazing, with and without lime application; hay cutting only, with and without lime application; hay cutting followed by aftermath grazing, with and without lime application; and a control treatment continuing the previous site management (liming, NPK application and continuous sheep grazing).

Defoliation type, irrespective of liming, was the key driver influencing plant species diversity (hay cutting followed by aftermath grazing > hay cutting > grazing). Grazing only managements supported grasses at the expense of forbs, and thus related plant species diversity significantly declined. Limed treatments had higher concentrations of Ca and Mg in the soil compared to those receiving no lime. However, no effects on species richness or plant species composition were found. Potassium was the only element whose plant-available concentration in the soil tended to decrease in response to cutting treatments with herbage removal.

Postponing the first defoliation to the middle of the growing season enables forbs to reach seed production, and this was the most effective restoration management option for upland grassland (as hay cutting only, and as hay cut followed by aftermath grazing). Although continuous low-density sheep grazing is often adopted as a means of improving floristic biodiversity, deleterious effects of this on plant diversity mean that it cannot be recommended as a means of long-term maintenance or restoration management of European temperate grasslands.

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1. Introduction

Grasslands are among the most important biotopes in Central Europe, providing habitats where almost two-thirds of endangered plant species occur (Jongepierová et al., 2018), and some type of defoliation is required for their management (Bakker, 1989). Both intensive farming practices (e.g. high application rates of N, P and K plus frequent offtake of biomass) and cessation of management can cause a significant decline in related biodiversity. Finding optimal ways to restore grassland floristic diversity is the subject of much debate among land managers, ecologists, and conservationists. The overall aim of fertilizer and/or lime application in intensive grassland management is to increase biomass yield, but increasing nutrient availability simultaneously reduces plant species richness (Bakker et al., 2002; Hejcman et al., 2010; Storkey et al., 2015; Goulding, 2016; Humbert et al., 2016; Titěra et al., 2020). Cessation of fertilization will not lead to immediate restoration of species-rich grassland due to a combination of a lack of diaspore sources together with high residual levels of available nutrients and changes in soil microbial activity (Pegtel et al., 1996; Smith et al., 2008; Pavlů et al., 2011).

There are several strategies that can be applied to reverse nutrient accumulation caused by intensive grassland management. Long-term biomass removal by hay-making or grazing without fertilization are both seen as potential ways to remove soil nutrients (Hansson and Fogelfors, 2000; Van Diggelen and Marrs, 2003) and increase plant species diversity. However, reducing concentrations of excess nutrients within the soil and increasing species richness through taking two or more annual cuts can be a lengthy and difficult process (Pavlů et al., 2011), clearly depending on the period over which any particular management regime has been adopted, on soil nutrient status, and vegetation structure. Consequently, conservation management after nutrient removal should include the traditional practices that have historically contributed to the formation of the biological diversity of semi-natural grasslands (Bonari et al., 2017).

According to some authors it is possible to reduce K concentrations in the soil relatively quickly (Parr and Way, 1988; Schaffers et al., 1998; Pavlů et al., 2013). However, P reduction is reported as being much slower (Perring et al., 2009). High concentrations of plant-available P in the soil are particularly associated with low species richness and dominance of highly productive species (Janssens et al., 1998; Hejcman et al., 2010; Klaus et al., 2011) while, in contrast, high soil K concentrations are compatible with high values of plant diversity (Janssens et al., 1998; Crawley et al., 2005). Although Janssens et al. (1998) conclude N is the main element limiting plant diversity, its availability is considered to be controlled by P as this is an important nutrient for the symbiotic fixation of atmospheric N in legumes and for the mineralization of organic matter in soils. The interdependency of P and N was highlighted by the studies of Crawley et al. (2005) and Klaus et al. (2013). Lime application to increase soil pH is another wide-spread agricultural practice undertaken to improve herbage production through enhancing nutrient availability in the soil (Holland et al., 2018) that has a long-lasting impact on grassland (Spiegelberger et al., 2006). Liming can have both negative and positive effects on plant diversity depending on the number of species with different pH optima in a species pool. However, in areas characterised by long-term acidification, liming is used as an important tool in the restoration of species-rich grassland habitats (de Graaf et al., 1998).

The restoration of species-rich grassland on previously agriculturally-improved pastures has several further specific abiotic and biotic constraints, including a degradation period. In general, re-establishment of species from the seed bank is considered to be poor if this degradation phase takes more than a few decades. However, if the lost species are still present in the locally surrounding vegetation there is a chance to restore degraded communities (Van Diggelen and Marrs, 2003 and citations therein). In situations where inappropriate abiotic conditions and lack of propagules are not barriers to re-establishment of desirable vegetation, management regime can be a key driver influencing floristic diversity (Pavlů et al., 2011). As indicated previously, two basic defoliation options are hay-making and grazing, which can also be used in combination (Van Diggelen and Marrs, 2003). However, the effects of grazing and hay-making on species richness and plant species composition differ in several ways (Hansson and Fogelfors, 2000; Krahulec et al., 2001; Moog et al., 2002; Mládková et al., 2015). During hay-making the above-ground biomass is non-selectively cut and removed at the same time, while factors affecting vegetation under grazing management include stocking rate, selective grazing, trampling and nutrient enrichment (WallisDeVries, 1998; Stewart and Pullin, 2008; Ludvíková et al., 2014; Pavlů et al., 2019). Sheep are more selective of forbs and legumes than cattle, with preferential grazing encouraged by low grazing pressure (Dumont et al., 2011). A combination of grazing with cutting is generally recommended to minimize conservation risks (Krahulec et al., 2001).

In this paper we describe grassland community status after 19 years of continual exposure to various alternative restoration regimes. We compare the species composition and richness, and soil chemical characteristics when managed according to one of seven regimes that represent the common and best practices in less favoured areas dominated by temperate European upland grassland. Within this context, we aimed to answer the following questions: (i) what are the effects of long-term restoration managements on species richness, plant species composition and soil chemical properties?, (ii) what is the effect of previous liming on species richness, plant species composition and soil chemical properties?, and (iii) can any regime can be recommended for restoring plant diversity of temperate upland grasslands?

2. Materials and methods

2.1. Experimental design

The experimental plots used (the Brignant plots) were set up in 1994 to test the effectiveness of various restoration management regimes in achieving reversion of upland improved permanent pasture to semi-natural vegetation (Hayes and Tallowin, 2007). They were established at the Pwllpeiran Upland Research Centre on permanent pasture that had been ploughed and reseeded in 1973, and which had received regular inputs of fertilizer and lime. Sown grass species still dominated the sward at the time the plots were established, particularly *Lolium perenne*, at 58% cover. The plots are located at 310 m a.s.l. (O.S. Ref: SN752757) on free-draining typical brown podzolic soils. The area receives a mean annual rainfall of approximately 1850 mm and has average minimum and maximum air temperatures of 5.2 °C and 11.9 °C respectively. The plots are arranged in a randomized block design with three blocks and a total of seven grassland management regimes imposed (see Supplementary materials Figs. S1 and S2). The treatments, which have been running continuously since 1994, are: sheep grazing, with (GL+) and without (GL−) lime application; hay cutting only, with (HL+) and without (HL−) lime application; and hay cutting followed by aftermath sheep grazing, with (HGL+) and without (HGL−) lime application. Control (CO) plots continuing the previous site management (i.e. limed, fertilized and continually grazed by sheep) are also included within each block. These receive an annual application of 60 kg ha^{-1} N and 30 kg P ha^{-1}, with K also applied as required to maintain an index of 2+ (ADAS, 1983). Soil samples are taken in spring and tested for K concentration. Additional K is then applied by hand if the concentration has fallen below that equivalent to an index of 2 (ADAS, 1983). All of the lime treatment plots received a single application of lime in 1998 with the intention of maintaining a soil pH of 6.0. Treatments are imposed on three replicate plots of 0.08 ha (hay cut only) or 0.15 ha (grazed) in size. The schematic block design of the experiments and an aerial photo are provided in Appendix A, Figs. S1 and S2.

From 1994 to 2012 the plots were stocked with ewes (usually Welsh Hill Speckled Faced yearlings) with numbers adjusted to maintain a sward surface height of approximately 4 to 6 cm. Turnout occurred late April/early May when there was sufficient biomass to sustain stock. There was no spring grazing of the HGL+ and HGL− treatments, to allow colonising forb species an opportunity to establish. The HL+, HL−, HGL+, HGL− plots had a single hay harvest taken annually after the 15th of July, as and when weather conditions allowed. Plots were subsequently restocked on the HGL+ and HGL− treatments after a short period of re-growth. All stock were removed at the end of September/early October, depending on seasonal climatic conditions and related biomass growth.

2.2. Measurements

Data and samples were collected in July 2012, 18 years after the treatments were imposed. Visual percentage cover of vascular plant species was estimated in ten randomly located (0.4 m × 0.4 m) quadrats per plot in July 2012. The mean of ten quadrats of botanical composition was used for statistical evaluation. The nomenclature of the plant species follows Kubát et al. (2002). The plant species diversity was evaluated by plant species richness, Shannon (H) species diversity index and Shannon (J) species evenness index (Begon et al., 2005).

Fifteen individual soil cores were taken to a depth of 7.5 cm from randomly located areas from within each plot. The soil cores per plot were bulked then air-dried, biomass residues and roots were removed and the samples were then ground in a mortar to pass a 2 mm sieve. All chemical analyses were performed in an accredited laboratory of the Crop Research Institute in Chomutov. Plant-available Ca, K, Mg and P were extracted by the Mehlich III method (Mehlich, 1984) and concentrations were determined by inductively coupled plasma optical emission spectrometry (GBC Scientific Equipment Pty Ltd., Melbourne, Australia). Determination of pH ($CaCl_2$) was done using a pH meter (Sentron Wellinq, Leek, The Netherlands). Total N soil concentrations were determined using the Kjeldahl method and organic C concentrations by the conventional oxidation procedure incorporating chromo-sulphuric acid and colorimetry (AOAC, 1984).

2.3. Data analysis

A linear mixed-effects model (LMM) with fixed effects of treatment and random effect of the block was used to analyse the effect of treatment on selected plant species, cover of graminoids and forbs, species richness, Shannon (H) species diversity index, Shannon (J) species evenness index, and soil chemical properties. If necessary, data was log-transformed to meet LLM assumption requirements. Benjamini-Hochberg's procedure was applied to control for false-discovery rate (FDR) (Verhoeven et al., 2005). To identify significant differences between individual treatments a post-hoc comparison using Tukey's range test was applied. All LMM analyses were performed in Statistica 13.1 (Dell Inc., Texas, 2016).

Redundancy analysis (RDA) in the CANOCO 5 program (ter Braak and Šmilauer, 2012) was used to evaluate multivariate vegetation and soil data. All cover, soil and herbage chemical properties data in RDA were logarithmically transformed [$y = \log(y + 1)$]. For multivariate data analyses, two approaches were taken: i) impact of liming (comparing Limed+ versus Limed- treatments, regardless of cutting management); ii) impact of defoliation management (comparing Grazing, Hay, and Hay + Grazing treatments, regardless of liming). For all analyses 999 permutations were performed, with blocks used as covariables to restrict permutations into blocks. To visualize the results of the RDA analyses standard biplot ordination diagrams were generated.

3. Results

3.1. Species richness

Significant differences in the mean numbers of all vascular plant species, the mean numbers of vascular plant species with cover ≥ 1%, and Shannon (H) species diversity indices were recorded between treatments (Table 1). Shannon (J) species evenness indices did not show significant differences between treatments. The mean numbers of all vascular plant species, the mean numbers of vascular plant species

Table 1
Cover (%) of the most abundant vascular plant species in alphabetical order; cover (%) of total graminoids and total forbs; number of all plants species and plant species ≥ 1%; Shannon index (H) and Shannon species evenness index (J) under the different treatments in 2012. Treatment abbreviations: CO = control, continuing the previous site management with liming, fertilizing and grazing by sheep, GL+ = sheep grazing with liming application, GL− sheep grazing without liming application, HL+ = hay cutting only with liming application, HL− = hay cutting only without liming application, HGL+ = hay cutting followed by aftermath sheep grazing with liming application, HGL− = hay cutting followed by aftermath sheep grazing without liming application. Numbers represent average of three replicates ± standard error of the mean (SE); *F*-ratio = *F*-statistics for the test of a particular analysis; *P*-value = corresponding probability value. In cases of significant differences obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction (highlighted in bold), a post hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments. Differences are indicated by different small letters within rows.

	F-ratio	*P*-value	Treatment						
			CO	GL+	GL−	HL+	HL−	HGL+	HGL−
Number of all plant species	15.43	**<0.001**	8.3 ± 1.9 c	9.3 ± 1.5 c	11.3 ± 0.3 bc	17.3 ± 1.2 ab	17.3 ± 0.9 ab	19.3 ± 1.2 a	20.3 ± 0.7 a
Number of plant species ≥1%	14.39	**<0.001**	6.0 ± 1.5 b	6.3 ± 0.3 b	6.7 ± 0.9 b	11.3 ± 1.2 a	11.7 ± 0.3 a	13.3 ± 0.3 a	14.0 ± 0.6 a
Shannon (H) diversity index	14.05	**<0.001**	1.4 ± 0.2 c	1.6 ± 0.1 c	1.7 ± 0.1 bc	2.2 ± 0.1 a	2.1 ± 0.1 ab	2.3 ± 0.1 a	2.4 ± 0.1 a
Shannon (J) evenness index	1.70	0.20	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.05	0.8 ± 0.03	0.7 ± 0.01	0.8 ± 0.01	0.8 ± 0.01
Total graminoids	101.76	**<0.001**	116.6 ± 1.9 a	117.3 ± 1.6 a	112.8 ± 3.9 a	52.1 ± 3.8 b	55.6 ± 4.1 b	58.6 ± 4.0 b	46 ± 3.9 b
Total forbs	159.35	**<0.001**	2.8 ± 1.5 b	2.5 ± 1.5 b	3.3 ± 1.1 b	65.7 ± 3.3 a	62.2 ± 2.3 a	59.5 ± 4.8 a	63.3 ± 2.7 a
Anthoxanthum odoratum	4.08	**0.018**	0.43 ± 0.4 b	13.3 ± 5.2 ab	17.7 ± 2.4 a	10.1 ± 5.5 ab	12.2 ± 4.4 ab	10.3 ± 2.2 ab	8.9 ± 4.8 ab
Agrostis capillaris	4.93	**0.009**	32.5 ± 4.9 ab	36.0 ± 5.1 ab	43.2 ± 9.6 a	11.1 ± 2.3 b	25.2 ± 8.5 ab	18.9 ± 4.4 ab	15.8 ± 3.6 b
Cynosurus cristatus	2.26	0.10	0.0	0.7 ± 0.7	0.3 ± 0.3	1.1 ± 0.95	0.4 ± 0.4	3.3 ± 1.3	0.4 ± 0.2
Festuca rubra	3.26	0.038	29.2 ± 4.5	28.7 ± 0.7	21.8 ± 3.8	18.6 ± 2.1	12.0 ± 6.1	16.9 ± 6.5	14.4 ± 5.0
Holcus lanatus	3.19	0.041	8.9 ± 2.9	10.2 ± 0.6	7.4 ± 0.4	6.1 ± 1.1	3.0 ± 2.02	6.7 ± 0.6	3.0 ± 1.5
Lolium perenne	1.84	0.171	1.8 ± 1.8	0.3 ± 0.3	5.6 ± 4.0	0.0	0.0	0.03 ± 0.03	0.003 ± 0.003
Poa pratensis	7.15	**0.002**	43.7 ± 14.2 a	28.2 ± 8.4 a	16.5 ± 5.6 a	2.0 ± 1.1 b	1.4 ± 0.9 b	1.1 ± 0.6 b	1.0 ± 0.3 b
Trifolium repens	1.01	0.457	0.4 ± 0.4	1.5 ± 1.2	1.1 ± 0.7	0.0	0.0	3.0 ± 2.3	0.8 ± 0.4
Cerastium holosteoides	3.33	0.036	0.0	0.0	0.0	0.5 ± 0.3	0.9 ± 0.3	0.13 ± 0.13	0.4 ± 0.4
Crepis capillaris	2.10	0.129	0.0	0.0	0.0	6.3 ± 4.1	1.7 ± 1.7	3.9 ± 1.9	5.3 ± 2.7
Leontodon autumnalis	1.97	0.148	0.0	0.0	0.0	3.9 ± 3.9	7.0 ± 3.6	1.1 ± 1.1	0.2 ± 0.2
Plantago lanceolata	10.53	**<0.001**	0.0 b	0.0 b	0.0 b	9.3 ± 3.9 ab	14.6 ± 2.1 ab	19.7 ± 6.0 a	22.0 ± 1.1 a
Ranunculus acris	8.98	**<0.001**	0.0 b	0.03 ± 0.03 b	0.0 b	2.7 ± 1.4 ab	1.4 ± 0.8 b	3.0 ± 0.4 ab	5.4 ± 1.3 a
Ranunculus repens	10.28	**<0.001**	0.1 ± 0.1 c	0.03 ± 0.03 c	0.4 ± 0.1 c	25.5 ± 7.0 a	18.6 ± 4.3 ab	13.1 ± 3.8 abc	8.3 ± 2.3 bc
Rhinanthus minor	1.24	0.35	0.0	0.0	0.0	0.6 ± 0.5	1.9 ± 1.9	0.3 ± 0.2	3.2 ± 2.6
Rumex acetosa	31.93	**<0.001**	1.0 ± 0.6 c	0.1 ± 0.1 c	1.4 ± 1.3 c	16.2 ± 2.4 a	14.7 ± 1.0 a	12.1 ± 2.6 ab	7.4 ± 1.6 b
Taraxacum spp.	2.33	0.099	0.0	0.0	0.0	0.4 ± 0.2	0.5 ± 0.2	1.3 ± 0.6	1.7 ± 1.0

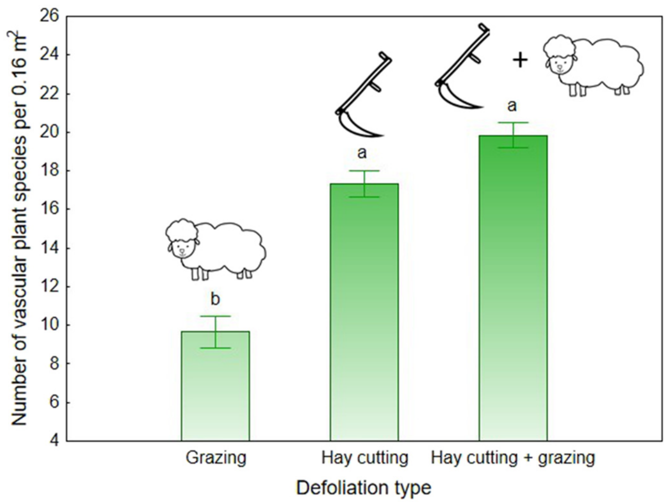

Fig. 1. Species richness under different defoliation management regimes: sheep grazing, hay cutting, hay cutting followed by aftermath sheep grazing. Error bars represent standard error of the mean. Significant differences ($P < 0.05$) according to the Tukey post-hoc test are indicated by different letters.

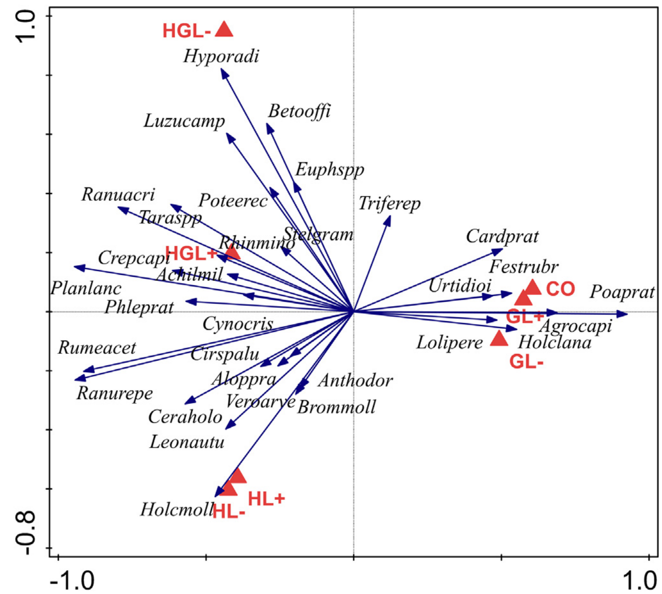

Fig. 2. Ordination diagram representing the results of redundancy analysis showing changes in plant species composition, treatments were used as predictors (see Table 2, Analysis 1 for details). Treatment abbreviations: CO = control, continuing the previous site management with liming, fertilizing and grazing by sheep, GL+ = sheep grazing with liming application, GL− sheep grazing without liming application, HL+ = hay cutting only with liming application, HL− = hay cutting only without liming application, HGL+ = hay cutting followed by aftermath sheep grazing with liming application, HGL− = hay cutting followed by aftermath sheep grazing without liming application. Species abbreviations are based on the first four-letter of genera and the four-letter of species name: Achilmile = *Achillea millefolium*, Agrocapi = *Agrostis capillaris*, Aloppra = *Alopecurus pratensis*, Anthodor = *Anthoxanthum odoratum*, Betooffi = *Betonica officinalis*, Brommoll = *Bromus mollis*, Cardprat = *Cardamine pratensis*, Ceraholo = *Cerastium holosteoides*, Cirspalu = *Cirsium palustre*, Crepcapi = *Crepis capillaris*, Cynocris = *Cynosurus cristatus*, Euphspp = *Euphorbia* spp., Festrubr = *Festuca rubra*, Holclana = *Holcus lanatus*, Holcmoll = *Holcus mollis*, Hyporadi = *Hypochoeris radicata*, Leonautu = *Leotodon autumnalis*, Lolipere = *Lolium perenne*, Luzucamp = *Luzula campestris*, Planlanc = *Plantago lanceolata*, Phleprat = *Phleum pratense*, Poaprat = *Poa pratensis*, Poteerec = *Potentilla erecta*, Ranuacri = *Ranunculus acris*, Ranurepe = *Ranunculus repens*, Rhinmino = *Rhinanthus minor*, Rumeacet = *Rumex acetosa*, Stelgram = *Stellaria graminea*, Taraspp = *Taraxacum* spp., Triferep = *Trifolium repens*, Veroserp = *Veronica serpyllifolia*, Veroarve = *Veronica arvensis*, Urtidioi = *Urtica dioica*.

with cover ≥ 1% and the Shannon species diversity indices were almost always significantly higher under treatments that included a cutting regime (HL+, HL−, HGL+, and HGL−) than those which included grazing management only (GL+, GL−, CO) (Fig. 1). Fertilizer applications and liming had no significant influence on species richness or diversity (Table 1).

3.2. Plant species composition

Based on RDA the effect of liming on plant species composition was negligible, and most of the variability in plant species composition was explained by defoliation regime (Table 2, Analyses 1–3). The first axis of the RDA of the vegetation data (Table 2, Analysis 1) displayed a gradient of defoliation management (Fig. 2). Three groups of treatments with similar plant species composition were identified on the ordination diagram: CO, GL+ and GL− treatments as the first group; HL+ and HL− treatments as the second group; and HGL+ and HGL− as the third group. The first group was species poor (number of species <12) and favoured grasses (especially *Poa pratensis*, *Agrostis capillaris*, *Festuca rubra*) and *Urtica dioica*. The second group was medium species rich (number of species about 17), and included *Alopecurus pratensis*, *Cerastium holosteoides*, *Cirsium palustre*, *Holcus mollis*. The third group was the most species rich (number of species >19) and included the forb species *Betonica officinalis*, *Hypochoeris radicata*, *Potentilla erecta*, *Ranunculus acris*, *Taraxacum* spp.

Based on LMM results the cover of graminoids was significantly supported by grazing only management regimes (CO, GL−, GL+) regardless of fertilizer and liming inputs (Table 1). The total cover of graminoids in these treatments ranged from 112.8 ± 3.9% (GL−) to 117.3 ± 1.6% (GL+), whereas the cover in the treatments that incorporated cutting ranged from 46.0 ± 3.9% (HGL–) to 58.6 ± 4.0% (HGL+). Grazing managements (CO, GL+, GL−) supported the dominant grasses *A. capillaris* and *P. pratensis*. While there was a tendency for *Anthoxanthum odoratum*

Table 2
Results of redundancy analyses for six different null hypotheses analyses (A1-A3 for vegetation, A4-A6 for soil); % expl. = explained variation by axis 1 (adjusted explained variation by all ordination axes), a measure of the explanatory power of the explanatory variables; *F*-ratio = *F*-statistics for the test of a particular analysis; *P*-value = corresponding probability value obtained by the Monte Carlo permutation test. Treatment abbreviations (CO, GL+, GL, HL+, HL−, HGL+, HGL−) are defined in Table 1.

Analysis	Expl. var.	Covariables	% expl.	*F*-ratio	*P*-value
Vegetation					
A1 Different grassland managed regimes have no effect on plant species composition	CO, HGL+, HGL−, HL+, HL−, GL+, GL−	Blocks	58.8 (77.2)	18.6 (7.3)	0.001 (0.001)
A2 Different defoliation management regimes have no effect on plant species composition	Grazing, Hay, Grazing + Hay	Blocks	58.6 (65.3)	24.0 (16.0)	0.001 (0.001)
A3 Liming has no effect on plant species composition	Limed+, Limed−	Blocks	5.8	1.1	0.309
Soil					
A4 Different grassland managed regimes have no effect on soil properties	CO, HGL+, HGL−, HL+, HL−, GL+, GL−	Blocks	44.8 (65.5)	10.6 (4.1)	0.002 (0.001)
A5 Different defoliation management regimes have no effect on soil properties	Grazing, Hay, Grazing + Hay	Blocks	13.5 (15.4)	2.7 (1.5)	0.167 (0.179)
A6 Liming has no effect on soil properties	Limed+	Blocks	42.3	13.2	0.001

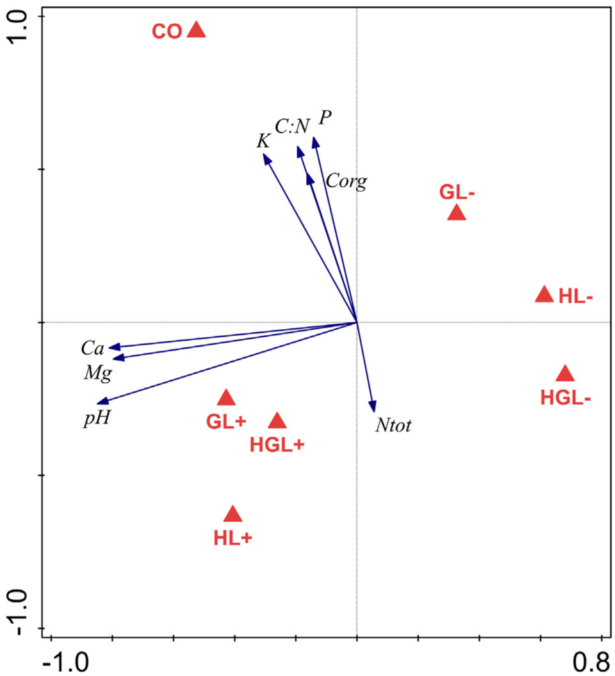

Fig. 3. Ordination diagram representing the results of redundancy analysis showing changes in nutrient concentrations in the soil at a depth of 0–7.5 cm, treatments were used as predictors. (see Table 2, Analysis 4 for details). Treatment abbreviations (CO, GL+, GL, HL+, HL−, HGL+, HGL−) are defined in Fig. 1. Abbreviations: pH-soil acidity, Corg-organic carbon; Ntot-total nitrogen in the soil; P, K, Mg, Ca-plant available nutrients; C:N-ratio in the soil.

to be suppressed by fertilizer applications (CO), *Festuca rubra* tended to be supported by a grazing only management.

Unlike graminoids, forbs were significantly suppressed by grazing only management (CO, GL−, GL+), regardless of fertilization and liming (Table 1). The total cover of forbs for the grazed treatments ranged from 2.5 ± 1.5% (GL+) to 3.3 ± 1.1% (GL−), whereas for the cut treatments the total cover ranged from 59.5 ± 4.8% (HGL+) to 65.7 ± 3.3% (HL+). The main species (*Plantago lanceolata*, *Ranunculus repens*, *Rumex acetosa*), as well as other less abundant species (such as *Ranunculus acris*, *C. holosteoides*, *Crepis capillaris*, *Leontodon autumnalis*, *Rhinanthus minor* and *Taraxacum* spp.), were supported by cutting management regimes, whereas *Trifolium repens* cover was not influenced by defoliation method.

3.3. Soil chemical properties

Redundancy analysis indicated that management regime explained the highest proportion of the soil nutrient content variability in the first and all axes (Table 2, Analyses 4–6). The first axis of RDA displayed a gradient of soil pH (Fig. 3). Soil from the plots that had received lime had a higher pH and plant available concentrations of Ca and Mg. Plant available concentrations of P, K and organic C were positively correlated with the CO treatment fertilized by N, P and K. Liming plot treatment was the second best explanatory predictor for soil chemical properties (Table 2, Analyses 4–6).

Results from LMM show that management regime did not influence the concentrations of N_{tot}, C_{org}, or the C:N ratio in the soil (Table 3). Concentrations of K in the soil had a tendency ($P = 0.040$) to be higher in the grazing only managements. Liming influenced concentrations of Ca and Mg in the soil. The concentrations of Ca were highest in all formerly limed treatments, and a similar response was found for Mg. The highest concentration of P was found in CO plots. Plant species richness was negatively correlated with soil K concentration ($P = 0.015$, $r = -0.52$), however, there was only a trend for P concentration in the soil to impact species richness. Concentrations of other soil nutrients did not influence species richness.

4. Discussion

4.1. Species richness and plant species composition

Our results showed that type of defoliation (cutting, grazing, and their combination) influenced plant species composition, species richness and Shannon (H) species diversity indices more than fertilizer applications (CO treatment) or liming. This is in accordance with previous studies that found that changes in species composition (Köhler et al., 2001) and species richness (Parr and Way, 1988) were more affected by type of disturbance than by minor changes in soil nutrients.

The grazing only management regimes encouraged an increase in grasses at the expense of forbs, regardless of fertilizer applications and liming, leading to decreasing species richness and Shannon (H) species diversity indices. In contrast, hay cutting and hay cutting followed by aftermath grazing showed the highest species richness, linked to increased forb cover, which was likely the result of there being no sward disturbance until the middle of July. Dominance by grasses is a typical response of temperate grassland to frequent sward defoliation (Louault et al., 2005; Pavlů et al., 2007; Ludvíková et al., 2015). Likewise, grazing management in comparison to cutting has been shown to promote dominance of grasses at the expense of forbs (Krahulec et al., 2001; Dumont et al., 2011; Mládková et al., 2015), and therefore mowing is generally advocated in situations where species richness maintenance is the main goal of sward management (Hansson and Fogelfors, 2000). However, our data show that similar outcomes can be obtained by hay cutting followed by aftermath grazing. Although liming has been shown to frequently have positive effects on species richness in grasslands (de Graaf et al., 1998; Holland et al., 2018), this was not evident during the current study. It is likely that this is a result of the vegetation found in the surroundings of the experiment being adapted to growth in the acidic brown podzolic soils at the site.

Table 3
Soil characteristics per plot under the different treatments in 2012. Treatment abbreviations (CO, GL+, GL, HL+, HL−, HGL+, HGL−) are defined in Table 1. Numbers represent average of three replicates ± standard error of the mean (SE); *F*-ratio = *F*-statistics for the test of a particular analysis; *P*-value = corresponding probability value. In cases of significant differences obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction (highlighted in bold), the post hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments. Differences are indicated by different small letters within rows.

	Characteristics	*F*-ratio	*P*-value	Treatment						
				CO	GL+	GL−	HL+	HL−	HGL+	HGL−
Soil up to 7.5 cm	pH	12.78	**<0.001**	4.73 ± 0.05 ab	4.89 ± 0.03 a	4.54 ± 0.10 bc	4.91 ± 0.01 a	4.41 ± 0.09 c	4.74 ± 0.05 ab	4.43 ± 0.04 c
	C_{org} (%)	1.10	0.430	8.28 ± 0.66	7.18 ± 0.77	7.46 ± 0.19	6.83 ± 0.18	7.02 ± 0.27	7.51 ± 0.16	7.27 ± 0.13
	N_{tot} (%)	0.41	0.854	0.51 ± 0.11	0.53 ± 0.05	0.56 ± 0.02	0.66 ± 0.11	0.55 ± 0.03	0.58 ± 0.01	0.57 ± 0.02
	P mg kg^{-1}	4.11	**0.018**	25.01 ± 5.38 a	13.78 ± 1.96 b	16.00 ± 1.67 ab	16.65 ± 1.02 ab	14.57 ± 0.24 b	13.16 ± 1.09 b	13.28 ± 0.73 b
	K mg kg^{-1}	3.22	0.040	172.53 ± 7.11	153.07 ± 17.27	175.91 ± 24.86	121.63 ± 9.26	121.72 ± 4.95	141.47 ± 3.99	122.62 ± 6.41
	Ca mg kg^{-1}	12.62	**<0.001**	1395 ± 136 a	1390 ± 115 a	813 ± 112 bc	1415 ± 123 a	773 ± 213 c	1261 ± 73 ab	714 ± 144 c
	Mg mg kg^{-1}	6.75	**0.002**	278.83 ± 17.92 ab	290.38 ± 25.82 a	207.31 ± 17.36 abc	302.16 ± 32.70 a	183.31 ± 32.39 bc	269.93 ± 20.57 abc	177.63 ± 17.81 c
	C:N	2.07	0.103	18.0 ± 3.6	13.7 ± 0.7	13.3 ± 0.8	11.6 ± 1.8	12.9 ± 0.5	12.9 ± 0.2	12.8 ± 0.4

4.2. Soil chemical properties

Although it is generally considered that P and N_{tot} concentrations in the soil have negative effects on grassland diversity (Janssens et al., 1998; Crawley et al., 2005; Hejcman et al., 2010; Pruchniewicz and Żołnierz, 2014), the results of our experiment show that N did not have any effect on plant species diversity. Low soil P concentrations in the locality were likely responsible for only a negative tendency of P concentration in the soil to influence species richness. Furthermore, Janssens et al. (1998) hypothesised that while N is the main nutrient limiting plant diversity, its availability is controlled by P.

It is assumed that K concentrations in the soil do not strongly influence plant species richness (Crawley et al., 2005) and that a higher K concentration is compatible with high levels of sward diversity (Janssens et al., 1998). In our experiment, K concentration in the soil had a tendency to be higher not only in the control treatment with fertilizer applications, but also in both other grazing only treatments, where a proportion of the nutrients removed were returned in sheep excreta. Cutting with biomass removal resulted in a decrease in soil K concentrations, as has been reported previously (Parr and Way, 1988; Schaffers et al., 1998; Alfaro et al., 2003, 2004; Hejcman et al., 2010; Pavlů et al., 2013; Pavlů et al., 2016). The negative relationship between plant-available K and species richness was likely due to the relatively high concentrations of K, even after the long-term removal of herbage biomass from cut plots. So, although it appears that reductions in species richness are connected to higher soil K concentrations, these decreases may be predominantly linked, once again, to defoliation management.

4.3. Management implications

Restoration of improved permanent pasture is a long-term process that can be successful if several conditions (as summarized by Van Diggelen and Marrs, 2003) can be fulfilled: (i) abiotic resource levels are within thresholds of target communities and species, (ii) sufficient viable propagules of target species are available at a rate that supports rapid establishment of desired species, and (iii) appropriate management regimes well adapted to target species requirements are in place.

Although it is generally believed that extensive grazing is a suitable management technique to maintain and restore plant diversity of temperate grassland (e.g. Van Diggelen and Marrs, 2003; Marriott et al., 2009; Jacquemyn et al., 2011; Ludvíková et al., 2015; Moinardeau et al., 2016) it is necessary to consider factors such as type of grazing animal and the grazing system. As indicated previously, sheep can be highly selective grazers and consistently sort the best quality components from within multi-species swards (Garcia et al., 2003). Thus, a low species richness is typical of swards managed by continuous sheep grazing, regardless of grazing intensity (Marriott et al., 2009). While grazing by less selective stock is preferable, economic and socio-economic pressures have led to a reduction in cattle numbers within marginal areas of the UK and similar regions within the EU, despite targeted support within agri-environment schemes.

While the current study has established the comparative benefits to plant species richness of hay cutting, the practicality of this management option in upland fringe areas is commonly compromised by topography, terrain and climatic conditions (especially rainfall). Alternatives based on delayed or rotational grazing systems should be explored if reliance on support payments to offset reduced productivity is to be avoided.

5. Conclusion

Measurements carried out on this long-term restoration experiment provide several clear messages. The first is that a higher species richness with a high proportion of forbs was observed on treatments in which cutting with biomass removal is included, whereas treatments with only grazing were linked to low species richness with a higher proportion of grasses in the sward. Secondly, K was the only element whose plant available concentration in the soil tended to decrease in response to cutting treatments with herbage removal. Higher concentrations of Ca and Mg in the soil in treatments with former liming had no effect on species richness and plant species composition. Finally, continuous sheep grazing is not recommended as a means of maintaining or restoring plant diversity within temperate grasslands. Postponing the timing of the first defoliation (hay cutting) to mid growing season, thus allowing forbs to reach the reproductive stage, would be the most effective restoration management option for upland grassland.

CRediT authorship contribution statement

Lenka Pavlů: Investigation, Conceptualization, Methodology, Writing - original draft. **Pavlů V. Vilém:** Investigation, Formal analysis, Visualization, Writing - original draft. **Mariecia D. Fraser:** Investigation, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142469.

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