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Long term defoliation by cattle grazing with and without trampling differently affects soil penetration resistance and plant species composition in *Agrostis capillaris* grassland

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ABSTRACT

There are no long-term experimental studies dealing with plant community responses to trampling. Here we report the results for soil penetration resistance and plant species changes in mesotrophic temperate Central European grassland after 12-years of grazing management with and without trampling by cattle. Five grazing treatments (intensive and extensive grazing; cut for hay in June followed by intensive or extensive grazing; and intensive grazing with no trampling under permanent electric fencing) with two replicate blocks have been applied since 1998. Species richness, species composition, sward height, nutrient availability in soil and soil penetration resistance were recorded and evaluated. For statistical analysis we used one way ANOVA and RDA with the Monte Carlo test. Long term grazing by large herbivores had a significant effect on soil penetration resistance with the lowest values in the 'not trampled' treatment. Legumes, particularly *Trifolium repens*, and short forbs (especially *Veronica serpyllifolia*) were supported by intensively defoliated and trampled treatments, whereas tall forbs (mainly *Aegopodium podagraria, Hypericum maculatum*) prevailed under the extensively managed treatments. The cover of tall and short graminoids was not dependent on applied treatments. The 'not trampled' treatment had the highest prevalence of bryophytes (domination of *Rhytidiadelphus squarrosus*) and was also the richest in a number of vascular plant species; however, it also had the lowest evenness index. Long-term defoliation by grazing animals without trampling does not lead to the creation of typical pasture swards. Species forming pasture communities are essentially dependent on regular defoliation by grazing and regular trampling by hooves, which causes a high degree of soil compaction as well as sward disruption.

1. Introduction

The sward under grazing management is mainly affected by (i) defoliation; (ii) manipulation of nutrient availability by removal of biomass or by deposition of urine and feces; and (iii) trampling (WallisDeVries, 1998). Studied effects of grazing management are often interpreted as a result of only defoliation and manipulation of nutrient availability, and trampling effects are frequently underestimated or ignored. Only a few studies have underlined the impacts of dung deposition (Dai, 2000; Gillet et al., 2010) and trampling (Curll and Wilkins, 1983; Kohler et al., 2004) on vegetation, and these have shown the marked impact of these disturbances on plant species patterns by increasing variation in vegetation composition and structure. Grazing animals re-distribute a high proportion of the nutrients removed in the vegetation via dung and urine, while removal of vegetation removes nutrients from grassland (Ausden, 2007). Trampling by large herbivores usually causes soil compaction (Witschi and Michalk, 1979; Taboada and Lavado, 1993; Betteridge et al., 1999), which afterwards increases moisture runoff, water erosion and thus evaporation. Animals also directly cause variation in sward through disturbances by hooves, they create patches of bare ground which subsequently lead to germination gaps (Ausden, 2007) where the higher temperatures at the disturbed soil surface are a very important factor limiting species establishment (Kobayashi et al., 1997). On the other hand, gaps favor seedling establishment through reduced competition by existing sward (Kitajima and

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Fenner, 2000) or they can support clonal spread of perennial species by clonal growth colonization (Bullock et al., 1995).

Generally, herbage growth declines with increasing trampling intensity. According to Pande and Yamamoto (2006) this is caused mainly by a decrease in tiller density, although higher grass tiller density was found under intensive than extensive grazing by Pavlů et al. (2006). Pasture cover and the impact of trampling damage is higher on wet soils and also in the summer, when many tillers are developing flowers (Edmond, 1963). Trampling generally promotes species with rosettes, prostrate, rhizomatous (e.g. Posse et al., 2000) or tussock growth-forms (Kobayashi et al., 1997) and thus reduces vegetation height (e.g. Bakker et al., 1983; Cole and Bayfield, 1993) and herbage yield (Edmond, 1964). Conversely, the absence of trampling supports a well-developed litter layer (Kauffman et al., 1983).

To date, the majority of studies dealing with the effects of cattle trampling have been short-term, and so the results can only show the typical temporary changes that mostly follow after introduction of a new management treatment, or they reflect an inter-seasonal dynamic (Kohler et al., 2004). No data exist on how long-term absence/presence of trampling affects plant species composition.

Fence-lines on pastures have previously attracted attention (e.g. Tanner and Mamaril, 1959; Cingolani et al., 2003). For example, the effect of fencing on plant diversity and community composition plots were studied in South Africa (Todd and Hoffman, 1999). However, there is an absence of studies in temperate grasslands on plant species composition directly under permanent electric fences where animals are not allowed to create disturbance with their hooves. According to our long term visual observations the vegetation under fences is conspicuously different in plant species richness and structure from other grazed plots, but this has not previously been recorded. Therefore we performed a comparative study inside the long-term Oldřichov Grazing Experiment (OGE) (Pavlů et al., 2007), in which trampling under electric fence lines had been completely absent throughout the 12-years since the OGE was established. There were additional treatments where defoliation (grazing as well as cutting and aftermath grazing) and trampling were applied to areas with different long term grazing intensities.

Through the following questions we analysed how the absence of cattle trampling together with the absence of feces deposition affect soil and vegetation in species-rich temperate grassland with a 12-year history of grazing: (i) can the long term effects of no trampling be detected by differences in soil penetration resistance; (ii) how does the absence of feces deposition affect soil chemical properties; and (iii) how does the absence of trampling affect vegetation height, plant species richness, abundance of main functional groups and plant species composition?

2. Material and methods

2.1. Study site

The study was performed in the long-term OGE in 2010. The OGE is situated in the Jizera Mountains, 10 km north of the city of Liberec, in the northern part of the Czech Republic (lat. 50°50′N, long. 15°06′E) in Oldřichov v Hájích village. The study site is on a northwest slope with an inclination of 5°; the bedrock is biotic granite overlain by Cambisol with pH KCl 5.45 and organic C content 4.53% in the upper 10 cm soil layer. Full plant rooting is apparent until 14 cm in the Ah horizon (0–24 cm). The Bv horizon (24–45 cm) contains 50–60% of the soil weathered skeleton of up to 22 cm. The average annual precipitation is 803 mm and the mean annual temperature is 7.2 °C (meteorological station in Liberec). The altitude of the study site is 420 m a. s. l. Prior to the introduction of experimental treatments in 1998 the grassland was classified as upland hay mesophile meadow (alliance *Arrhenatherion*) (Chytrý, 2007). Lately, the alliance *Cynosurion cristati* with about 24 species per m^2 has been successively developing under long term grazing management.

2.2. Experimental design

The trampling study was established and conducted in 2010 after 12-years of different management under OGE. The experimental site was established in 1998 on formerly abandoned grassland (Pavlů et al., 2007). Since then the experimental pasture has been continuously stocked every year with young heifers from May to October and no additional fertilizers have been applied. The experiment was arranged in two completely randomized blocks. One block (Fig. 1a) was formed by four paddocks with different grazing regimes (treatments). Each experimental paddock was approximately 0.35 ha.

We took advantage of the existing permanent electric fencing around each paddock and areas under the permanent electric fencing. In the border line between neighboring paddocks we have identified as a new treatment, labeled as cutting and grazing with no trampling (CGN). Thus the experimental area of this treatment was formed by a 1.0 m wide strips of vegetation between all neighboring paddocks (see Appendix A.1). For 12-years, these areas followed the regime of intensive defoliation by cattle grazing and no feces deposition throughout all the grazing seasons and cutting in June. The electric fences have been fixed on woody posts by two horizontal wires 50 cm and 100 cm above ground level which allowed animals to graze beneath the line but not to tread and fully defecate there (see Appendix A.1). Although the CGN treatment is always situated between the treatments with different grazing intensities, we can exclude that the area under the fencing was also grazed by varying intensity, because animals from both paddocks could graze it. To test that the differences in vegetation under fencing (CGN treatment) are caused only by the absence of trampling and not by cutting or by different stocking rate, we have compared CGN treatment with all existed treatments that were applied on our experimental pasture since 1998 as follows: (i) extensive grazing (EG) – stocking rate adjusted to achieve a mean target sward surface height more than 10 cm; (ii) first cut in June followed by extensive grazing for the rest of the growing season. (ECG); (iii) intensive grazing (IG) – stocking rate adjusted to achieve a mean target sward surface height less than 5 cm; (iv) first cut in June followed by intensive grazing for the rest of the growing season (ICG). The spatial arrangement of the trampling study was a set of 0.33 m × 1 m study plots (Fig. 1b) in each treatment in 2010.

The forage harvest in ECG and ICG treatments was performed by tractor with three machines: cutting at 3–5 cm, haymaker and pick-up hay-loader. The excessive biomass extending to wires of permanent electric fencing (CGN) between all treatments was cut to 3–5 cm by a brush cutter and then removed by raking in June. The mean stocking rates were at about 500 kg liveweight (two heifers) per 1 ha and 1000 kg liveweight (four heifers) per 1 ha for EG (ECG) and IG (ICG) treatments, respectively. The sward surface heights were measured weekly across each experimental plot (100 measurements) using a rising plate meter (Correll et al., 2003), and stocking density was adjusted accordingly by increasing or decreasing the area available for grazing.

2.3. Soil penetration resistance measurements

Soil penetration resistance (MPa) measurements were performed by certificated penetrometer reading according to European Standard No. EN ISO 22476-2:2005 (CEN, 2005) using the dynamic cone penetrometer among studied treatments. Over the entire area of all study paddocks, not corresponding to trampling study plots, and under the electric fencing five sites were randomly selected

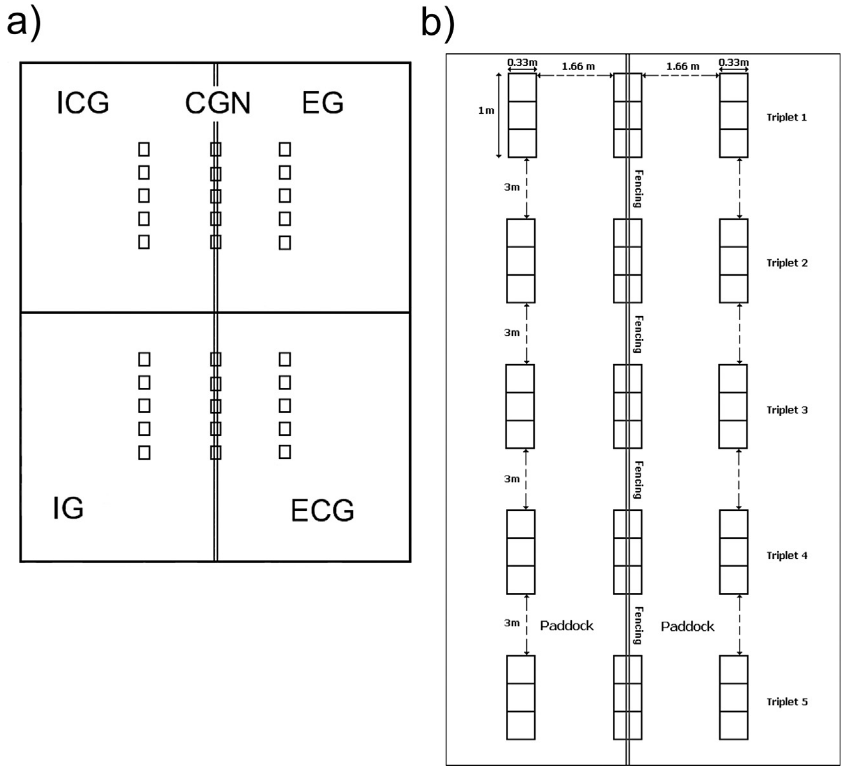

Fig. 1. Design of one experimental block (a) and spatial arrangement of study plots (b). Study plots (0.33 m × 1.00 m) were divided into three 0.33 m × 0.33 m subplots for monitoring. There were three study plots (one triplet) in one row. In total there were five triplets within each of four experimental paddocks (2 blocks × 4 paddocks × 5 triplets × 3 study plots × 3 subplots = 360 subplots in total). Abbreviations: CGN = intensive defoliation by grazing and cutting with no trampling; EG = extensive grazing with trampling; ECG = extensive grazing and cutting with trampling; IG = intensive grazing with trampling; ICG = intensive grazing and cutting with trampling.

where six measurements within each site were performed in October 2010. The mean of the six measurements were used for statistical analysis. This resulted in 300 penetrometer measurements in total (five treatments × two blocks × five sites × six measurements). Because penetrometer readings are dependent on soil moisture content (Vaz et al., 2001) we supplemented this field sampling with information on volumetric water content in soil. The absolute soil moisture u (%) was measured at 30 cm depth using an electronic humidity meter GMH 3850 (GREISINGER electronic GmbH, Germany) simultaneously with soil penetration resistance measurements in all sites in the same day. Mean absolute soil moisture u was 16.7% (S.E. 2.14) during these measurements.

2.4. Soil sampling

Soil cores (96 mm diameter) were taken at 0–15 cm depth from each subplot within the study data set plots (see below) in May 2013. One representative sample consisted of three separate soil samples from each study plot. The soil samples were air-dried, biomass residues and roots were removed and the sample was then ground in a mortar and sieved to 2 mm. All chemical analysis were performed in an accredited laboratory of the Crop Research Institute in Prague. Basic soil chemical analysis were performed using the Mehlich III extraction (Mehlich, 1984) to determine soil reaction (pH/$CaCl_2$), and the content of plant-available P, K, Mg and Ca. Total N was analysed by the Kjeldahl method (AOAC, 1984).

2.5. Sward measurements

Prior to grazing in May three relevés were collected in the study data set plots, as specified above, using a grid of 0.33 m × 0.33 m subplots in five distant triplets along the permanent fence (Fig. 1). The distance between plots within the triplet was 1.66 m, with a gap of 3 m between the next two triplets. This distance of 1.66 m from the permanent fences was chosen to avoid the effects of excessive trampling by heifers, because the animals prefer to walk up and down fences lines. The percentage canopy cover for all vascular species, bryophytes, bare ground, and feces was visually estimated up to more than 100% (Whalley and Hardy, 2000). The sward height of each subplot was measured after finishing all relevés collection at the beginning of June. Till this time the subplots were protected to grazing. The method of 'compressed sward height' (CSH) by rising plate meter was used (Castle, 1976; Correll et al., 2003). All vascular plant species present within the study were categorized according to their main traits based on mean height descriptions in the regional flora (Kubát et al., 2002). The functional groups were: tall graminoids (>50 cm), short graminoids (<50 cm), tall forbs (>50 cm), short forbs (<50 cm), legumes and bryophytes (Table 1). To show the effect of the absence of trampling on the species richness of the community, the mean number of species, the Hill's N_1 diversity index and evenness index, expressed as Hill's ratio (Hill, 1973), were calculated for each treatment.

2.6. Data analysis

The mean of three subplots (of all study parameters) for one study plot was used for statistical evaluation. To obtain normal distribution Hill's evenness index were arcsine transformed and total percentage cover of vascular plant species and bryophytes were log-transformed ($y' = \log_{10}(y+1)$) before data analysis. One-way ANOVA followed by *post-hoc* Tukey comparison was

Table 1
Functional groups of the study sward.

Short graminoids	Tall graminoids	Short forbs	Tall forbs	Legumes
Agrostis capillaris	*Alopecurus pratensis*	*Alchemilla* sp.	*Aegopodium podagraria*	*Lathyrus pratensis*
Anthoxantum odoratum	*Dactylis glomerata*	*Campanula patula*	*Achillea millefolium*	*Lotus uliginosus*
Luzula campestris	*Deschampsia caespitosa*	*Cardamine pratensis*	*Cirsium palustre*	*Trifolium pratense*
Poa annua	*Elytrigia repens*	*Cerastium holosteoides*	*Galium album*	*Trifolium repens*
	Festuca pratensis	*Galium uliginosum*	*Heracleum sphondylium*	*Vicia cracca*
	Festuca rubra agg.	*Glechoma hederacea*	*Hypericum maculatum*	*Vicia sepium*
	Holcus lanatus	*Leontodon autumnalis*	*Ranunculus acris*	
	Holcus mollis	*Lychnis flos-cuculi*	*Rumex acetosa*	
	Poa pratensis	*Plantago lanceolata*	*Rumex obtusifolius*	
	Poa trivialis	*Plantago major*	*Urtica dioica*	
	Trisetum flavescens	*Ranunculus repens*		
		Stellaria graminea		
		Stellaria media		
		Taraxacum spp.		
		Veronica arvensis		
		Veronica chamaedrys		
		Veronica serpyllifolia		

performed to identify significant differences in the variability of soil chemical properties, soil penetration resistance, sward heights, species richness, Hill's diversity and evenness indices. Pearson correlation analysis was performed for testing relationship between cover of vascular plant species and bryophytes. Statistica 10.0 was used to perform all univariate statistical procedures (StatSoft, 2002). To reveal the effects of grazing intensity and trampling on plant species composition a redundancy analysis (RDA) followed by a Monte Carlo permutation test in the CANOCO program (ter Braak and Šmilauer, 2002) was used. Each analysis was performed with 999 permutations. Species data were log-transformed ($y' = \log_{10}(y+1)$). The blocks were treated as covariables. Ordination diagrams constructed in the CanoDraw program (ter Braak and Šmilauer, 2002) were used to visualize the results of the analysis. Bryophytes, bare ground, feces and dead biomass were not included as active response variables in the RDA. They were displayed as passive response variables in the CanoDraw program.

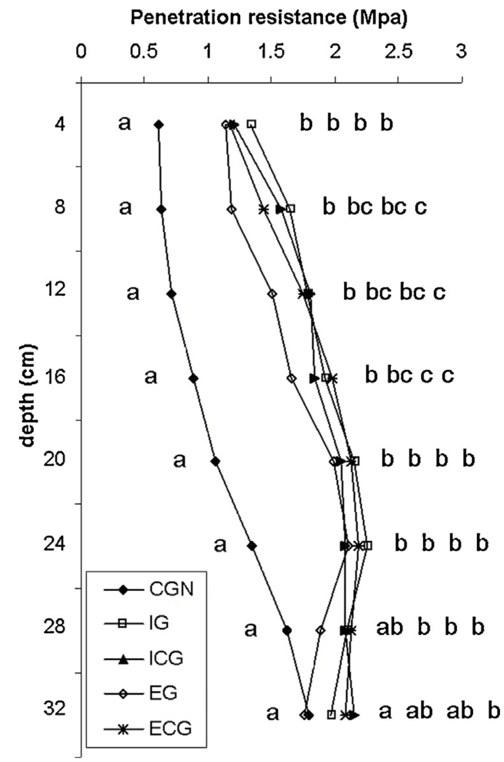

Fig. 2. Soil penetration resistance measurements. The results of soil penetration resistance (MPa) measurements among treatments from depths of 4–32 cm. Significant differences ($P < 0.05$) according to the Tukey's *post-hoc* test are indicated by different letters in the soil layers. For treatment abbreviations see Fig. 1.

3. Results

3.1. Soil penetration resistance

Soil penetration resistance showed significant differences among investigated treatments (Fig. 2). The subsurface layer of the soil under trampled treatments had significantly higher penetration resistance up to c. 30 cm in comparison with the CGN treatment. The highest penetration resistance was apparent at a depth of 24 cm (2.25, 2.08, 2.10 and 2.2 MPa) in all trampled treatments (IG, ICG, EG and ECG respectively) and at a depth of 32 cm (1.79 MPa) in the CGN treatment.

3.2. Soil chemical properties

Except for pH ($CaCl_2$), plant available concentrations of P, K, Ca and Mg and also N in soil were affected by treatments (Table 2). In the CGN treatment, P and Ca concentration in the soil did not differ from

Table 2
Results of basic soil chemical analysis and ANOVA analysis of treatments. pH, content of total N and plant available (Mehlich III) concentrations of P, K, Ca and Mg in 0–10 cm layer. Numbers represent average values of treatments, ±values represent standard error of the mean (SE), degrees of freedom in all analysis = 4. Significant differences ($P < 0.05$) between treatments according to the Tukey's *post-hoc* test are indicated by different letters in column. Abbreviations: Treat = Treatment; for treatment abbreviations see Fig. 1; d*f* represents degree of freedom, *F*-ratio represents a value derived from *F* test in repeated measurements and *P*-value represents related probability value.

Treat	pH/$CaCl_2$	N mg kg^{-1}	P mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	Ca mg kg^{-1}
CGN	5.2 ± 0.2	0.34 ± 0.06bc	41 ± 10.2ab	94 ± 29.0c	122 ± 65.9b	1779 ± 439ab
IG	5.3 ± 0.7	0.34 ± 0.03bc	45 ± 21.6ab	228 ± 204.6ab	158 ± 52.4ab	1367 ± 204b
ICG	5.2 ± 0.3	0.39 ± 0.03a	35 ± 11.5b	104 ± 25.8c	135 ± 54.8ab	1720 ± 508ab
EG	5.4 ± 0.2	0.39 ± 0.03ab	56 ± 16.5a	259 ± 101.4a	198 ± 68.6a	2081 ± 357a
ECG	5.1 ± 0.2	0.32 ± 0.03c	38 ± 16.9ab	116 ± 52.7bc	103 ± 38.2b	1466 ± 303b
F-ratio	1.21	6.41	2.90	7.35	4.15	5.34
P-value	0.316	<0.001	0.029	<0.001	0.005	0.001

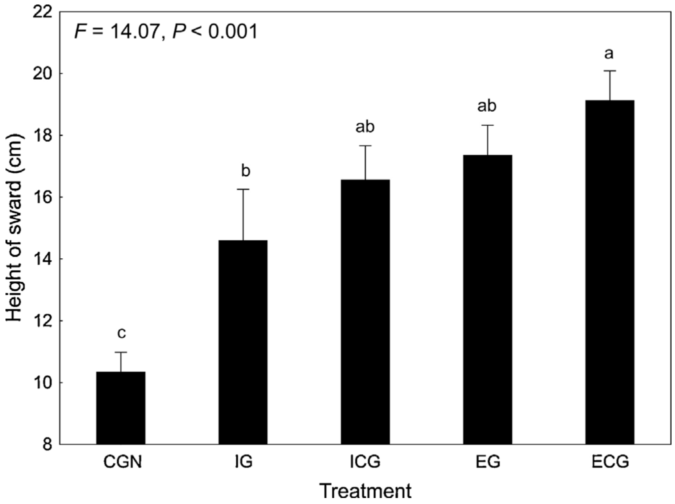

Fig. 3. The mean sward height (cm) in each treatment F is a value derived from F statistics in one-way ANOVA, and P is the probability value. Significant differences ($P < 0.05$) according to Tukey's *post-hoc* test are indicated by different letters. Error bars represent standard errors of the mean. For treatment abbreviations see Fig. 1.

other treatments. In the case of plant available K and Mg, CGN treatment did not differ from ICG and ECG treatments. The highest content of plant available K and Mg was revealed under EG treatment. The lowest N total content in the soil was in ECG treatment.

3.3. Sward height

Measurements of the actual mean sward heights, tested by one-way ANOVA, showed significant differences between the treatments. The shortest sward height was recorded in the treatment with an absence of trampling (CGN), then in IG treatment, whereas the highest sward was recorded in the ECG treatment (Fig. 3).

3.4. Species richness of vascular plants

Significant differences in the number of vascular plant species were detected between treatments. In the CGN treatment, there was a significantly higher number of species compared to the trampled treatments (Fig. 4a). The Hill's N_1 diversity index and Hill's ratio N_1/N_0 (in brackets) for plant species richness and evenness index respectively were 7.02 (28.63), 7.48 (43.32), 8.08 (44.50), 8.26 (53.62) and 8.96 (48.13) for CGN, IG, ICG, EG and ECG treatments, respectively. The differences among the treatments were not significant for N_1 ($F = 2.23$, $P = 0.078$) but were significant for N_1/N_0 ($F = 17.01$, $P < 0.001$), see Fig. 4b.

3.5. Plant functional groups

A significant effect of treatment on the cover of short graminoids, tall graminoids, short forbs, tall forbs, legumes and bryophytes was found (see Table 3, Analysis A1 for details).

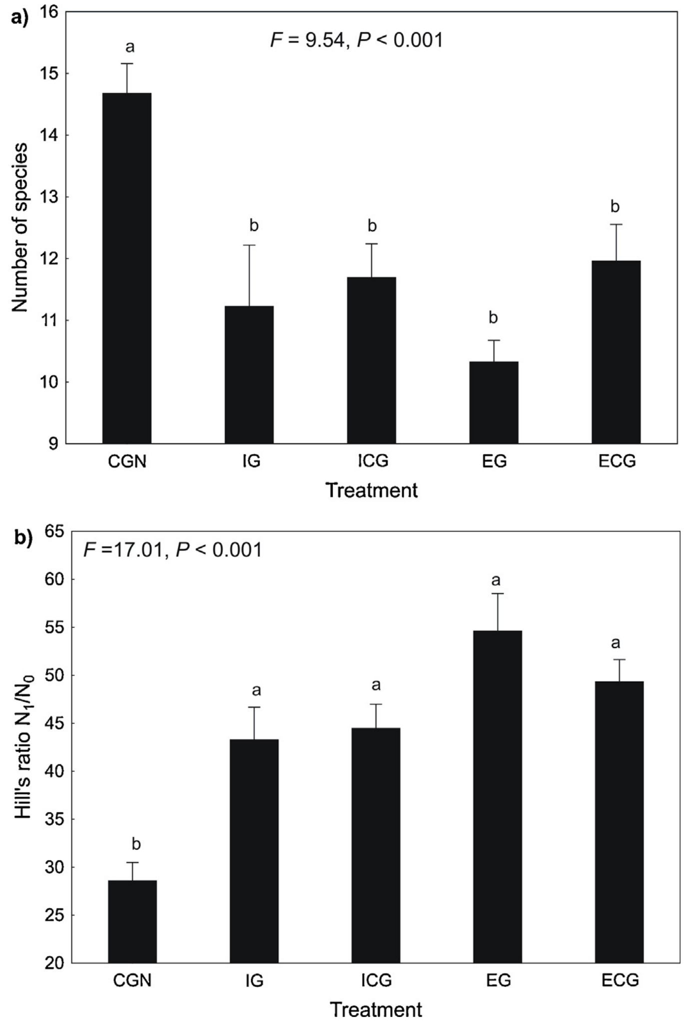

Fig. 4. Species richness. (a) The mean number of vascular plant species in each treatment, (b) Hill's ratio N_1/N_0 showing evenness of the plant species community. F is a value derived from F statistics in one-way ANOVA, and P is the probability value. Significant differences ($P < 0.05$) according to Tukey's *post-hoc* test are indicated by different letters. Error bars represent standard errors of the mean. For treatment abbreviations see Fig. 1.

Graminoids (regardless of height classification) profited from the presence of cattle trampling (Fig. 5a) whereas bryophytes became dominant with the CGN treatment. Legumes were supported by intensively defoliated and trampled treatments (IG, ICG), whereas tall forbs responded in the opposite way. Short forbs responded negatively to both extensive treatments (EG, ECG).

3.6. Plant species composition

Negative correlation (Pearson's correlation coefficient = −0.59; P-value <0.001) was revealed between the percentage cover of bryophytes and vascular plants. The lowest ($P < 0.001$) cover (97, S.

Table 3
Results of the RDA analysis. % Expl. = explained by axis 1 and all ordination axes (in brackets) – measure of explanatory power of the explanatory variables; F-ratio = F-statistics for the test of particular analysis; P-value = corresponding probability value obtained by the Monte Carlo permutation test.

Analysis	Expl. var.	Covariables	% Expl.	*F*-ratio	*P*-value
A1: Different grazing regimes have no effect on the abundance of plant functional groups composition	Grazing regimes	Blocks	39.7 (50.6)	114.6 (44.9)	0.001 (0.001)
A2: Different grazing regimes have no effect on plant species composition	Grazing regimes	Blocks	16.8 (30.4)	5.2char_dot (19.0)	0.001 (0.001)

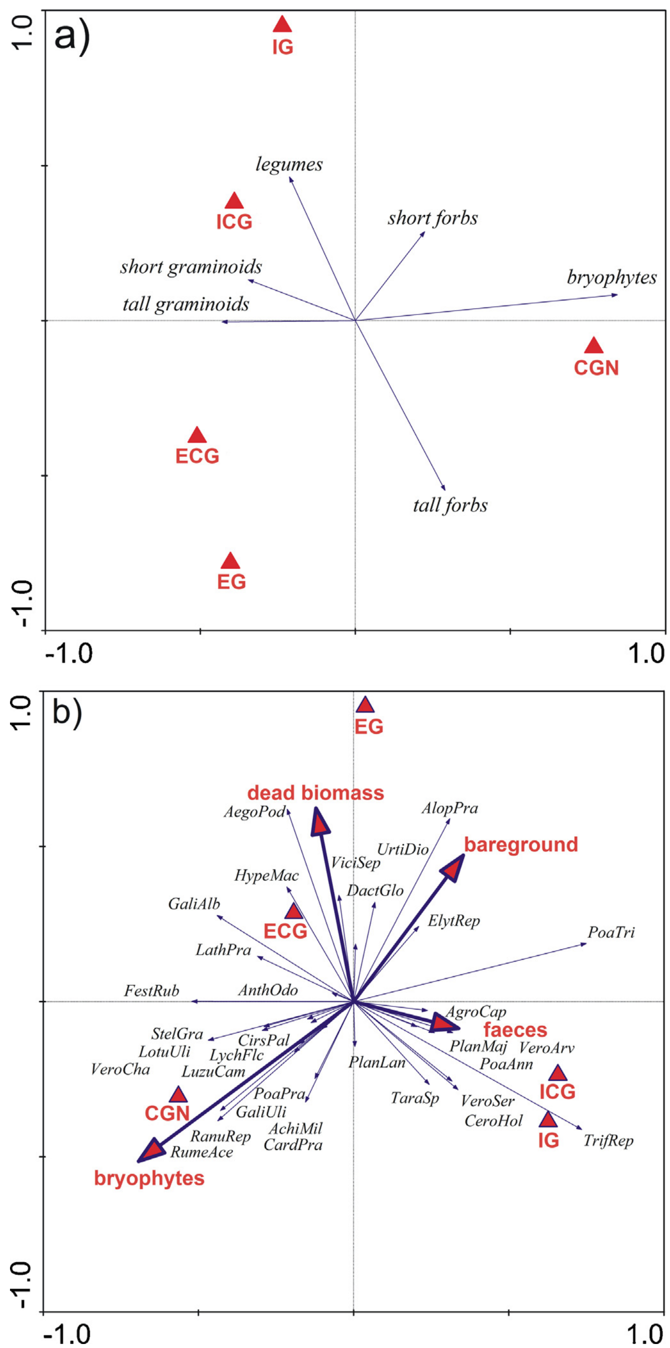

Fig. 5. Ordination diagrams showing the results of redundancy analysis of plant species composition data. Abbreviations: AchilMil = *Achillea millefolium*; AegoPod = *Aegopodium podagraria*; AgroCap = *Agrostis capillaris*; AlopPra = *Alopecurus pratensis*; AnthOdo = *Anthoxantum odoratum*; CardPra = *Cardamine pratensis*; CeraHol = *Cerastium holosteioides*; CirsPal = *Cirsium palustre*; DactGlo = *Dactylis glomerata*; ElytRep = *Elytrigia repens*; FestRub = *Festuca rubra*; GaliAlb = *Galium album*; GaliUli = *Galium uliginosum*; HypeMac = *Hypericum maculatum*; LathPra = *Lathyrus pratensis*; LotuUli = *Lotus uliginosus;* LuzuCam = *Luzula campestris*; LychFlc = *Lychnis flos-cuculi*; PlanLan = *Plantago lanceolata*; PlanMaj = *Plantago major*; PoaAnn = *Poa annua*; PoaPra = *Poa pratensis*; PoaTri = *Poa trivialis*; RanuRep = *Ranunculus repens;* RumeAce = *Rumex acetosa;* StelGra = *Stellaria graminea*; TaraSpp = *Taraxacum* spp.; TrifRep =*Trifolium repens*; UrtiDio = *Urtica dioica*; VeroArv = *Veronica arvensis*; VeroCha = *Veronica chamaedrys*; VeroSer = *Veronica serpyllifolia*; ViciSep = *Vicia sepium*. For treatment abbreviations see Fig. 1.

E. 4.8) of vascular plant species and significantly highest ($P < 0.001$) cover of bryophytes (75, S.E. 3.4) occurred in the CGN treatment, where more than 95% of bryophytes were represented by *Rhytidiadelphus squarrosus* (see Appendix A.2). In trampled treatments were cover of vascular plant species 130 (S.E. 5.1), 140 (S.E. 2.6), 157 (S.E. 6.2), 130 (S.E. 3.8) and cover of bryophytes 11 (S.E. 3.5), 3.8 (S.E. 1.5), 7 (S.E. 3.8), 0.2 (S.E. 0.1) under IG, ECG, ICG and EG treatment, respectively. According to *post-hoc* Tukey comparison there were no differences among all trampled treatments for cover of vascular plant species or bryophytes. In all of the trampled treatments the cover of *R. squarrosus* accounted for was less than 25% of the bryophyte species. The RDA showed significant differences in plant species composition among treatments (see Table 2, Analysis A2 for details). Species became associated with three groups according to defoliation by grazing and trampling intensities: extensive grazing and trampling (EG, ECG), intensive grazing and trampling (IG, ICG) and intensive defoliation by grazing and no trampling (CGN) (Fig. 5b). The ordination diagram (Fig. 5b) also identifies species dependent on trampling but independent of grazing intensity. Those species were *Agrostis capillaris*,*Poa trivialis* under intensive treatments (IG, ICG), and *Elytrigia repens* under extensive treatments (EG, ECG). The species that benefited from the absence of trampling (CGN treatment) were bryophytes and *Ranunculus repens*, *Rumex acetosa*, *Lychnis flos-cuculi*, *Luzula campestris*, *Galium uliginosum*, *Cirsium palustre, Cardamine pratensis* and *Poa pratensis*. In the IG and ICG treatments the species with the highest abundance was *Trifolium repens*. Other species which were supported by the intensive grazing treatments were *Cerastium holosteoides*, *Veronica serpyllifolia*, *Veronica arvensis*, *Taraxacum* spp., *Poa annua* and *Plantago major*. Also, the highest numbers of heifer feces were recorded in these two treatments. In the EG and ECG treatments the species with the highest abundance were *Aegopodium podagraria*, *Hypericum maculatum*, *Galium album*, *Dactylis glomerata*, *Vicia sepium*, *Urtica dioica, Anthoxantum odoratum* and *Alopecurus pratensis*. A large amount of dead above-ground biomass was accumulated in these two treatments.

4. Discussion

Although the lowest soil penetration resistance was recorded in the CGN treatment, a significant difference was also found between different grazing intensities with the result that lower penetration resistance was observed under extensive (EG, ECG) treatments in comparison to intensive (IG, ICG) treatments. The soil compaction expressed by penetration resistance reflected the intensity of trampling management. This is in accordance with the study by Novák (2009), where the penetration resistance was higher under higher stocking rates even in a short term experiment.

The chemical composition of soil under the fencing was very similar to pastures which were cut and grazed (ICG, ECG); in particular, we confirmed the decrease in K availability under the management regime with herbage removal by cutting revealed by several authors, e.g. (Koerselman et al., 1990; Schaffers et al., 1998; Alfaro et al., 2003, 2004; Hejcman et al., 2010a; Pavlů et al., 2013). Therefore, it seems that in our experiment dung and urine deposition did not significantly affect nutrient availability in the soil and thus it seems that they have not such an important role in resulting plant species composition on grazed grasslands, as similarly reported by Mikola et al. (2009). Probably permeable bedrock and soil type in our experiment was responsible for the leaching of nutrients from dung and urine deposition to groundwater.

The no trampled treatment significantly diverged from the others, not only in soil penetration resistance but also in all other studied characteristics: species richness of vascular plants, plant species composition, cover of bryophytes and sward height. Bryophytes were found to be a major component of the vegetation in the CGN treatment. They were observed to form 'a carpet' (especially *R. squarrosus*) with a sparse density of vascular plants. Similarly, Chappell et al. (1971) found that *R. squarrosus* was reduced with increasing trampling pressure on chalk grassland. In accordance with previous studies (Common et al., 1991; Virtanen et al., 2000; Bergamini et al., 2001; Hejcman et al., 2010b) there was a negative correlation between the abundance of bryophytes and vascular plants in grasslands. Although bryophytes are considered to be poor competitors with vascular plants (van Tooren et al., 1988), they can prevent seed germination or seedling survival (Kotorová and Lepš, 1999). In particular, if there is an absence of any bare ground disturbance, which would create germination gaps and low nutrient availability, then bryophytes can prevail over vascular plants. However, during grazing in the CGN treatment cattle could pull up bryophytes with their mouths together with vascular plants. Usually they spit these out and tufts of bryophytes are visible on pasture surfaces, especially in autumn (Vilém Pavlů, personal observation). This factor probably causes small disturbances, which can lead to the formation of germination gaps. The prevalence of bryophytes in the 'not trampled' treatment resulted in the lowest compressed sward height in this treatment. A previous study (Kobayashi et al., 1997), found that trampling itself significantly suppresses vegetation height, but the trampling in this study was not separated from grazing. Therefore these results are consistent only with our IG treatment, where vegetation was defoliated by grazing and trampled.

Although the CGN treatment was found to be richest in the number of vascular plant species, it had the lowest evenness index, especially because the bryophytes prevailed there and they were accompanied by lower cover and density of vascular plant species. On the contrary the lowest rate of bryophytes with regard to the abundance of vascular plants occurred under all grazed treatments where vegetation was defoliated and trampled. However, there was a tendency toward lower bryophyte cover under the ECG and ICG treatments, where the biomass of vascular plants was removed by regular harvests at the end of spring. As the bryophytes achieve their highest growth and abundance from autumn to spring (van Tooren et al., 1988) the absence of vegetation removal in spring appeared to limit light availability, as similarly reported by Al-Mufti et al. (1977). The animal hoofs compacted and disturbed soil surface in all trampled treatments which dramatically reduced bryophytes presence. It was particularly visible when we compare ICG and CGN treatments only, which consistently differed in trampling presence/absence. Therefore we can argue that absence of trampling in CGN treatment is responsible for the high cover of bryophytes.

There were no species closely connected with the CGN treatment which did not occur in the other treatments. The occurrence of other vascular plant species seems to be random and the community response might be dependent on the presence of the surrounding species pool. Species with the highest abundance in the CGN treatment were semi-rosettes which distribution is centered on plant communities in which competition is limited by moderate impacts of disturbance, and which are mostly absent from heavily disturbed habitats (Grime et al., 1988). Species like *Luzula campestris* or *Lychis flos-cuculi* found in the CGN treatment are species which are usually present on meadows rather than in pastures, and are better adapted to vegetation removal solely than in combination with other type of disturbances such as trampling or deposition of feces. *T. repens* (the dominant legume species) had the highest cover, especially in the IG and ICG. From previous studies performed in comparable climatic and soil conditions it can be concluded that *T. repens* is restricted to intensively grazed swards with good light conditions (Ter Heerdt et al., 1991; Adler et al., 2001; Correll et al., 2003; Hejcman et al., 2010c; Pavlů et al., 2011). From our study it appears that not only an intensive defoliation, which ensures suitable light conditions, is important for the prevalence of *T. repens*, but also the disturbances associated with trampling. Kohler et al. (2004) and Kobayashi et al. (1997) also acknowledged that the abundance of legumes (especially *T. repens*) was related to trampling. Furthermore, short graminoids (including dominant *A. capillaris*) were negatively correlated with the CGN treatment. In general *A. capillaris* is described as a species promoted by regular defoliation in low-production temperate grasslands (Hellström et al., 2003; Louault et al., 2005; Pavlů et al., 2007; Mašková et al., 2009; Gaisler et al., 2013). However, the present study demonstrates that only the interaction of defoliation with trampling promotes the abundance of such species. This means that not only defoliation by itself, but also the disturbance associated with trampling is one of the key factors responsible for supporting typical pasture species such as *T. repens* and *A. capillaris*.

5. Conclusions

In conclusion, long-term defoliation by grazing animals without trampling does not lead to the creation of typical pasture communities of temperate grasslands (Chytrý, 2007). In our study we demonstrated that the species present in pasture swards do not profit only from regular defoliation resulting in good light conditions for remaining species. They also need bare ground disturbances and soil compaction which favor them in strong intraspecific competition with other plant species. Species forming pasture communities are essentially dependent on two types of disturbances (i) regular defoliation by grazing and (ii) regular trampling by hooves.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2014.07.017.

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