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The influence of long-term grassland management on sward parameters in a semi-natural grassland

(Ph.D. Dissertation Thesis)

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Prague 2021

Declaration of originality

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In Prague, October 2021 ----------------------------------

Acknowledgement

PhD study was by far the hardest challenges I have taken so far. But I was not alone facing the numerous challenges. First and foremost, I praise the almighty God, who gave me strength to finish my study and my thesis.

My warmest gratitude to my supervisor (Vilém Pavlů) for seeing the potential in me when no one did, providing support and inspiration throughout the PhD study period. My gratitude extends also to his family and colleagues (Lenka Pavlů, Bara Pavlů and Klára Kajzrová) who have been kind and supportive.

Special thanks to my friends Friedrich ZurHeide and Christian Sefrin for their unreserved support and encouragement. I want also to thank my colleagues, Jan a Titěra, Chukwudi Nwaogu, Franta Paška, Honza Gaisler, Věra Ismanická, and Irena Jonášová.

Big love to Kassahun and Blen, thank you for your patience and willingness to listen to my frustrations, happy stories and strange experiences from the Czech Republic. I am also indebted to Svane for believing in me, supporting my work and being a light during the dark days.

I am indebted to my Czech family, Mr Zrnik, Eva, Pava and Veronika for their hospitality and familyhood. Without them, much of my research and stay in Czech Republic would have been difficult. And yoni my boy we did it !

Finally, I would like to thank the Internal Grant Agency – Faculty of Environmental Sciences, Czech University of Life Sciences Prague (Project No. 20194211) for financial support.

Abbreviations

ADF – Acid Detergent Fibre

AES – Agri-environmental Schemes

ANOVA – Analysis of Variance

CP – Crude Protein

DM – Dry Matter

DMSB – Dry Matter Standing Biomass

EG – Extensive Grazing

ECG – Extensive Cutting Grazing

GLM – Generalized Linear Model

IG – Intensive Grazing

ICG – Intensive Cutting Grazing

NDF – Neutral Detergent Fibre

SH – Sward Height

UAA – Utilized Agricultural Area

Contents

Summary

Semi-natural grassland especially those that are located in upland areas and less accessible are important part of European landscape. They have significant natural as well as cultural-historical significance, due to the influence of human. But following the 1990s post communism, several Central European countries (e.g. Czech Republic and Slovakia) with large areas of grassland in the upland areas were lost or converted to forest lands. These problems were further exacerbated with the declining livestock population that traditionally grazed on these lands. These lands are generally managed by grazing or mowing depending upon the site condition. And to keep these habitats open, maintain biodiversity in them and avoid abandonment management is needed. Therefore, the overall aim of this thesis was to use a long-term experiment data and investigate the effects of the different management on various sward parameters (biomass production, forage quality, nutrient in the soil etc.). More specifically, effects of different grazing intensities and cutting management, were investigated using data from existing long-term grazing experiment in Czech Republic (Oldrichov Grazing Experiment) and Slovakia (experiment located at National Park of Nízké Tatry) with the aim of using the results to solve existing grassland challenges and providing potential management and methodological recommendations.

Chapter 1 gives insight to temperate grasslands with a focus on semi-natural grasslands. It provides literature review on the different management strategies, challenges as well as the effect of management on sward parameters. **Chapter 2** investigated the effect of grazing and cutting management on plant functional groups at two different vertical layers. Analyses of the 15-year data revealed intensity of management to be the key driver affecting the vertical distribution of functional groups, while type of defoliation (grazing or cutting) had less effect. Furthermore, a high proportion of living biomass was found in contrast to lower layer which is filled will dead

and ungrazed biomass. This suggests for adjustment of our methodological approach especially when sampling for forage quality or productivity analysis.

Chapter 3 and **4** studied the effects of different management methods on the herbage and soil nutrient concentrations and other sward parameters. **Chapter 3** analyzed the effects of dung presence on the nutrient concentration of soil and herbage under sward height patches exposed to different grazing intensities. The analyses revealed intensity of grazing as the key driver for nutrient concentration (N, P, K) in the herbage while dung presence had no significant effect on soil nutrient concentrations. In contrast, **Chapter 4** analyzed the restoration measures of upland meadows infested with expansive weedy species. The result indicates cutting management and herbicide application coupled with cutting management had affected both the soil and herbage nutrient concentrations. But the excessive presence of nutrients in the soil meant more management that can remove the excess nutrient from the soil while also removing the weedy species is necessary.

Chapter 5 and **6** deals with the forage quality, biomass production and performance of heifers using a long-term experiment data from Oldrichov Grazing Experiment, Czech Republic. **Chapter 5** focused on finding optimum period for introducing management (first cut or early grazing) in order to meet cattle nutritional as well as mineral concentrations need. This analysis is especially essential for grasslands that are protected under agri-environment schemes. The 13-year data analysis revealed that up to the first seven weeks of the vegetation season the forage quality is suitable for cattle even as the only source of feed, but after that the forage quality is very low and it is only suitable for low productive cows and beef cattle. This suggests the need to maintain the agri-environment schemes to compensate for loss in forage quality while meeting the nature conservations aims. Similarly, **Chapter 6** revealed that extensive management applied for almost two decades can meet the cattle requirements without compromising heifer performance and at the same time contribute to landscape management of upland grasslands.

The published papers included in this thesis suggest different defoliation management (cutting/grazing) methods have different effect on the sward parameters of semi-natural grassland. One of the most important factors that influenced the sward parameters is grazing and its intensity. Compared to the traditional cutting management, grazing management seems to offer a higher biomass production, influences the nutrient cycle of the grassland via dung and urine return to the system as well as influencing the species composition of the grassland in the long run. In contrast the cutting management, played important role especially in upland areas that are typically neglected of management and under threat of encroachment by shrubs or dominance of weedy species. It especially helped to remove excess amount of nutrients from the soil as well as decrease the dominance of weedy species that are prevailing due to lack of any management Therefore, choosing the appropriate method for specific sites, must consider the previous management history of the site, the existing condition, the future plan or objective and the cost implication for management.

Chapter 1

1. General introduction

1.1 Grassland

Grasslands give a different meaning to different authors. It can refer to as a plant community which is opposite to forest, or to an ecosystem consisting of soil, domestic/wild animals, vegetation, and management. Others also define it as a plant community in which grasses are dominant and shrubs are rare and trees are not available at all. However, on a global scale, they are areas which are covered by grasses, which are used for livestock production or as game reserves, consisting of woody species. UNESCO-UNEP-FAO, (1979), defines grasslands as "a plant community in which woody species do not exceed 40% of the total cover".

After tropical forest, grasslands form the greatest terrestrial biome, in terms of biomass. They can be natural or manipulated (by a human). In terms of ecology, grasslands are considered as pure or areas free of wooded vegetation types controlled by several factors such as soil, climate, biotic factors, and topography. Natural grasslands are in general more common in areas where climatic conditions are either too cold or too dry for forests to occur. They are also quite common in areas that are burnt or in heavy textured soils. Man-made or manipulated grasslands are common in humid and sub-humid climates, because these areas do not have the necessary climatic conditions needed for grasslands to prevail naturally (Mannetje and Jones, 2012).

We find temperate grasslands in regions where the climatic conditions (mid-altitude) are favorable for dominant perennial grasses. The Eurasia, steppes covers 250 million ha of the plain extending from Hungary to Northeast China. These grasslands are important buffer zones between forest and deserts and can act as a frontier for expansion between the forest and desert depending on the dominant climatic conditions (Shinoda et al., 2011). In the context of European grasslands, they have a rich flora and can develop a very high small-scale species density compared to other community types. For example, the largest vascular plant species numbers are found at the smallest scale of a few square centimeters to one square meter in temperate grasslands. European grasslands are also famous for their richness in terms of genetic variability within plant species. They possess several threatened species and show diverse landscape patterns (Pärtel et al., 2005). In central Europe, the importance of grasslands is even bigger. In the past, they played a significant role especially in the mountain region where they are used as a source of fodder for ruminant animals, mostly for sheep.

In temperate regions of Europe, grasslands are a major component of the landscapes as they play a vital role in the economic activity for animal production. It represents the only crop that has a well-developed homeostatic mechanism and stable even without any additional input of energy (Rychnovska, 1993). Due to the large variation in soil condition, climate and history, we can easily distinguish grasslands across Europe, as permanent and temporary grasslands, as the latter includes some proportion of forage legumes. Both grassland types contribute differently to the proportion of utilized agricultural areas (UAA) in many parts of the temperate region (Fig 1) Although the percentage cover of permanent as well as temporary grassland is quite different in most of the temperate region, they play a vital role as an important component of the agricultural landscape (Huyghe, 2014).

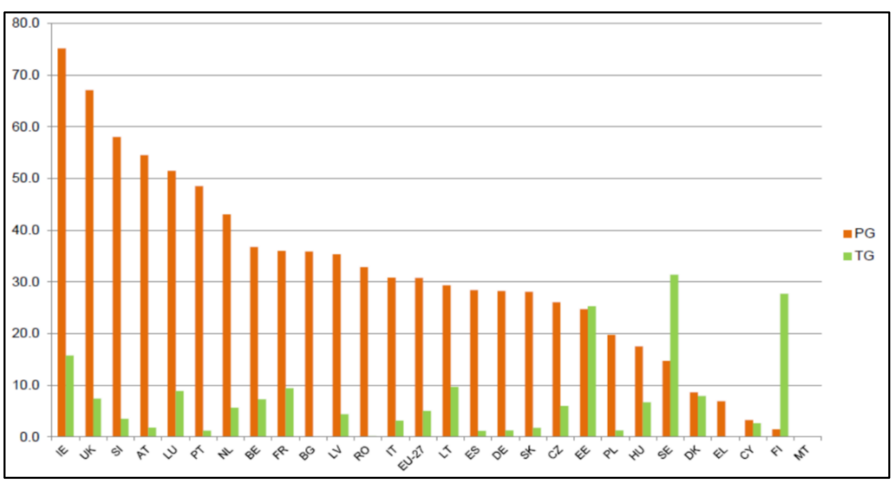


Figure 1: Share of permanent and temporary grassland in 2009, expressed as a percentage of Utilized Agricultural Area in the countries of the temperate region. (Source: Eurostat, 2009).

When it comes to the potential for biomass production temperate region performs better due to good soil quality and adequate climate conditions (Fig 2). There is, however, a slight difference from West to East gradient, with higher potential in the western part due to longer growing season because of oceanic climate. This difference could also be related to the species that are sown in temporary grasslands or just being naturally productive permanent grassland.

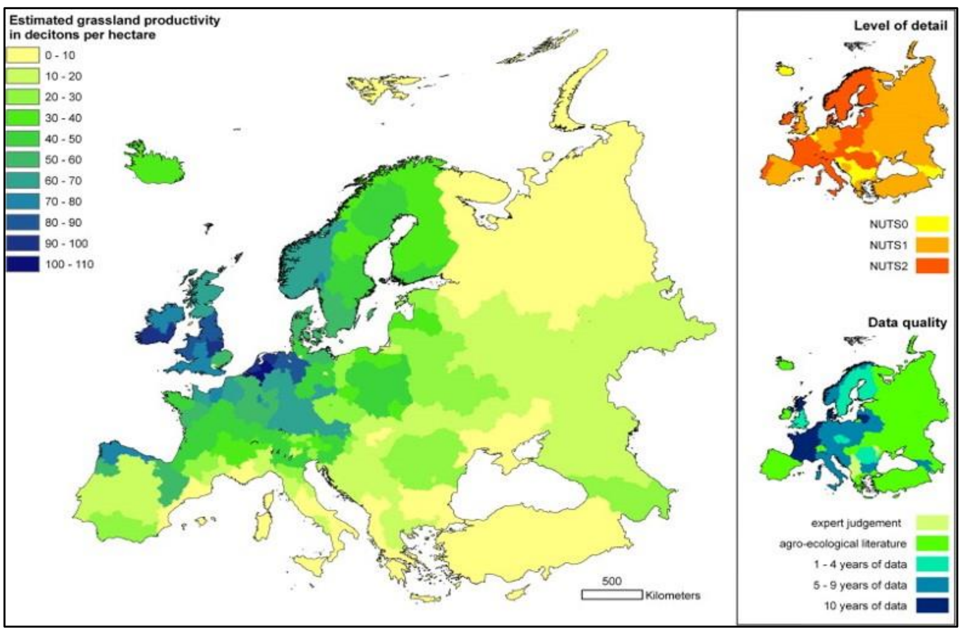


Figure 2: Spatial distribution of grassland productivity (dt ha^{-1}) in Europe. NUTS, Nomenclature of Territorial Units for Statistics (Source: Smit et al. 2008).

The highest productivity is achieved in the Atlantic North (Countries such as North Western Spain, Western France, Ireland, South Western part of Norway and Wales and England) were more than 10 t ha^{-1} is achieved. In addition, to favorable climatic conditions, high use of fertilizer in this part are also a major determinant for higher yield. The lowest productivity is located in the Mediterranean region, were annual yield is limited to only 1.5 t ha^{-1}. The tundra system is also another low productive zone. The countries in the Central Europe are comparatively high yield zones, with annual production between 4 to 6 t ha^{-1}. Overall the variation in productivity between years could be significantly different and this could be due to variability in climate (Smit et al., 2008).

The existence of temperate grasslands could be attributed to moderate disturbances such as grazing, mowing or fire incidences. Most of them are sub-climax communities, hence they require periodic defoliation to avoid succession that could lead to being converted into scrubs and woodlands (Rook et al., 2004). During the last millennia, temperate European grasslands have been largely managed by grazing of domestic animals or by hay making activities. This is one of the main reasons why this ecosystem is mostly described as semi-natural. It just implies the importance of grazing be it wild or domestic animals. In general, they are dependent on some kind of disturbance that inhibits dominance of woody plant species (Pärtel et al., 2005).

In central European condition, most of the grasslands we find do not represent climax communities as they were largely created after large-scale deforestation and maintained by agriculture activities. In general, grazing and mowing have been the most widely used management strategy for centuries, or even in some areas possibly up to Neolithic or Bronze age. These grasslands possess not only natural values, but also have huge cultural-historical value, as they have been under the influence of human for several generations (Jongepierová et al., 2012).

A review by Hejcman (2013) divides grasslands in central Europe, into three broad categories based on their origin:

(1) Natural grasslands: differentiated by the climatic condition like shortage of moisture which is common for a steppe region on the eastern border of Central Europe and low temperature with shorter growing season for higher mountains above the upper tree limit;

(2) Semi-natural grasslands: These grasslands are mostly linked to human interaction starting from the beginning of agriculture during the Mesolithic-Neolithic transition. They have also a wide range of species richness of vascular plants ranging from 1 to 67 species and herbage production from 1 to 10 ton dry matter. Semi-natural grasslands can also be further divided based on the management system they are in as pastures, meadows, and grazed meadows. Livestock grazing is the key management for pastures, regular cutting for meadows and cutting in spring and grazing in summer/autumn for grazed meadows;

(3) Intensive grasslands are the result of intensive agriculture, which includes sowing of highly productive forage grasses and legumes.

During the last 100 years, we have observed a significant decline of grassland areas across temperate regions of Europe. Humans have played a tremendous role in these changes. We have changed various land uses and grasslands have been one of the major expansion areas for arable land. Highly productive grasslands were converted to artificial pastures, arable land, and mixed farming. Although conversion of grasslands came more prominent in temperate grasslands before the 1950s, the conservation efforts dedicated for this biome compared to other biome is relatively small (Dixon et al., 2014).

The decline in grassland diversity and overall biological diversity has been ongoing for the last hundred years. Among several reasons changes on agricultural management such as intensive milk husbandry in cowsheds is top of the list leaving only a few portions of grassland to be used and the vast amount of them to be abandoned. The situation is much more serious in less accessible areas such as mountainous areas that have low productivity, were semi-natural grassland is common. Extensification in terms of avoiding or minimizing the intensive application of fertilizers as well as a change in the frequency and timing of defoliation can be beneficial. But in reality, it can be challenging as it can bring various risks due to the temporary or total abandonment of the grasslands. The absence of grassland defoliation leads to a decline in plant species diversity (Pavlů et al., 2005), and abundance of tall species as more litter on the ground promotes the nutrient availability and restricting seedling emergence (Hejcman et al., 2009). As more intensification of livestock production with larger and more specialized farm units continue to develop, the more the role of grasslands in livestock production diminishes (Kristensen et al., 2005). This trend probably will continue as an intensification of cattle production with highly digestible forages from arable lands and concentrates is applied (Isselstein et al., 2005; Pavlů, et al., 2007).

1.2 Grassland and livestock feed

The main function of grasslands is its role as a source of feed for ruminants. They provide forage for browsing and grazing animals, be it domestic or wild. We can define forage as any plant material that is provided to livestock as feed, excluding concentrates (Gibson, 2009). The value of grasslands can be determined based on the quantity of biomass it produces and the forage quality. Here forage quality could be defined as the potential of the feed to produce the intended response from the livestock, such as milk production or weight gain etc. One can have several criteria to describe the quality of forage: such as protein content, energy concentration, and digestibility. It is also worth to remember the quality and quantity of the forage normally changes during a season. The value of grassland may also be dependent on how it is used. Some farmers could decide to use their grassland by allowing their livestock to graze directly or the grass will be mowed and used as either hay or silage (Taugourdeau et al., 2016).

Several factors can be considered as a potential challenge that are affecting forage quality and ultimately livestock productivity. Based on a meta-analysis conducted by Dumont et al. (2015), climate change comes at the front as it can impact forage which also means livestock in two ways: (i) directly affecting the animals intake and digestion process and (ii) affecting the physical and chemical characteristics of the forage. The review was done based on existing knowledge on different forage quality parameters and how it is impacted by elevated CO2, increased temperature and drought. The review showed elevated CO2, decrease the forage N by 8% and increased the total non-structural carbohydrate of the forage tissue by 25%. Although there is high variability on water soluble carbohydrate, its content also showed an increase. Elevated CO2, was also able to affect the forage quality by changing the morphology or heading date of the species which is different to the effect of warming were advanced flowering time was observed. Although the review did not show any clear effect of warming on forage quality, another meta-analysis on experimental warming by Bai et al. (2013), indicated plant N content increasing due to warming as higher mineralization in warmer soil increases soil N availability. When it comes to drought effect, an average increase of 5% in forage N concentration and a 3.5% decline in plant cell was observed. Overall there is no clear effect of drought on digestibility, which may have been due to small amplitude Nitrogen and neutral detergent fiber. Nevertheless, the different studies which are trying to show the effect of drought on forage production and quality must consider the real management practices undertaken in grassland-based livestock farming. Because most drought studies are conducted on permanent pastures were cutting is the main method used to simulate grazing. Failing to consider the grazing aspect will distort our result or conclusion, as grazing affects the spatial structure of the vegetation via feeding preference and trampling (Kohler et al., 2005).

Phenology or maturity of plant species is one of the most important factors that influences the forage quality. As the age of the plant and its maturity increases within a growing season, the quality of the forage declines. This is basically observed by a decline in digestibility of plant component and a decrease of nitrogen content. This decline could be attributed to change in leaf/stem ratio and rise in fiber content (Bruinenberg et al., 2002). However, this difference is even more complex when we consider functional groups as they differ in their phonological development and their feeding value plus digestibility. When we compare grasses with legumes, the forage quality and digestibility of the later declines much slower than the former (Duru et al., 2008). As forage quality is mostly dependent on abiotic factors such as temperature, water availability and soil nutrient status, changes to this factors will directly impact the quality of forage (Andueza et al., 2010). For example, rising temperature is likely to increase the development of the plant, reduces the leaf/stem ratio and digestibility (Ansquer et al., 2009). The rise of temperature is more devastating in spring than in summer as a rise in spring will lead to faster plant maturity and faster decline in nutritive value. Overall climate change does not only affect forage quality directly by altering the abiotic factors that are crucial for plant growth and development, but also by affecting the plant composition (Kreyling et al., 2011). The composition of plant strongly affects the nutritive value of grasslands because of strong variation in species identities, chemical composition, functional groups and photosynthetic pathways. Nutritive value of grassland is more affected by species composition than species richness, although strong richness ensures good biomass yield (Baumont et al., 2008).

1.3 Defoliation managements

The breakup of state farms following political change in the 1990s in Central Europe had brought a tremendous change in grassland management. Traditional agriculture management practice has been the main method how majority of grasslands used to be managed, and the main practices were regular defoliation using grazing animals or hay making (Hejcman et al., 2013). However, in the last two decades, restoration of species-rich grassland has been gaining momentum. The techniques that are used mainly are reintroduction of grazing management, changing cutting frequency, mulching and even depletion of excess nutrient from soils. Of course, the management techniques introduced depend on the objective and target of the outcome. For instance, if the plan is to achieve a desirable grassland community, then regular cutting or grazing becomes vital, although cutting is more preferred if the objective is maintaining high species diversity (Hansson and Fogelfors., 2000). Others could follow a more traditional management that was applied for a generation like mulching. This management was introduced in the Czech Republic at the beginning of the 90s (Gaisler et al., 2013). It has been recommended as an alternative method for management of species-rich grasslands and also as a substitute for cutting without significant loss of plant species richness and diversity (Gaisler et al., 2013). However, the absence of any defoliation can lead to a decrease in plant species diversity (Pavlů et al., 2005; Pykälä, 2004).

1.3.1 Grazing management

When we are referring to management of temperate grasslands, we should not forget the roles played by grazing animals. In sward management, we can divide grazing methods in two broad categories: continuous and rotational grazing. The main difference between them is capital cost, labor needed to operate, easiness of operation, degree of control of the stock and interaction between stock and sward. Under continuous grazing we let the animals to graze the area for the whole grazing season. Nevertheless, in rotational grazing the area is divided in to paddocks that will be grazed in sequences, giving each paddock a rest period. In the Czech Republic, the main pasture management before 1989 was rotational grazing. However, in 1980s, due to the decline in capital cost continuous stocking was introduced (Pavlů et al., 2003). Grazing is very important in temperate grasslands especially to control succession to scrubs or woodlands. We can still have these defoliations in places that are not conducive for livestock such as steep slopes or uneven grounds, using mechanical harvesting equipment. This has been clearly demonstrated in hay meadows that have evolved to such management.

Grazing is vital to maintain and enhance structural heterogeneity of the sward canopy, which can also influence floral and faunal diversity (Rook and Tallowin, 2003). It also helps to create heterogeneous sward structure with heterogeneous height, which in turn affects the floristic composition and heterogeneity of species in grasslands (Sasaki et al., 2005). The selective defoliation, which is mainly due to dietary choice, is one of the main mechanism in which grazing animals create sward heterogeneity. Firstly, it changes the competitive advantage between species plant species due to direct removal of plant biomass (Bullock and Marriot, 2000), secondly, it opens up spaces which will be colonized by gap colonizing species and thirdly, the nutrient cycling which occurs through dung and urine (Rook et al., 2004). It is well documented that grasslands communities in Europe depend on several kinds of physical disturbances that inhibit shrub and tree. Evidences show grassland management by livestock grazing at moderate level can help to maintain species diversity by suppressing the abundance of competitive species. The disturbance in the soil and the sward structure is also important as it enables species establishment through niches (Klimek et al., 2007).

In temperate grasslands grazing intensity and animal preference have an influence on the floristic composition and heterogeneity of vegetation resulting in the patchy structure of swards. This so-called patch grazing (Adler et al., 2001) ultimately results in tall and short patches, which also creates difference in quality of biomass since ungrazed patches tend to be more mature and therefore difficult to digest than that of short frequently grazed patches. Hence, Cattle graze shorter patches compared to taller patches that are mostly left ungrazed. This trend of selective grazing gets stronger over the course of the grazing season (Ludvíková et al., 2015). Under this system, the amount of neglected patches is dominant due to excess supply of forage availability than herbivores demand. Therefore, the effect of patch grazing in this case is low pasture productivity per hectare compared to intensive or high stocking rates. Though pasture productivity is low, individual animal live weight gains can be as high as those found under heavily stocked grazing systems. This is possible because the available short patches allow livestock to graze high quality forage regardless of the average quality in the pasture or paddock (Isselstein et al., 2007; Dumont et al., 2007).

Since recently, extensive grazing is also being recommended more and more especially for management of semi-natural hay meadows and pastures, as they create and maintain sward structure heterogeneity, which is attractive outcome for nature conservation. It is also characterized by strong variable sward height and species composition. Under extensive grazing, patches that are neglected by herbivores are quite a lot, as the amount of forage available for the herbivores is higher than their demand, hence these non-grazed patches can increase total species diversity (Pavlů et al., 2006a). Selective grazing also leads to uneven distribution of grazing pressure both within and between plant communities. For a country like the Czech Republic, were continuous decline in livestock number and an area with more than 30% is unmanaged meadows and pasture, grazing becomes very crucial (Pavlů et al., 2006b).

Grazing animals also affect the nutrient content of the soil. By grazing and removing vegetation from the grasslands, they remove nutrients. At the same time, high amount of nutrient is returned via dung and urine deposition. A cow produces roughly 15 dung pats per day with each pat covering an area of around 0.5 m^2 (Marsh and Campling, 1970). Based on several factors such as water content, climatic conditions and soil fauna, it can take few weeks to several years to completely decompose a cattle dung pat (Marsh and Campling, 1970; Dicknson and craig, 1990). According to Pavlů et al. (2019) the amount of nutrients supplied from dung on an individual patch are 40–60 g N/m^2, 14–20 g P/m^2, 16–25 g K/m^2, 40–60 g Ca/m^2 and 10–14 g Mg/m^2. Hence, dung deposition has a significant effect on the chemical status of the soil and thus presents a potential source of available nutrients for plants (Shepherd et al., 2000; Aarons et al., 2004). Similarly, urine is another source of nutrient especially N, which occurs primarily as a hydrolyzed urea, and is easily plant-available after deposition (Haynes et al., 1993) and enables increased plant biomass N uptake and biomass productivity (Decau et al., 2003; Marsden et al., 2016). Of course, the joint effect of dung and urine deposition has an effect on the behavior of grazing animals, creating ungrazed areas or patches around the dung or urine. The combined effect of dung/urine deposition coupled with the avoidance of contaminated areas by the grazers and the nutrient enrichment will have a direct effect on the sward structure and dynamics (Gillet et al., 2010).

1.3.2 Cutting

Abandonment and intensive farming have been a major threat for semi-natural grasslands, although they are high conservation value because of high species richness (Soussana and Duru, 2007). When we include the continuous decline of livestock population across Europe, the situation becomes more challenging. Hence, finding alternative management that could replace grazing is necessary to avoid degradation and loss of diversity. Cutting is one of the methods that is effective as grazing in maintaining species diversity in grasslands (Hansson and Fogelfors, 2000).

Of course, several studies are conducted comparing different effects of grazing versus cutting on herbage production. For example, a study by Binnie and Chestnutt, (1991) observed a higher herbage yield under cutting compared to grazing management. In the contrary, Creighton et al. (2012) observed no difference in herbage production under the cutting and grazing. These contrasting differences could be attributed to different effects of the treatments on plant species, which ultimately affects the sward structure. Nevertheless, cutting has been actively promoted for restoring the declining species richness of semi-natural grasslands. In general, there is a negative correlation between species richness of semi-natural grassland and high content of soil nutrient, which is also related to biomass productivity (Hejcman et al., 2007). Hence, one way of restoring these grasslands is by decreasing grassland productivity, and one way of achieving this is by imposing long-term cutting management with biomass and nutrient removal (Niinemets and Kull, 2005).

1.3.3 Mulching

Among the different defoliation techniques mulching has been largely used as an alternative or low cost method in Czech Republic since the 1990s (Gaisler et al., 2013) to maintain grasslands without agricultural utilization. Mulching is a method were swards are cut into smaller pieces, and spread all over the site for decomposition to take place which helps to release the mineral nutrient content of the sward (Gaisler et al., 2004). This method has been used in other ecosystems such as vineyards and other agriculture crops for suppression of weeds and to improve soil and water conservation (Doležal et al., 2011). It is also considered as one of the least expensive method to apply. For agriculturally maintained grasslands that are considered as valuable for biodiversity alternative method such as mulching could be considered, as it is economically viable and maintain biodiversity.

Several studies have documented the effects of different defoliation treatments such as cutting and grazing, but little attention has been given to the effect of mulching. The little available information indicates species richness and composition to be significantly affected by mulching treatment (Gaisler et. al., 2004). In separate study Gaisler et al. (2013) reported that mulching could be a good substitute for cutting management without seriously compromising the species richness and diversity. With regards to impact on biomass production results are not straightforward. For example, Mašková et al. (2009) reported biomass production under long-term management mulching to be somehow intermediate between cutting and abandonment. Although management regimes and soil chemical properties are key factors that influence biomass production, conditions at experiment site and vegetation type could also play a vital role (Römermann et al., 2009). Analysis of long-term experiment data from Jizerské Hory Mountains found no significant effect on nutrient concentration of soil as well as herbage with different mulching regimes. Nevertheless, for temperate grassland in Central Europe, with increasing unutilized agricultural lands, mulching with two or three times per year could be the best option to maintain ecosystem functions and replace the conventional agriculture cutting regime (Pavlů et al., 2011).

1.4 Vertical and horizontal sward structure

Defoliation process are mainly influenced by sward structure (Coleman, 1992; Ungar, 1996) through the spatial distribution of different forage species (Tainton et al., 1996), the sward height (Armstrong, et al., 1995) and the density of leaves (Flores et al., 1993). However, several questions could be raised about sward structure; such as what is sward structure and why we need to know about it. Sward structure is normally defined and measured as "the distribution and arrangement of above ground plant parts within the community". We try to measure sward structure in order to understand and provide a reasoning to several topics like growth rate, light interception by canopies, forage quality and intake rate by herbivores. Furthermore, sward structure measurements are critical factor in determining primary and secondary productivity in grazed ecosystem. We must also consider both vertical as well as horizontal patterns when we study sward structure. This is mainly because herbivores select forage vertically and horizontally from bite to landscape scale (Laca and Lemaire, 2000). Furthermore, both vertical and horizontal distribution of vegetation are essential to understand plant-animal interactions especially in grazed plant communities (Marriot and Carrere, 1998).

Grasslands (natural as well as semi-natural) main role for millennia has been producing fodder for animals (Emanuelsson, 2009). In homogeneous swards where no horizontal or vertical selection by grazing animal occur, bite dimensions results from the interaction of sward height, stiffness of plant unit and grazing behavior of the animal (Laca and Lemaire, 2000). A study by Kassahun et al. (2021) showed the upper layer of the sward, which is typically grazed and considered as highly digestible material, has high proportion of live biomass, whereas the lower sward layer is largely representative of dead biomass that is mainly avoided by grazers. Hence, the nutritive value of herbage ingested by grazers considerably varies vertically (Fig 4) with in a sward (Barrett, 2000). According to Delagarde et al. (2000), the chemical composition of herbage changes vertically due to increase in organic matter and with increasing depth in sward a decrease in organic matter digestibility (Johnston et al., 1993).

Horizontal patterns in the sward strongly influences herbivores in their forage selection as well as competition among plants. It is well documented that grasslands are heterogeneous spatially due to resources patchiness and plants characteristics differ within these patches. Given the same herbage biomass as well as species composition, grasslands will still differ broadly in horizontal spatial structure (Laca and Lemaire, 2000). Defoliation such as grazing intensity coupled with preference of animals (Pettit et al., 1995; Sasaki et al., 2005) are largely responsible for floristic composition and vegetation heterogeneity in temperate grasslands. For instance, taller patches are less favored by cattle compared to short patches, because shorter patches tend to have higher quality biomass than taller patches (Dumont et al., 1995; Correll et al., 2003).

2. Objectives and research questions

Semi-natural grasslands, especially which are low productive and species rich are found in upland and mountain areas of temperate regions. In Central Europe, they are often part of protected areas that need special management for protecting diversity of flora and fauna. Typically, they are managed by grazing, cutting and sometimes by mulching. This PhD research assess the response of different sward parameters under contrasting management. Based on existing long-term data and field experiments, this PhD research explores the effect of different defoliation management (especially grazing and cutting) strategies and its effect on herbage production, as well as the nutrient concentration in the soil and herbage. In more detail the aim was to find an answer to the following practical questions that arise in semi-natural grassland management:

1. How does long-term grazing and cutting management affect plant functional groups found at two different vertical layers?
2. In what way is nutrient concentration of soil and herbage under sward height patches exposed to different grazing intensities are affected by the presence of dung?
3. Does Cutting and herbicide application coupled with cutting affect biomass productivity as well as nutrient concertation in soil and herbage of grassland covered with invasive weedy species?
4. When is the appropriate period to introduce management (grazing or cutting) in order to meet cattle nutritional and mineral requirements in a semi-natural grassland?
5. What is the effect of intensive and extensive grazing on biomass production and heifers' performance?

3. Study area

Site 1

Four of the case studies data for this thesis were collected in Jizera Mountains in the northern part of the Czech Republic, 10 km north from the township of Liberec (50°50'

N, 15°06' E) in Oldrichov v Hájích village (Fig 3). The first record about the village was in 1651 when identification for agricultural areas was conducted. Then four years later another record shows a census on livestock population that was used as a reference to establish tax payment system. In 1651 the total agricultural area was roughly 150 ha, but continued to increase and became more than 400 ha during the 18th and first half of 20th century (Hejcman et al., 2013).

Currently there is a site with ongoing long-term grazing experiment established in 1998 (Oldrichov Grazing Experiment) and managed by the Crop Research Institute Liberec. The experiment site is underlain by granite bedrock and medium deep brown soil (cambisol) with the following attributes: pH/KCl = 5.1, available P content = 64 $mg.kg^{-1}$, available K content = 95 $mg.kg^{-1}$ and available Mg content = 92 $mg.kg^{-1}$. The altitude is 420 m a.s.l., the average annual precipitation is 803 mm and the mean annual temperature is 7.2 °C (Liberec meteorological station).

Highly productive grass/clover was reseeded after the experiment site was drained and ploughed in the 1980s, followed by intensive management using cutting and grazing. At the beginning of the 1990s mulching was applied around august and then the grassland was abandoned once again. Until 1998, there was no agricultural management in this experiment site. Before the start of the experiment, the site was classified as upland hay meadows. The dominant species of the unmanaged sward were *Agrostis capillaris, Alopecurus pratensis, Festuca rubra* agg., *Aegopodium podagraria* and *Galium album*. No fertilizer has been applied since the 1980s.

Site 2

In 2004, a randomized block experiment was set up at 1140 m a.s.l. in the National Park of Nízké Tatry, Slovakia. At the study site, the mean annual precipitation and temperature were 800 mm and 8°C respectively. The snow cover, which is higher than 10 mm, is 160 days per year. The soil type is classified as cambisol, and as the depth of the soil increases the lower the proportion of clay and silt fraction and the higher the proportion of sand fraction. The most dominant species recorded in the experiment plots were *U. dioica*, and *R. obtusifolius*. The total cover (%) of forbs, grasses, legumes and the mean value of the most abundant species in the experiment site under each treatment for the year 2004 (start of the experiment) and 2011 (end of the experiment) are shown in Table 1.

The experimental site was previously used for grazing and then for herding of heifers. However, during the last decade before 2004, it was abandoned without any grazing or cutting management. The experiment was arranged in three randomized blocks each with the following treatments: (i) Unmanaged (U), (ii) Cutting twice per year (2C), and (iii) Herbicide application and, after three weeks, it was reseeded with a grass mixture of 18 species (list of species see Table 2) and subsequently cut twice per year (2CH). Glyphosate (active substance– IPA 480 g.l.; Roundup; Monsanto) herbicide was applied on to the leaves of plants at 3 l ha^{-1} (0.30 ml agent + 20 ml water on 1 m^2) with a sprayer in the spring of 2004. The area of individual plots was 15 m^2.

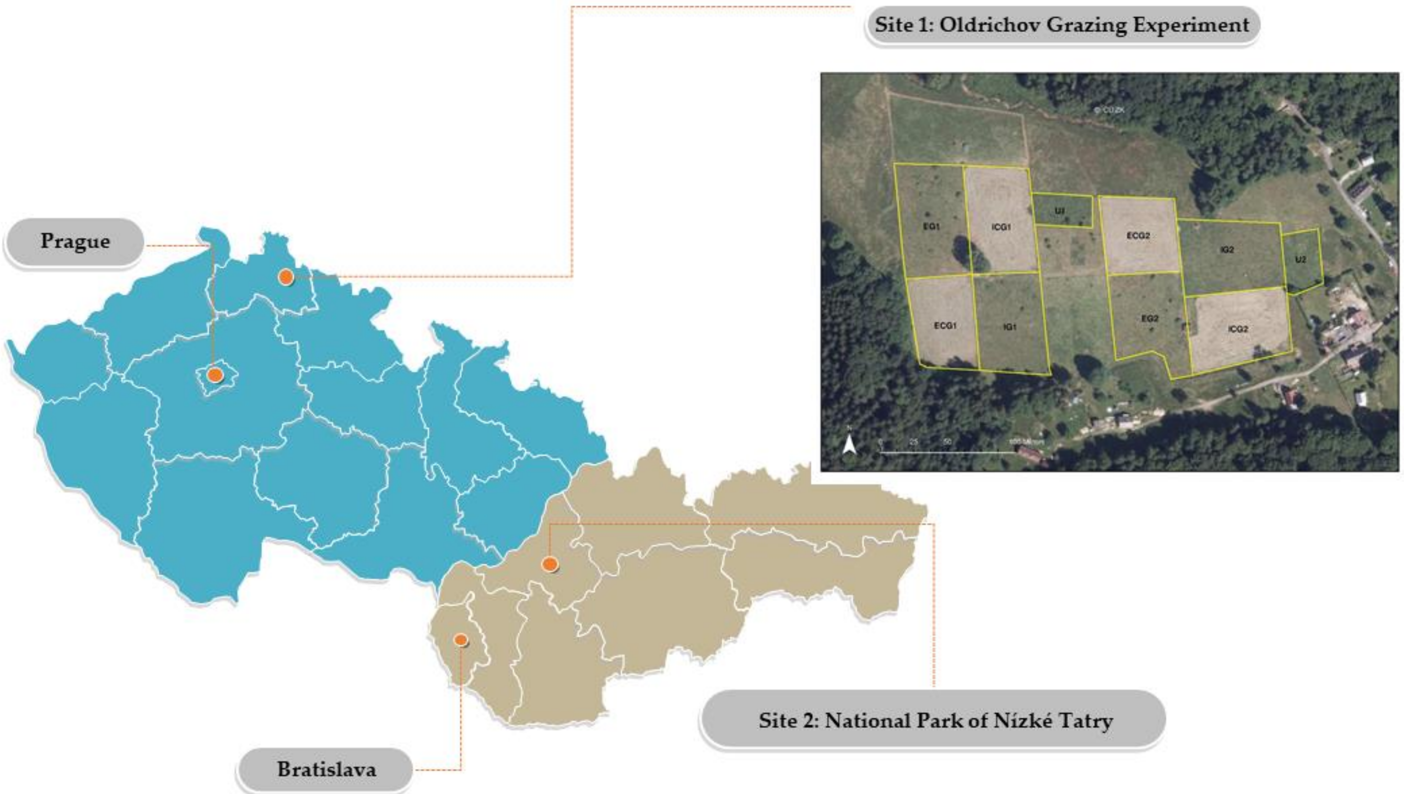


Figure 3: Map of study areas

Outline of the thesis

The objectives and research questions of the thesis are framed on individual case study/literature and are structured accordingly. The thesis consists of seven chapters.

Chapter 1: introduces the background of the study and introduces grassland especially in temperate regions of Europe. Furthermore, it gives the main concepts of defoliation management and their effect on selected sward parameters. Finally, it gives the overall objective and presents a brief information of study areas.

Chapter 2: addresses the question of how different plant functional groups under long-term contrasting management respond? It analyses the responses from two different vertical sward layers. Finally, it discusses the successional development or trajectories of vegetation over the experimental period.

Chapter 3: this chapter explores the effect of dung on patches created under different grazing intensities, especially on nutrient concentration in soil and herbage. It discusses other potential effects on other sward parameters such as on dry matter standing biomass, dead biomass and dry matter content. Finally, it will highlight any relationship that may exist between nutrient concentration in soil and herbage.

Chapter 4: asks if an upland grassland covered with expansive weedy species that was previously used as cattle, resting place can be restored using cutting, herbicide application and combination of this techniques. Using long-term data (8 years), it discusses the effect of the measures taken to restore the grassland and its effect on nutrient concertation in herbage and soil.

Chapter 5: asks how 13 years of different grazing intensities affect the forage quality of a semi-natural grassland. Furthermore, it attempts to find the best time to introduce management in order to meet the nutritional requirements of cattle.

Chapter 6: briefly examines the effect two contrasting grazing intensities (extensive and intensive grazing) on biomass production and heifers performance using a 20-year long experimental data.

Chapter 7: summarizes the significant results from the different case studies presented in the thesis and provides practical interventions to safeguard semi-natural grasslands.

4. Statement of contribution

Teowdroes Kassahun (TK) contributed significantly in formulating the research questions, selecting appropriate methods for data collection, analysis and interpretation of the results. For chapter 1, the entire review was done by TK. For Chapter 2 and 4, TK was responsible for data analysis and drafting of the manuscript. Chapter 3 and 5, TK contributed in the data analysis, funding acquisition and drafting of manuscript. Chapter 6 TK was responsible for partially collecting the data and conducting the data analysis. Overall TK contributed in the data collection fully or partially at the long-term experiment site for all papers except for Chapter 4.

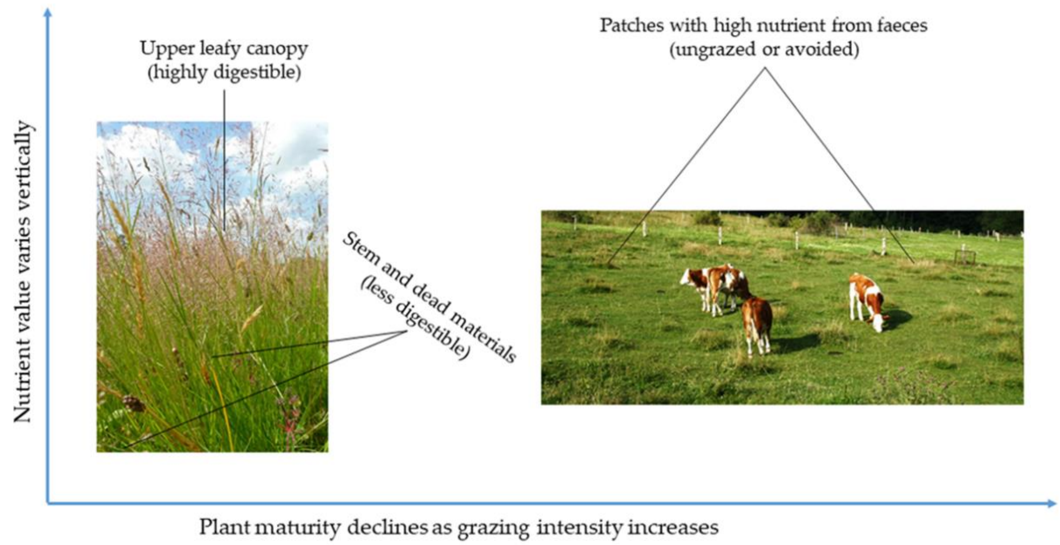
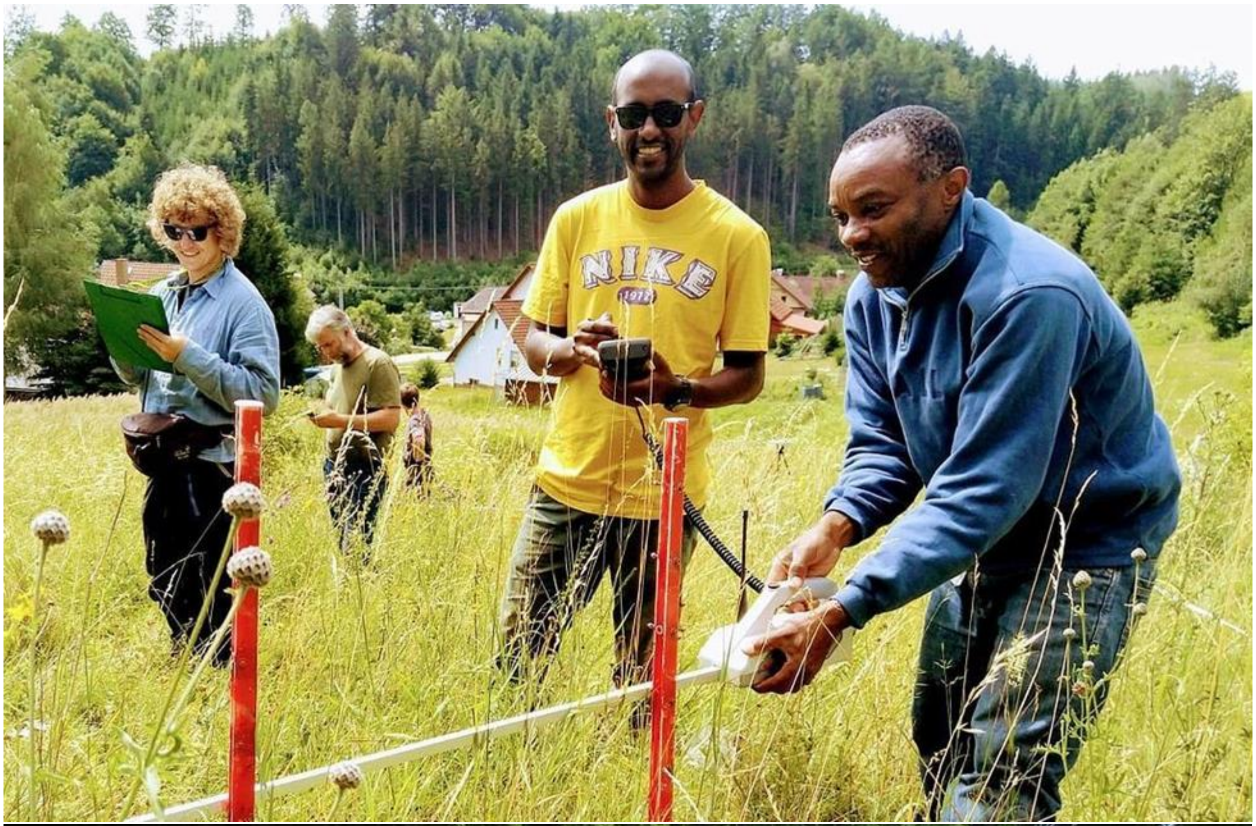


Figure 4: vertical and horizontal sward structure in a semi-natural grassland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Chapter 2

Effect of 15-year sward management on vertical distribution of plant functional groups in a semi-natural perennial grassland of central Europe

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Published as:

Kassahun T, Pavlů K, Pavlů VV, Pavlů L, Blažek P. Effect of 15-year swardmanagement on vertical distribution of plant functional groups in a semi-natural perennial grassland of central Europe. Appl Veg Sci. 2021;24:e12568. https://doi.org/10.1111/avsc.12568

Received: 12 October 2020 | Revised: 12 January 2021 | Accepted: 26 January 2021
DOI: 10.1111/avsc.12568

RESEARCH ARTICLE

Applied Vegetation Science IAVS

Effect of 15-year sward management on vertical distribution of plant functional groups in a semi-natural perennial grassland of central Europe

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Funding information
This research was conducted with support from the Ministry of Agriculture of the Czech Republic (RO0417). This paper was finalized with support from the Internal Grant Agency – Faculty of Environmental Sciences, Czech University of Life Sciences Prague "Vertical and Horizontal sward structure of species rich upland grassland under long-term grazing management - No. 20194211".

Co-ordinating Editor: Flavia Landucci

Abstract

Aims: The nutrient concentration in herbage and biomass productivity analyses are dependent on the vertical distribution of different sward layers where the sampling is done. Notably, a majority of studies indicate clipping biomass to the ground level without any consideration of the vertical distribution. This study examined the effect of cutting and grazing intensities on the vertical distribution of plant functional groups.

Location: Oldřichov Grazing Experiment, northern Czechia.

Methods: During a 15-year experiment: (a) intensive and (b) extensive grazing without cutting; (c) cutting in June followed by intensive and (d) extensive grazing; and (e) undefoliated treatment were applied throughout the vegetation season. Biomass data were collected at two layers in the sward (below and above 3 cm) and separated into five functional groups. Biomass data were analysed to examine the succession and effects of treatments on vertical distribution of functional groups.

Results: Treatment effects were differentiated after 2–3 years from the introduction of management, but the composition of functional groups fluctuated over time. Treatments significantly affected total biomass of all functional groups and the vertical distribution within swards of most groups. Particularly intensive grazing significantly decreased the total biomass of graminoids, forbs, and dead biomass in favour of legumes (which increased). This led to a shift in the relative biomass distribution from the upper sward layer to the lower layer for most functional groups except for legumes and mosses.

Conclusion: The high proportion of dead biomass in the lower sward layer suggests the need for a methodological approach that considers clipping of biomass only above 3 cm when sampling for productivity and forage quality analysis. This approach would avoid including biomass from below 3 cm or the lower layer, which would be ungrazed by cattle. Many previous studies may have reported a distorted or inflated value in herbage productivity or forage quality results.

KEYWORDS
Central Europe, cutting, functional groups, grassland management, grazing intensity, heifers, sward structure

1 | INTRODUCTION

Although the relationship between biodiversity and grassland productivity remains a passionately contested topic (Adler et al., 2011; Grace et al., 2012), it is still assumed that in agricultural settings higher plant diversity has lower economical value for farmers as it is often associated with lower forage quality and biomass yield (Bruinenberg et al., 2002; Isselstein et al., 2005). Therefore, several studies have been conducted focusing on grassland productivity and forage quality. However, accurate measurement with a clear methodology is critical when it comes to productivity and forage quality assessments. Unfortunately, which layer to incorporate during sampling and analysis of data from grazing or cutting experiment remains vague or largely unanswered.

Grazing management is a highly complex process that affects the grazers as well as the sward structure. Vegetation structure (where height of the sward is the main criterion determining structure) is one of the main factors that affects the quantity as well as the quality of available forage resources for grazing animals. Therefore, the performance of the grazers is directly affected by their nutrient intake from the vegetation structures (Fleurance et al., 2016). In mixed-species swards, the vertical structure also affects grazing intake as well as influencing inter-species competition for light (Schulte & Lantinga, 2002). For instance, in temperate grasslands the biomass intake by cattle is significantly influenced by sward structure through several factors of grazing behaviour including bite mass (Casey et al., 2004), intake rate (Barrett et al., 2003) and amount of energy utilized during grazing (Illius et al., 1995). Intake and grazing behaviour are also influenced by morphological changes in sward structure, which was observed as having a direct relationship between grazing activity and sward structure of lucerne (*Medicago sativa*) and cocksfoot (*Dactylis glomerata*) in a New Zealand silvopastural site (Peri et al., 2001). Hence, greater emphasis on the management of sward structure has increasing relevance in the context of grassland utilization, as the direct influence of sward structure on herbage intake ultimately affects animal production (Gordon & Benvenutti, 2006).

The promotion of certain managements such as grazing or long-term exclusion of grazing could lead to a change in dominance of above-ground biomass (different plant functional groups) ultimately affecting the proportion of palatable grasses and unpalatable forbs in temperate ecosystems (Zhao et al., 2019). However, plant competition (del-Val & Crawley, 2005), and grazing behaviour of the animals also influence the composition and perenniality of plant communities (Matches, 1992), the functional groups and the spatial heterogeneity of vegetation (Adler et al., 2001; Bullock et al., 2001; Díaz et al., 2007; Fernández-Lugo et al., 2013) as well as soil physical and chemical properties (Augustine and Frank, 2001; Steffens et al., 2008).

Grassland managers often consider grazing to be one of the most important management tools for manipulating the vegetation, yet the respective management decisions should always be based on a clear set of criteria that includes sward structure. According to Hodgson and Maxwell (1981), sward measurements such as height and growth stage are important for managing grazing systems, and can greatly improve grassland productivity and utilization as well as improving the sward structure (Milchunas & Lauenroth, 1993), by matching sward condition and herbage availability to the requirements of animals. For instance, the relative proportion of flowers, stems and dead material in the different horizons (vertical structure) is one of the main components of structural variation that is open to manipulation (Tallowin et al., 2005). However, sward structure could be very different depending on the types of plant species present. For example, Hodgson (1985), described the vertical structure for a legume (white clover, *Trifolium repens*) and grass (perennial ryegrass, *Lolium perenne*), and observed that the upper horizons of the sward canopy are made up primarily of living leaves, whereas leaf sheaths, stems and dead biomass are concentrated in the lower horizons. In a sub-humid grassland type in Argentina, Sala et al. (1986) investigated the effect of grazing management on plant community structure in seven sward layers and found that in grazed grassland most of the plant material was concentrated in the bottom layer, whereas in undefoliated plots the largest proportion of the leaf area was in the upper layer.

Previous studies and documentation of the vertical structure of swards in temperate areas have mainly considered homogeneous swards of legumes and grasses, such as perennial ryegrass and white clover. The structure of these swards has been shown to consist typically of leaves in the upper layer, while the lower layer mostly comprises stems and dead biomass (Hodgson, 1985). However, grasslands of central Europe, which often have a high species diversity, have been very little studied, and particularly long-term data of mixed-species swards composed of graminoids, forbs, mosses and legumes are lacking. In this paper, we present the results of a long-term study that started in 1998 in the Jizera Mountains (Czech Republic) with the main objective of investigating the effects of different levels of grazing intensity on different functional groups of grassland species at two vertical sward layers. Against this background, and using long-term data, we seek to answer the following questions:

1. What is the successional development of functional groups in different layers of the sward under contrasting grazing intensity and cutting management?
2. What is the effect of treatments on the vertical distribution of functional groups? Is grazing intensity or cutting management the key driver?

2 | METHODS

2.1 | Study site

The study was conducted at the site of the "Oldřichov Grazing Experiment" in the Jizera Mountains, northern Czech Republic

(50°50.34′ N, 15°05.36′ E; elevation 420 m a.s.l.). The site has an average annual precipitation of 803 mm, and a mean annual temperature of 7.2°C (Liberec Meteorological Station). For monthly rainfall and mean monthly temperatures, see Appendix S1.

The geological substratum is granite underlying a low, deep, brown soil (cambisol). The content of plant-available P, K, and Mg at the start of the experiment analysed according to the Mehlich III method (Mehlich, 1984) was 64, 95 and 92 mg/kg respectively (Pavlů et al., 2006a). For plant-available P, K, Mg, Ca and pH/$CaCl_2$ under each treatment for the year 2016 see Appendix S2. In the early 1980s, the area was drained, ploughed and reseeded with productive grasses, namely *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, and *Phleum pratense*. Between 1987 and 1992, rotational grazing was introduced, and fertilizer was applied over the entire experimental site as follows: N (40–140 kg/ha as NH_4NO_3), P (40 kg/ha as $Ca(H_2PO_4)_2$), and K (120 kg/ha as KCl). No fertilizers have been applied since 1992 (Pavlů et al., 2003), and the site remained abandoned until 1998.

The botanical diversity at the experimental site can be considered as high with up to 24 vascular plant species per m^2. The dominant species are *Agrostis capillaris*, *Festuca rubra* aggr., *Trifolium repens* and *Taraxacum* spp. (Ludvíková et al., 2015).

2.2 | Experimental layout and grazing trial

The experiment was established in two adjacent completely randomized blocks in 1998 (Pavlů et al., 2007). Each block consisted of five treatment paddocks, each of 0.35 ha, except the undefoliated plot, which was 0.12 ha. Different management regimes were applied in each paddock. The treatments were (Table 1): (a) extensive grazing (EG), where the stocking rate (SR) was adjusted to achieve a mean target sward surface height >10 cm; (b) intensive grazing (IG), in which SR was adjusted to achieve a mean target sward surface height <5 cm; (c) cutting in June followed by extensive grazing (ECG) for the rest of the growing season; (d) cutting in June followed by intensive grazing for the rest of the growing season (ICG); and (e) the undefoliated control (U). The percentage cover (%) of the graminoids, forbs and legumes under different treatments for the years 2001–2012 are shown in Appendix S3.

In order to adjust the stocking density for IG and EG treatments, while also keeping the stock numbers constant, the size of grazed areas was adjusted by moving the fences continuously throughout the grazing season. Since its establishment, the design of the experiment, its layout and SR remained unchanged. All paddocks of treatments (a) and (b) were continuously stocked with young heifers with initial live weights of about 200 kg from early May until late October, and from mid-June to late October in the case of treatments (c) and (d).

2.3 | Measurements and sward structure

In early May (before cutting or the start of grazing) from 1998 to 2012 (15 years), six samples were collected from a 50 cm × 25 cm steel frame randomly placed within each treatment plot (paddock). In each, the biomass from two vertical sward layers was collected using electric clippers within the area of the steel frame: (a) lower 0–3 cm (stable non-grazed layer) and (b) upper >3 cm (grazed layer). Experimental evidence (Laca et al., 1994; Ungar, 1998) indicated that animals favourably graze the highest or upper part of the sward. For instance, in our experimental site under IG treatment, the average sward during the grazing season is typically between 3 cm and 4 cm, which was identified from weekly measurements of compressed sward heights across the experiment's plots (100 measurements per plot). Hence, the lowest layer, which is left ungrazed in our experiment, is considered under 3 cm in all plots.

Accordingly, the plant material that were collected from the two layers was then sorted into different functional groups: living biomass, separated into forbs (without legumes), graminoids, legumes and mosses, and undifferentiated dead material. Total living biomass of vascular plants was calculated as the sum of graminoids, forbs and legumes (hereafter referred to as living biomass). Finally, the samples were oven-dried for 48 hr at 70°C and weighed. In total, 1,800 samples (120 samples per year) were analysed over the 15-year experimental period. The experimental site can be classified as a low-productive site with herbage biomass production in the years 1998 to 2001 ranging from 3.33 t/ha to 3.90 t/ha under IG and 2.20 t/ha to 3.35 t/ha under EG (Pavlů et al., 2006a).

TABLE 1 Description of treatments at the study site

Treatment description	Sward height	Start of cutting	Start of grazing	One-way design: Treatment	Two-way design: Intensity	Two-way design: Management
Extensive grazing	>10 cm	No cut	Mid-May	EG	E	N
First cut followed by extensive grazing	>10 cm	Early June	Late June	ECG	E	C
Intensive grazing	<5 cm	No cut	Early May	IG	I	N
First cut followed by intensive grazing	<5 cm	Early June	Mid-June	ICG	I	C
Undefoliated	Uncontrolled	No cut	No grazing	U	–	–

Abbreviations: C, Cut; E, Extensive; G, grazing; I, Intensive; N, No cut; U, Undefoliated.

2.4 | Data analysis

The succession in the composition of functional groups in the two vertical layers was analysed using a partial principal components analysis (pPCA) with blocks as covariate and excluding the variable living biomass, as it is the sum of other variables. pPCA was performed using Canoco 5 (ter Braak & Šmilauer, 2012).

To investigate the effects of the treatments on differences between the vertical sward layers, the ratio of the biomass in the upper layer to the sum of the biomass in both layers was calculated for each functional group. The effects of the treatments on total biomass and on the upper biomass of each functional group, and their ratios were analysed using two sets of general linear models (GLMs). The first set of models included all five treatments (including undefoliated — U) in one factor, and the Tukey honestly significant difference test was applied to identify the differences between them. The second set excluded treatment U, thereby enabling us to test for the effect of grazing and cutting separately, including their interaction. In addition, "year" and all its interactions were included as random factor in both sets of models to account for the large between-year fluctuations. The first three years of data were excluded from this analysis due to the substantial change in vegetation that followed the introduction of management at the site. Block was excluded from the models, as it had no significant effect. The total biomass was log-transformed [$X' = \log_{10}(X + 1)$] and the proportion of the upper layer was arcsin-transformed {$X' = \text{asin}[\text{sqrt}(X)]/\text{asin}(1)$} to improve normality of the data. We applied Benjamini–Hochberg's procedure to control for false discovery rate (FDR; Verhoeven et al., 2005). Additional GLMs were used to evaluate the effect of treatments on the ratio of living to dead biomass for the two sward layers separately. The ratio was log-transformed [$X' = \log_{10}(X)$] and "infinity" ratios in samples with zero dead biomass were replaced by the maximum value of each respective treatment. The same model setting was applied as in the GLMs with all treatments described above (year as random factor, Tukey post-hoc test). GLMs were conducted using Statistica 13.1 (Dell Inc., 2016).

3 | RESULTS

3.1 | The successional development

The pPCA shows the overall differentiation in vegetation composition through the course of the experiment (main pattern in Figure 1, detailed successional trajectories in Appendix S4). The first axis (explaining 36% of variation) differentiated the intensive grazing (IG, ICG) from extensive grazing or no management (EG, ECG, U) with additional slight differences within the latter group. The start of all successional trajectories is close to the undefoliated control, and rapid changes in vegetation were observed for the two-year period following the establishment of management, especially in the intensively grazed plots. The vegetation of the undefoliated or extensively grazed treatments is largely characterized by large amounts of dead biomass in both layers, and graminoids and forbs dominate in the upper layer, while legumes were mostly absent. Intensive grazing treatments are characterized by lower amounts of dead biomass in both layers, while legumes were largely present in both layers. The second axis (explaining 19% of the variation) represents mostly random between-year fluctuations, which were generally consistent in all treatments. Most of this variation is attributed to mosses and forbs in the lower layer, and these are generally more abundant in ECG.

3.2 | Effect of all treatments on the biomass and its vertical distribution

The five treatments had significant effects on total biomass of all functional groups and on the vertical distribution of most groups except legumes and mosses (Table 2). Except for mosses, treatment also had a significant effect on the upper layer of all functional groups. Compared to the managed treatments (explored in more detail in the next section), the undefoliated treatment in the total showed the lowest biomass of graminoids (shared with intensive grazing) and legumes, but the highest amount of dead biomass. The sum of living biomass was only marginally different and the undefoliated treatment had intermediate values. Regarding the vertical

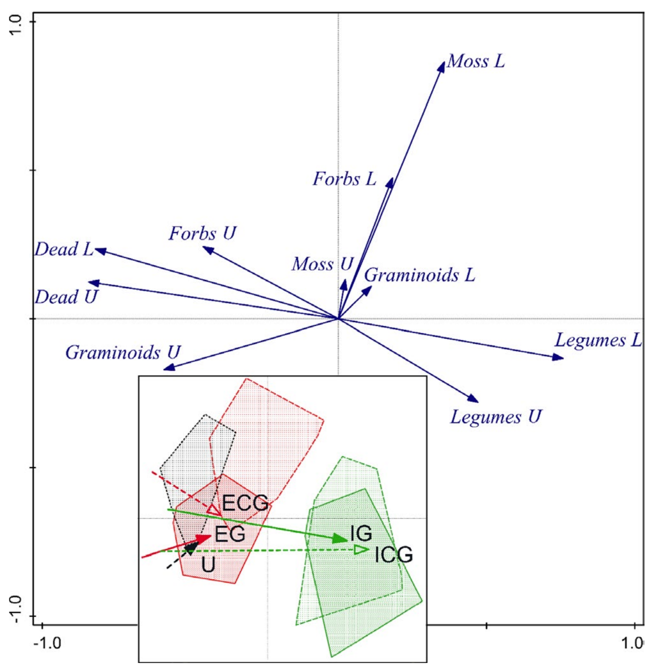

FIGURE 1 Partial principal components analysis (pPCA) of the plant functional groups for the upper (>3 cm) and lower (<3 cm) sward layers. Arrows indicate the main successional direction of treatments (from 1998 to 2012); envelopes encompass the region where the treatments were fluctuating after their initial divergence (from 2001 to 2012, i.e. excluding the first three years consistently with our general linear models). For detailed plot, see Appendix S4. Group labels include group name and layer abbreviation: L – lower, U – upper. For treatment abbreviations, see Table 1

distribution, in the undefoliated treatment the living biomass was present in the upper layer more than in the case of managed treatments. This holds for both the graminoid and forb components (legumes were almost absent in U, preventing reliable evaluation of their vertical distribution). Dead biomass was low in the U treatment, in contrast to extensive grazing.

Furthermore, our results showed an overall higher proportion of living biomass in the upper layer than in the lower layer. The IG and ICG treatments provided a significantly higher proportion of living biomass relative to EG, ECG and U treatments (Figure 2). In terms of dry standing matter, graminoids provided the highest amount among all functional groups throughout the entire experimental period (Appendix S5).

3.3 | Effect of grazing intensity and cutting management on the biomass and its vertical distribution

Grazing intensity significantly affected the upper layer biomass (>3 cm) of all functional groups except for mosses (Table 3). Extensive grazing increased biomass in graminoids, forbs, sum of living biomass (mostly composed of graminoids and forbs) and dead matter, while legumes decreased (Figure 3a). Apart from forbs, management and its interaction with grazing intensity had no effect on the functional groups of the upper layer. A higher ratio of biomass in the upper layer to total biomass was also observed under extensive treatment for graminoids, forbs, living and dead (Figure 3c), which in combination with higher total biomass in extensive treatment in these groups (Figure 3b) results in a more pronounced pattern in the upper layer alone (Figure 3a) compared to the pattern observed with total biomass. Overall, very little dead biomass was present in the upper layer while mosses were effectively absent from the upper layer (Figure 3a).

Grazing and cutting treatments significantly affected the total biomass of most functional groups (some of the main effects were only marginally significant; grazing intensity was not significant for mosses due to the contrasting effect of interaction; Table 3; Figure 3b). Intensive grazing suppressed both living and dead biomass, specifically through its effect in decreasing graminoids and forbs, while there was higher moss biomass in the cut plots. In contrast, intensive grazing resulted in a marked increase in legumes, and it also led to an increased occurrence of moss in the uncut plots. Cutting supported forbs (largely), legumes (slightly, and only when combined with intensive grazing), and mosses (largely, only when combined with extensive grazing), and suppressed graminoids and resulted in less dead biomass (only in extensive grazing).

TABLE 2 Result of general linear model for the effect of all treatments on the total biomass, on the upper layer biomass (>3 cm) and on the ratio of biomass in the upper layer (>3 cm) to total biomass for all functional groups

	df	*F*	*p*	IG	ICG	EG	ECG	U
Functional group total biomass								
Graminoid	44	6.51	**<0.001**	bc	bc	a	b	c
Forb	44	10.69	**<0.001**	d	c	c	a	bc
Legume	44	63.74	**<0.001**	a	a	c	b	d
Living	44	3.6	0.012	c	bc	ab	a	bc
Dead	44	113.96	**<0.001**	d	d	b	c	a
Moss	44	7.2	**<0.001**	b	b	c	a	bc
Ratio: >3 cm/total biomass								
Graminoid	44	9.82	**<0.001**	c	c	b	b	a
Forb	44	19.41	**<0.001**	c	c	ab	b	a
Legume	46.1	1.15	0.344	-	-	-	-	-
Living	44	15.95	**<0.001**	c	c	b	b	a
Dead	44	6.97	**<0.001**	b	b	a	a	b
Moss	44.9	0.94	0.448	-	-	-	-	-
> 3 cm biomass								
Graminoid	44	6.5929	**<0.001**	b	b	a	a	a
Forb	44	19.0508	**<0.001**	d	c	b	ab	a
Legume	44	27.80724	**<0.001**	a	a	b	b	c
Living	44	10.6314	**<0.001**	b	b	a	a	a
Dead	44	40.608	**<0.001**	c	c	a	b	a
Moss	44	1.276594	0.293	-	-	-	-	-

Results are summarized by denominator degrees of freedom *df* (numerator *df* was 4 in all tests), *F* ratio and *p*-value. Significant results (after table-wise Benjamini–Hochberg false discovery rate correction) are highlighted in bold. Significant differences between treatments (for abbreviations, see Table 1) in a Tukey test are indicated by different lowercase letters (alphabetic order represents decreasing values of means, i.e. a represents the largest mean).

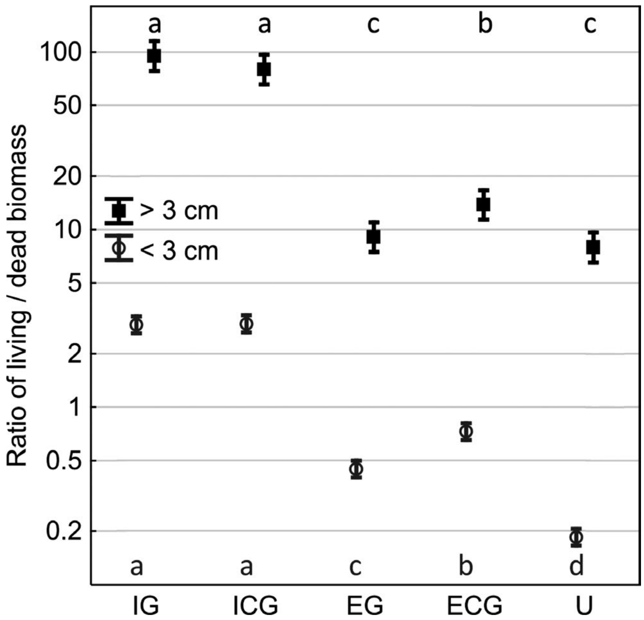

FIGURE 2 The effect of treatments on the herbage ratio of living biomass and dead biomass in two different sward layers (<3 and >3 cm). Error bars indicate model-based 95% confidence intervals. Different lowercase letters indicate significant differences between treatments in a Tukey test. For treatment abbreviations, see Table 1

Similar to the upper-layer biomass, cutting and its interaction with grazing intensity had no effect on the vertical biomass distribution for any of the functional groups (Table 3). In contrast, intensive grazing significantly suppressed the proportion of biomass in the upper layer in the functional groups of graminoids, forbs, living and dead biomass (Figure 3c). Neither grazing intensity nor cutting management had any significant effect on the vertical distribution of legumes or of mosses.

4 | DISCUSSION

4.1 | Successional development

The pPCA demonstrated large temporal fluctuations of functional group composition. It is interesting that these fluctuations were similar in all treatments, suggesting that they were not just random. A possible explanation could be changes and fluctuations in the environment (Lepš et al., 2018), such as weather conditions within seasons or climatic differences between years, which may benefit or suppress individual functional groups regardless of the treatment. Similarly, a study by Fischer et al. (2020) reported year-to-year changes in species composition due to seasonal fluctuations in temperature and precipitation, confirming weather is a dominant driver of local vegetation dynamics. For instance, *Festuca rubra* (one of the dominant grass species in our experimental site), which has many ecotypes (Grime et al., 1988) in comparison with other common grass species, is well adapted to various abiotic conditions including drought. Its variability in time can be explained by compensatory dynamic (Lepš et al., 2018) in which cover of species like *Festuca rubra* can increase even under dry conditions (Titěra et al., 2020) while compensating for the possible decline in cover of other species like *Poa trivialis*, which is less tolerant to drought (Peeters et al., 2004). Gaisler et al. (2018) also reported similar results from a long-term experiment (13 years) in which different functional groups such as tall graminoids and tall forbs fluctuated without any clear stable trend for any particular treatment. Despite the large variability in the present study, the main patterns found by pPCA largely overlap with GLM results, and in just 2–3 years after the introduction of management the succession was close to that of the final composition.

4.2 | Composition of total biomass

Several studies have reported on the impacts of grazing on plant communities, especially in terms of the role of long-term grazing in eliminating those species that are less resistant to the effects of grazing (Dorrough et al., 2004). Therefore, the ability of plant communities to respond to changes in the environment is heavily affected by the grazing history, including changes to grazing intensity (Mack & Thompson, 1982). After 15 years of different treatments of grazing and cutting management at our experimental site, a clear pattern was seen: both the IG and ICG treatments had a positive effect on total biomass of legumes, whereas forbs and graminoids, and also dead biomass, were present in greater amounts and were apparently supported by the management of the ECG and EG treatments.

Graminoids showed a remarkable dominance in terms of dry-matter standing biomass throughout the 15-year experimental period. This outcome can be explained by two effects: (a) the ability of graminoids to suppress other functional groups like forbs, because of their superior competitive ability (del-Val & Crawley, 2005); and (b) the dominance of *Agrostis capillaris*, which is largely promoted by grazing especially in grasslands of low productivity (Louault et al., 2005). Hence, it outcompetes species that are less tolerant of frequent defoliation (Gaisler et al., 2013). Grazing is generally expected to increase the dominance or abundance of graminoids (Puchetaet al., 1992). Frequent removal of the biomass of graminoids, as occurs under grazing, stimulates sward regrowth by increasing the amount of available light reaching the base of the sward (Deregibus et al., 1985).

The highest amount of total dead plant biomass was found in the undefoliated treatment. In the treatments with grazing, the frequent cutting leads to regrowth and reduces the opportunity for senescence of plant tissue. This outcome is not only unique to temperate grasslands. Altesor et al. (2005) for grasslands of Uruguay and Sala et al. (1986) for the Argentine Pampa also reported similar findings, where grazed and ungrazed treatments were compared. In addition, intensive grazing was able to reduce the standing dead biomass in both layers and shift its allocation to the lower layer, which ultimately helped to increase the living biomass proportion by promoting overall growth (Balph & Malechek, 1985).

TABLE 3 Result of general linear model for the effect of grazing intensity and cutting management in factorial design on the total biomass, on the upper layer biomass (>3 cm) and the ratio of biomass in the upper layer (>3 cm) to total biomass for all functional groups

	Functional group total biomass			Ratio: >3 cm/total biomass			>3 cm biomass		
Effect	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Graminoid									
Intensity	11	9.11	**0.01**	11	14.99	**0.003**	11	15.22	**0.002**
Management	11	7.05	0.02	11	0.44	0.522	11	4.19	0.065
Intensity × management	11	0.37	0.55	11	0.39	0.544	11	0.002	0.960
Forb									
Intensity	11	19.15	**<0.001**	11	20.78	**<0.001**	11	41.57	**<0.001**
Management	11	184.6	**<0.001**	11	4.68	0.053	11	52.64	**<0.001**
Intensity × management	11	0.21	0.65	11	2.54	0.139	11	1.92	0.192
Legume									
Intensity	11	61.26	**<0.001**	14.1	3.2	0.095	11	38.46	**<0.001**
Management	11	8.24	**0.015**	14.7	0.43	0.52	11	1.49	0.246
Intensity × management	11	3.04	0.108	14	0.00	1.000	11	2.89	0.117
Living									
Intensity	11	7.52	0.02	11	18.83	**<0.001**	11	17.60	**<0.001**
Management	11	4.09	0.07	11	0.57	0.466	11	0.18	0.681
Intensity × management	11	0.38	0.55	11	0.02	0.896	11	0.23	0.641
Dead									
Intensity	11	98.95	**<0.001**	11	18.4	**<0.001**	11	75.61	**<0.001**
Management	11	6.51	0.03	11	0.2	0.663	11	4.30	0.062
Intensity × management	11	8.68	**0.01**	11	0.1	0.76	11	2.25	0.161
Moss									
Intensity	11	0.01	0.9	11	2.04	0.18	11	1.66	0.224
Management	11	20.68	**<0.001**	11	1.37	0.266	11	1.85	0.200
Intensity × management	11	36.81	**<0.001**	11	0.00	0.954	11	0.48	0.501

Results are summarized by denominator degrees of freedom *df* (numerator *df* was 1 in all tests), *F* ratio and *p* value. Significant results (after table-wise Benjamini–Hochberg false discovery rate correction) are highlighted in bold. See Figure 3 for effect directions.

The total amount of legumes (mainly *Trifolium repens*) found in the undefoliated plots was very low. This may be attributed, at least in part, to their low ability to compete for light unless their leaves can reach the upper canopy of the sward. Thus, in the present study, white clover occurred predominantly in the IG and ICG treatments (Appendix S3). The explanation for its very low presence in undefoliated plots may, however, be highly complex as several factors, including winter survival as well as competition for light and nutrients, are known to affect clover growth, flowering and survival (Parsons and Chapman, 2000). Furthermore, when legumes are present in swards under intensive management, some may be annual species (such as *Trifolium dubium* in our experiment) that have the advantage of continuing to survive by producing new seedlings after established plants die or are removed by grazing livestock. The strategy of annuals provides a survival advantage relative to perennial plants that are grazed during their longer life cycle (Díaz et al., 2007). This is, however, in contradiction with other studies such as Matches (1992), who found that legume content was lower under increased grazing intensity, whereas light grazing favoured legumes rather than forbs or grasses (Qu et al., 2016). These disparities between different studies may be explained by differences in experimental sites' environments such as nutrient supply, water or light/shade conditions (Milchunas & Lauenroth, 1993; Borer et al., 2014). Especially leguminous species are generally known for their positive response to P and K and negative response to the high inputs of N, NP or NPK (e.g., Čop & Eler, 2019; Titěra et al., 2020).

The total biomass of mosses under the ECG treatment was relatively high compared to that in the other managed treatments, and this is attributed mainly to the inability of mosses to tolerate the effects of trampling by grazing heifers (Ludvíková et al., 2014), especially for *Rhytidiadelphus squarrosus*, which is the dominant species at the experimental site. Our result also showed treatment had no effect on the upper layer (>3 cm) for mosses, which could imply they are not really present in the upper layer neither in the managed nor in the undefoliated plots (Table 2; Figure 3). Similarly, total forbs and total graminoids were also more abundant, and total living biomass

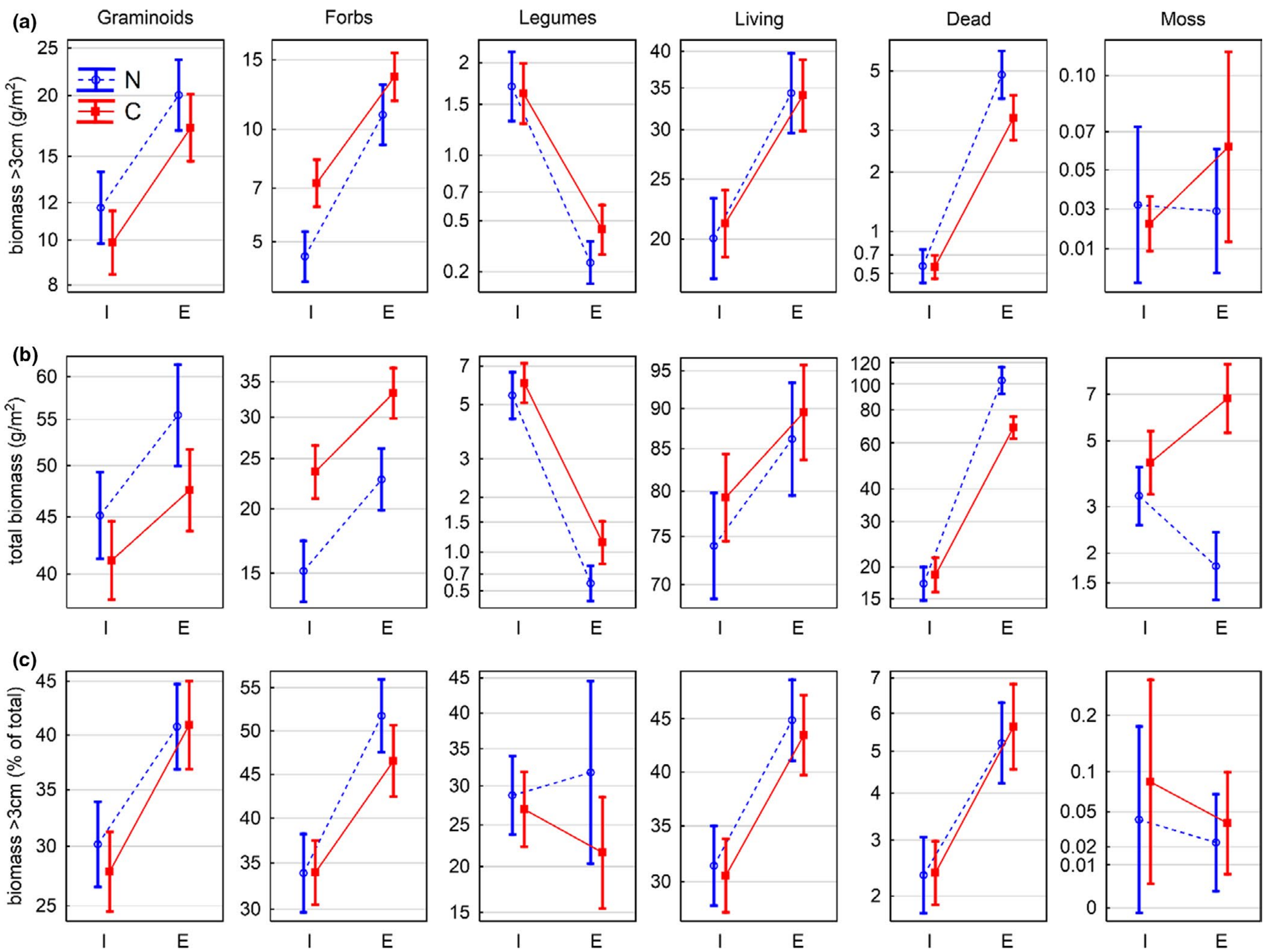

FIGURE 3 The effect of grazing intensity and cutting management on (a) upper layer biomass (>3 cm), (b) total biomass and (c) ratio of biomass in upper layer (>3 cm) to total biomass of each functional group. Abbreviations on the *x*-axis and in legend: N = No Cutting, C = Cutting, I = Intensive, E = Extensive. Error bars indicate model-based 95% confidence intervals. See GLM results in Table 3

was greater, in the extensive treatment. These findings are consistent with results of Correll et al. (2003), who also found a higher proportion of forbs under extensive grazing. It is well understood that many forb species typically benefit from reduced grazing intensity (Wahren et al., 1994). In our experiment, the most dominant forb species are *Taraxacum* spp., which are generally shade-intolerant species (Grime et al.,1988). In tall-growing swards, as represented by the undefoliated plots in this study, the growth of forbs like *Taraxacum* spp. is adversely affected by reduced light at lower sward depths. However, many forbs are able to develop well under management with frequent defoliation (Louault et al., 2005; Pavlů et al., 2007), and can adapt quickly to the changing trophic regime of soil under extensive management.

4.3 | Vertical distribution of sward

Grazing intensity had strong and significant effects on the vertical distribution of several functional groups. A high proportion of living biomass in the upper layer was revealed by the analysis (Figure 2) and this is a common phenomenon. As growing herbage gradually reaches maturity, a greater proportion of green matter will be found in the upper layer and dead biomass accumulates at the bottom layer. The higher proportion of living biomass in the upper layer, compared to the lower layer, raises another crucial issue in relation to experimental procedures and field assessments. Several reported studies have followed procedures of cutting or hand plucking close to the ground level, or clipping biomass at the soil surface, as the basis for determining herbage biomass per unit area (e.g., Grant et al., 1996; Fleurance et al., 2016) or when sampling for forage quality (e.g., White et al., 2014). Procedures that include herbage samples from the bottom layer that would normally be left ungrazed (<3 cm) could potentially result in disputable conclusions being drawn with regard to overestimation of the available biomass or the accuracy of forage quality.

The higher allocation of dead material to the upper sward layer under extensive grazing, with the resulting taller sward, was consistent with Wright and Whyte (1989), who also found a higher proportion of dead material with increasing sward height, and also with Bircham and Hodgson (1983), who identified higher rates of senescence in tall swards, typical for extensive grazing management and for ungrazed plots. In contrast to the high proportion of total biomass of forbs under extensification, more forbs were allocated to the upper layer in undefoliated plots and there was lower forb biomass under both the intensively grazed treatments (ICG > IG). This can be attributed to more grazing-tolerant species occurring within the forbs group and appearing frequently in the grazed areas (Bermejo et al., 2012). This different response by forbs as a functional group may possibly be explained by the heterogeneous features and wide range of morphological traits of the group, thereby enabling species within the group to respond to the various disturbances or conditions (Bermejo et al., 2012).

Similarly, more graminoids were found in the bottom layer of almost all treatments except the undefoliated plot. This could be explained by the effects of long-term grazing on the study site, as grazing results in the removal of leaf material from the upper layers of the sward, thereby reducing the canopy height, and in the long term it affects the competitive balance within the community so that shorter-growing species replace taller species (Fahnestock & Detling, 2000). Thus, the sward composition evolves with selection for species that are well suited to survive or are adapted to intensive grazing.

In contrast, management (cutting or non-cutting) had no discernible effect on the vertical distribution or on the upper-layer biomass (except for forbs) of any of the functional groups, although it had significant effects on the total harvested biomass. This is mainly because the increased frequency of defoliation rather than the type of defoliation (such as cutting in spring) influences total biomass more, increasing the densities of all sward components like grass tillers (Pavlů et al., 2006b).

A limitation to the study is the choice of a broad functional group approach for the samples collected in the two layers. Although all species within a functional group will not behave the same, it was not possible to collect the data at the species level. This is mainly because identifying species in the lower layer is nearly impossible after the top layer is already cut or sampled. Due to this, it was not possible to evaluate the species richness and detailed botanical composition in relation to the vertical distribution. However, a study by Pavlů et al. (2007) and Pavlů et al. (2016) conducted at the same experimental site concluded that grazing and cutting management has changed the plant species composition, leading to an increased proportion of short grasses and prostate forbs. Specifically, tall forbs (such as *Aegopodium podagraria*, *Galium album*, *Senecio* aggr.) and tall grass (such as *Alopecurus pratensis*, *Elytrigia repens)* were more abundant under U treatment. *Dactylis glomerata*, *Festuca rubra* aggr. and *Phleum pratense* were largely supported by both grazing treatments (IG and EG), while *Agrostis capillaris*, *Taraxacum* spp., *Trifolium repens*, *Ranunculus acris* and *Cirsium vulgare* were supported by both cut treatments (ICG and ECG). Overall, this study benefits from the long-term experimental data. Due to the multifunctionality of grasslands, environmental and biodiversity outputs require long-term studies, since processes in soil, vegetation and microorganisms are long-term in relation to any change in management (Lemaire, 2007).

Regarding the applicability of our results to other grazing animals in different grassland types, more research may be necessary due to differences in site conditions such as climate, plant composition, biomass productivity and anatomy of the grazing animal. For instance, cattle and sheep have different requirements for forage quality and selectivity which can be influenced by vegetation composition and diversity (Wrage et al., 2011). Furthermore, characteristic anatomical differences such as in the mouth and tongue allow sheep to graze close to the ground on top of their considerable selectivity for high-quality plants (Rook et al., 2004). Hence, these grazing differences between different grazers may have different effects on the vertical distribution and require further investigation.

5 | CONCLUSION

The final composition of functional groups 15 years after the introduction of management at the experimental site was similar to that reached in the first three years, although large temporal fluctuations were still observed subsequently. Long-term studies are therefore needed to evaluate changes in community structure. Treatments significantly affected total biomass and upper-layer biomass of all functional groups and the vertical distributions within swards of most groups. In addition, large proportions of biomass from all functional groups (except mosses and legumes) were allocated to the upper layer in undefoliated swards and swards under extensive management. Intensity of management was found to be the key driver affecting the vertical distribution of the groups, whereas type of defoliation (grazing or cutting) had little effect. Although similar patterns were observed between upper biomass, total and the ratios, the trends are much more pronounced in the upper layer when the bottom layer biomass was excluded from the analysis. Given the high proportion of live biomass in the upper layer and the high proportion of dead biomass in the lower layer, we suggest that careful biomass sampling procedures are needed to take account of differences in the different layers of a sward, and thereby ensure accurate results are provided to support appropriate management strategies for both agricultural utilization and other objectives such as nature conservation.

AUTHOR CONTRIBUTIONS

VP and LP conceived the study; PB, VP and TK designed the methodology; VP and LP collected the data; TK and PB analysed and interpreted the data; TK, VP, KP, and PB wrote and edited the manuscript. All authors contributed critically to the manuscript and gave approval for publication.

DATA AVAILABILITY STATEMENT

Data necessary to reproduce all results and figures are available in Appendix S6.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix S1. (a) Monthly precipitation and (b) mean monthly temperature recorded at the study site

Appendix S2. Plant-available pH/$CaCl_2$, P, K, Ca, Mg under each treatment for the year 2016

Appendix S3. Mean botanical composition (%) of the most abundant graminoids, legumes and forbs for the years 2001–2012

Appendix S4. Partial principal components analysis (pPCA) of the plant functional groups for the upper (>3 cm) and lower (<3 cm) sward layers, from 1998 to 2012

Appendix S5. The effect of treatments on dry matter standing biomass for the different functional groups showing changes over the period 1998 to 2012

Appendix S6. Primary data to reproduce all results and figures

How to cite this article: Kassahun T, Pavlů K, Pavlů VV, Pavlů L, Blažek P. Effect of 15-year sward management on vertical distribution of plant functional groups in a semi-natural perennial grassland of central Europe. *Appl Veg Sci*. 2021;24:e12568. https://doi.org/10.1111/avsc.12568

Chapter 3

Effect of grazing intensity and dung on herbage and soil nutriernts

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Published as:

Pavlů K., **Kassahun T**., Nwaogu C., Pavlů L., Gaisler J., Homolka P., Pavlů V. (2019): Effect of grazing intensity and dung on herbage and soil nutrients. Plant Soil Environ., 65: 343–348. https://doi.org/10.17221/177/2019-PSE

https://doi.org/10.17221/177/2019-PSE

Effect of grazing intensity and dung on herbage and soil nutrients

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Citation: Pavlů K., Kassahun T., Nwaogu C., Pavlů L., Gaisler J., Homolka P., Pavlů V. (2019): Effect of grazing intensity and dung on herbage and soil nutrients. Plant Soil Environ., 65: 343–348.

Abstract: Dung deposited by grazing animals is a key driver affecting sward structure and nutrient cycling in pastures. We tested herbage and soil properties in three types of tall sward-height patches (> 10 cm): (i) patches with dung under intensive grazing; (ii) patches with dung under extensive grazing; and (iii) patches with no dung under extensive grazing. These patches were compared with grazed swards under intensive and extensive grazing. Analyses indicated no significant effect of different types of patches on plant available nutrients. Herbage nutrient concentrations from the different types of patches differed significantly. The highest concentrations of nitrogen (30.65 g/kg), phosphorus (4.51 g/kg) and potassium (22.06 g/kg) in the herbage dry matter were in the tall patches with dung presence under intensive grazing regime because of nutrients from dung utilized for sward regrowth. Regardless of dung presence, similar herbage nutrient concentrations were revealed in non-grazed tall sward-height patches in extensive grazing regime. The presence of dung did not have any effect on the plant available nutrients in any type of patches, therefore we suppose that non-utilized nutrients were probably leached, volatilised or transformed into unavailable forms and thus soil nutrient enrichment was low.

Keywords: heifer grazing; faeces; grassland; grazing management; plant-soil relationship

Selective defoliation by grazing, which is mainly due to dietary choice, is one of the main mechanisms by which grazing animals contribute to sward heterogeneity. Grazing changes the competitive advantage among plant species through the selective removal of plant biomass (Bullock and Marriot 2000), it opens spaces for gap-colonizing species, and there is contamination of the sward surface by the animals' dung and urine which decreases the amount of forage available for grazing (Bokdam 2001). Furthermore, as the level of contamination increases, there is increased rejection by grazing animals, especially in the immediate vicinity of dung pats (Forbes and Hodgson 1985). Dung deposition, in combination with other grazing-related effects such as trampling, is an important factor that can explain the structure of vegetation in the pasture (Kohler et al. 2004). It also has a significant effect on the chemical status of the soil and serves as a potential source of available nutrients for plants (Aarons et al. 2004).

Supported by the Ministry of Agriculture of the Czech Republic, Project No. RO0417. This paper was finalized under support from Interreg VA SN/CZ (100264999-DiverGrass) and by the Internal Grant Agency – Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Project No. 20194211. The contributions of Klára Pavlů and Petr Homolka were supported by the Ministry of Agriculture of the Czech Republic, Project No. RO0717.

https://doi.org/10.17221/177/2019-PSE

Cattle generally show a grazing preference for shorter (< 10 cm) herbage patches rather than taller (> 10 cm) patches, which are mostly left ungrazed as their biomass is usually of lower feed value. This differentiation of patches into short and tall height is commonly observed in temperate grasslands (Ludvíková et al. 2015). Cattle avoid areas with tall-stem herbage where the leafy components of the sward are difficult to graze (De Vries and Daleboudt 1994) and also areas that have been contaminated by dung (MacDiarmid and Watkin 1972b). Several studies have been conducted that have focused on the effects of dung patches about botanical composition and nutrients (MacDiarmid and Watkin 1971, 1972a, Aarons et al. 2009, White-Leech et al. 2013). However, there has been little research focusing on patches of different heights in swards in terms of the concentrations of nutrients in the herbage and the soil, particularly in Central Europe, where only preliminary analyses are available (Pavlů et al. 2018).

Therefore, our goal was to determine the effects of different intensities of grazing by heifers on the nutrient concentrations in the herbage and the soil under tall sward-height patches in Central European *Agrostis capillaris* grassland. We aimed to answer the following questions: (i) what is the effect of the presence of dung on nutrient concentrations of soil beneath tall sward-height patches under intensive and extensive grazing management?; (ii) what is the effect of the presence of dung on dry matter standing biomass, dry matter (DM) content, dead biomass, and nutrient concentrations in the herbage?, and (iii) is there any relationship between soil nutrient concentrations and herbage nutrient concentrations under the tall sward-height patches?

MATERIAL AND METHODS

Study site. The study site of the 'Oldřichov Grazing Experiment' is located in the Jizerské hory (Jizera Mountains) in the northern Czech Republic, 10 km north of the city of Liberec (50°50.34'N, 15°05.36'E; 420 m a.s.l.). The experimental site was established in 1998 and had a mean annual temperature of 7.2°C and average annual precipitation of 803 mm (Liberec Meteorological Station). The site has a medium deep (10–15 cm) brown sandy soil (Cambisol, with less than 10% of clay, i.e., particle size fraction < 0.01 mm) and is underlain by granite bedrock. The sward on the experimental site has a high diversity of plant species, with about 24 vascular plant species per m^2. The dominant species are *Agrostis capillaris*, *Festuca rubra* agg., *Trifolium repens*, and *Taraxacum officinale.*

Experimental design and plot management. The experimental site was established as two completely randomized blocks. Each block consisted of four paddocks with different grazing regimes, and each experimental paddock was approximately 0.35 ha (Ludvíková et al. 2015). For this study, we selected two paddocks in each block, with two contrasting levels of grazing intensity: (i) extensive grazing (EG), with a mean target sward surface height of greater than 10 cm; and (ii) intensive grazing (IG) with a mean target sward surface height of less than 5 cm. Target sward heights were achieved by increasing or decreasing the area available for grazing by moving fences with a set number of stock per plot for IG or EG. All paddocks were grazed under continuous stocking by young heifers (Czech Fleckvieh) of initial live weights of about 200 kg, from early May until late October.

Herbage and soil data collection. Sward height measurement, herbage biomass, and soil samples were taken late in the grazing season on 18 September 2013. For this study, we identified three types of tall sward-height patches and two types of grazed patches: (i) IG_TF – tall patches in IG with presence of residual spring dung; (ii) EG_TF – tall patches in EG with presence of residual spring dung; (iii) EG_T0 – tall patches in EG without presence of residual spring dung; (iv) IG_C – grazed patches in IG; (v) EG_C – grazed patches in EG (for details see Table 1). For the IG regime, we were unable to find any presence of the tall sward-height patches without dung.

Four replications of the presented sward-height patches were randomly taken in each of two paddocks in the block. A total 40 of soil (each in 10 subsamples) and 40 herbage samples were then collected. Since the sward had a canopy height of > 10 cm in the EG regime, visual identification of dung presence was required. In spring, fresh dung deposits were 20–30 cm in diameter and weighed about 1 kg, with 15–20% DM content. The mean values of nutrient concentrations in the spring dung of heifers regardless of treatment were 21.1, 6.6, 7.7, 18.5 and 4.3 g/kg for N, P, K, Ca and Mg, respectively (V. Ludvíková unpublished data). To characterize sward height and patch type distribution in IG and EG, 100 measurements were taken along a transect in four paddocks of both regimes (400 measurements in total). At each sward height measurement, visual identification of the patch type was carried out simultaneously.

https://doi.org/10.17221/177/2019-PSE

Table 1. Description of the sward height patches and their management

Patch abbreviation terms used in text	Grazing management	Target average sward height (cm)	Patch type	Dung presence	Stocking rate (kg live weight per ha)	Patches percentage of total area
IG_C	intensive grazing	< 5	grazed	–	1000	95.0
IG_TF	intensive grazing	< 5	non-grazed or infrequently grazed tall sward patches > 10 cm	+	1000	5.0
EG_C	extensive grazing	> 10	grazed	–	500	92.5
EG_TF	extensive grazing	> 10	non-grazed or infrequently grazed tall sward patches > 10 cm	+	500	4.5
EG_T0	extensive grazing	> 10	non-grazed or infrequently grazed tall sward patches > 10 cm	–	500	3.0

The height of the sward along a transect in four paddocks and selected patches was measured using a rising plate meter (Correll et al. 2003). Using a circular ring of 30 cm in diameter on each type of patch, the proportion (as %) of dead plant biomass was assessed by visual observation; herbage biomass was then cut to ground level. The harvested herbage was weighed fresh, oven dried at 80°C, and the DM content and dry matter standing biomass (DMSB) were determined. Under each patch, any dung deposits present were removed, and soil samples were taken from the upper 10 cm of the soil profile using an auger, and the biomass residues and roots were removed. The soil samples were air dried and then ground to pass a 2 mm sieve.

The herbage concentrations of N, P, K, Ca, and Mg were determined after digestion of DM herbage in *aqua regia* by inductively coupled plasma-optical emission spectrometry (GBC Scientific Equipment Pty Ltd, Melbourne, Australia). Plant available P, K, Ca, Mg were extracted by Mehlich 3 (Mehlich 1984). Total nitrogen (N_{tot}) was determined by the Kjeldahl method and organic carbon content (C_{org}) by means of colorimetry (AOAC 1984). Determination for pH_{CaCl_2} was done using pH meter acidometer (Sentron, Wellinq, Leek, the Netherlands). All chemical analyses for soil and herbage were performed in an accredited laboratory at the Crop Research Institute in Chomutov.

Data analysis. A linear mixed-effects model with fixed effects of treatment and random effect of the block was used to analyse the effect of different type of patches on concentrations of each individual nutrient in the soil and the herbage, DMSB, sward height (SH), DM content, and proportion of dead biomass. Post hoc comparison using the Tukey *HSD* (honestly significant difference) test was applied to identify significant differences among different types of patches. In some cases, normality and homogeneity in data were achieved by applying the logarithmic transformation. Finally, linear regression analysis was used to identify the relationship between plant available nutrients in the soil and the nutrient contents in the herbage. All univariate analyses were performed using Statistica 13.1 (Dell Inc. 2016).

RESULTS AND DISCUSSION

Frequency of distribution of sward heights during the sampling under IG and EG is shown in Figure 1 and reflected the presence of different patches under the various types of management (Tonn et al. 2019). The highest values for SH, DM content and DMSB were found under EG_T0 and EG_TF patches, and the highest values for dead biomass under EG_T0 and EG_C (Table 2).

Based on the average amount of dung, their nutrient concentrations and area of coverage, the amounts of nutrients supplied in individual dung patches were calculated as follows: 40–60 g N/m^2, 14–20 g P/m^2, 16–25 g K/m^2, 40–60 g Ca/m^2 and 10–14 g Mg/m^2. These values are approximately half than those reported for cows by Whitehead (2000), differences which may be explained by the different types of grazed sward, supplementary feeding, weight, and age of animals and breed. However, this over-fertilization by faeces had a significant effect on herbage but not on soil properties.

https://doi.org/10.17221/177/2019-PSE

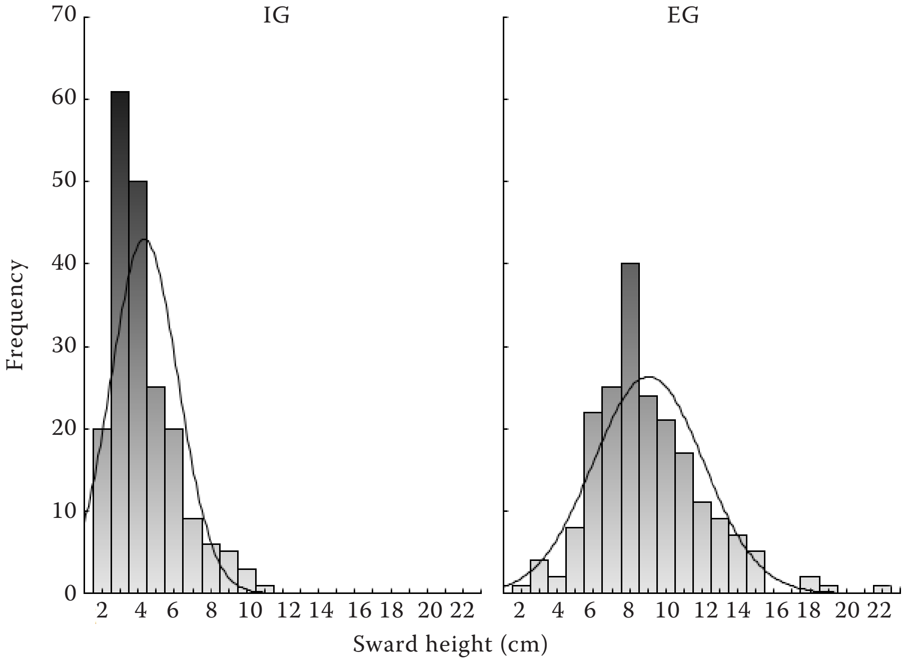

Figure 1. Frequency of distribution showing sward height variation in intensive grazing (IG) and extensive grazing (EG) treatments

Table 2. Sward characteristics and herbage nutrient concentrations of different sward height patches

	Tall sward-height patches			Grazed patches		*F*-ratio	*P*-value
	IG_TF	EG_TF	EG_T0	IG_C	EG_C		
SH (cm)	10.00 ± 0.46[b]	14.00 ± 0.98[a]	15.37 ± 0.98[a]	3.63 ± 0.26[c]	10.38 ± 0.63[b]	39.00	< 0.001
DM (%)	18.09 ± 0.68[b]	24.13 ± 0.72[a]	27.41 ± 1.27[a]	10.48 ± 0.32[c]	18.53 ± 1.14[b]	58.46	< 0.001
DMSB (g/m^2)	358.58 ±77.93[b]	548.29 ± 57.42[a]	707.43 ± 90.73[a]	79.03 ± 8.18[c]	254.91 ± 12.23[b]	47.37	< 0.001
Dead biomass (%)	8.38 ± 2.38[c]	24.38 ± 2.58[b]	32.50 ± 0.94[a]	1.63 ± 0.26[c]	28.75 ± 1.83[ab]	53.28	< 0.001
Herbage nutrient							
N (g/kg DM)	30.65 ± 2.96[a]	18.68 ± 0.40[cd]	16.68 ± 0.34[d]	25.49 ± 0.67[ab]	22.56 ± 0.39[bc]	21.48	< 0.001
P (g/kg DM)	4.51 ± 0.28[a]	2.75 ± 0.08[bc]	2.40 ± 0.09[bc]	2.96 ± 0.05[b]	2.75 ± 0.07[bc]	34.89	< 0.001
K (g/kg DM)	22.06 ± 1.66[a]	14.73 ± 1.30[b]	11.87 ± 0.63[b]	11.79 ± 0.92[b]	12.53 ± 0.68[b]	12.25	< 0.001
Ca (g/kg DM)	6.14 ± 0.37[b]	7.24 ± 0.63[ab]	6.12 ± 0.46[b]	9.14 ± 0.70[a]	6.92 ± 0.51[ab]	4.97	0.003
Mg (g/kg DM)	2.69 ± 0.17[a]	1.97 ± 0.15[b]	1.75 ± 0.11[b]	2.84 ± 0.19[a]	2.01 ± 0.12[b]	11.41	< 0.001
N:P	6.81 ± 0.57[c]	6.83 ± 0.20[c]	6.98 ± 0.22[bc]	8.62 ± 0.22[a]	8.27 ± 0.32[ab]	6.82	< 0.001
N:K	1.39 ± 0.09[b]	1.34 ± 0.11[b]	1.43 ± 0.07[b]	2.28 ± 0.22[a]	1.84 ± 0.10[ab]	9.62	< 0.001
K:P	4.97 ± 0.41	5.41 ± 0.54	4.97 ± 0.28	3.98 ± 0.29	4.56 ± 0.26	2.23	0.086
Ca:P	1.38 ± 0.08[b]	2.64 ± 0.23[a]	2.54 ± 0.15[a]	3.09 ± 0.23[a]	2.52 ± 0.18[a]	12.27	< 0.001
Total amount of nutrients in herbage per area							
N (g/m^2)	10.66 ± 2.76[ab]	10.30 ± 1.18[a]	11.82 ± 1.58[a]	2.01 ± 0.20[c]	5.74 ± 0.27[b]	30.52	< 0.001
P (g/m^2)	1.52 ± 0.27[a]	1.49 ± 0.14[a]	1.72 ± 0.26[a]	0.24 ± 0.03[c]	0.70 ± 0.04[b]	24.82	< 0.001
K (g/m^2)	8.10 ± 2.33[a]	7.97 ± 1.13[a]	8.42 ± 1.19[a]	0.92 ± 0.11[c]	3.22 ± 0.26[b]	33.59	< 0.001
Ca (g/m^2)	2.16 ± 0.44[b]	3.97 ± 0.52[a]	4.37 ± 0.73[a]	0.73 ± 0.10[c]	1.77 ± 0.16[b]	21.76	< 0.001
Mg (g/m^2)	0.95 ± 0.20[ab]	1.11 ± 0.17[a]	1.23 ± 0.17[a]	0.23 ± 0.04[c]	0.51 ± 0.04[bc]	13.65	< 0.001

Numbers represent average values of patches; ± values represent standard error of the mean. *F*-ratio – *F*-statistics for the test of a particular analysis; *P*-value – corresponding probability value. Significant differences ($P < 0.05$) between patches according to Tukey's post-hoc test are indicated by different letters in the row. Abbreviations for the type of patches see Table 1. SH – sward height; DM – dry matter content; DMSB – dry matter standing biomass

https://doi.org/10.17221/177/2019-PSE

The highest N, P, K concentrations in the herbage were revealed in IG_TF patches, whereas the highest Ca and Mg concentrations were found in IG_C patches (Table 2). The presence of dung under tall sward-height patches in extensive grazing had no influence either on the DM content and DMSB or on N, P, K concentrations in the herbage (Table 2). We can suppose that released nutrients from dung were predominantly leached from the sandy soil and partly volatized as NH_3 from this type of dung patch. The youngest sward was under IG_C patches with the lowest SH, DM, DMSB, and dead biomass. Although herbage at early stages of maturity usually has very high nutrient concentrations (Duru and Ducrocq 1996, Pavlů and Velich 1998), the highest N, P, K concentrations in the herbage were found not in IG_C but IG_TF patches. It was caused by the nutrients released from dung under the IG_TF patches. Therefore, regardless of maturity, the key driver for N, P, K concentrations in the herbage under intensive grazing was the presence of faeces.

The highest concentrations of Mg in the herbage in both patches under intensive grazing regardless of dung presence (IG_C and IG_TF) as well as the highest Ca concentration in IG_C patches could be connected to a higher proportion of white clover (*T. repens*) and dandelion (*T. officinale*) in the sward (Ludvíková et al. 2015). These prostrate herbs have been reported to have high concentrations of Mg and Ca in the herbage (Whitehead 2000). Therefore, higher uptake of Mg and Ca by plants could also be the reason for the tendency of lower Ca and Mg concentrations in the soil under IG_C patches. Herbage in all tall sward-height patches accumulated more nutrients (N, P, K, Mg) on a per-m^2 basis (Table 2) than herbage in frequently grazed patches as nutrients were removed from tall patches by grazing animals only marginally.

Type of patch did not show any significant effect on the concentrations of N_{tot}, C_{org}, and plant available nutrients P, K, Ca, and Mg in the soil (Table 3). The higher C:N ratio and lower pH in the soil, and ratios of N:P and N:K in the herbage of both types of grazed patches is probably connected with higher amounts of nitrogen used for sward regrowth after grazing. The regression analysis showed no relationship between the concentrations of nutrients in the soil and the herbage. Similarly, Dickinson and Craig (1990) suggested nutrient losses from dung are not necessarily associated with increases in nutrients in the soil and argued that the nutrients might have been used immediately by the plants under the dung as soon as they were released from the dung. However, other studies have reported direct positive effects of dung-derived nutrients on the nutrient concentrations in the soil (MacDiarmid and Watkin 1972a, Aarons et al. 2009, Yoshitake et al. 2014) or herbage (Scheile et al. 2018). The inconsistencies in results might be attributed to nutrient mobility through the soil sampling depth, or to differences among types of grassland ecosystems, grazing management, soil type, differences in plant species, and environmental factors.

We can conclude that the intensity of grazing management can influence the utilization of nutrients released from dung. The intensive grazing supported

Table 3. Soil chemical properties under different sward height patches: pH_{CaCl_2}, total nitrogen (N_{tot}), organic carbon (C_{org}), plant available (Mehlich 3) concentration of P, K, Ca, Mg and C:N ratio in 0–10 cm layer

Soil chemical properties	Tall sward-height patches			Grazed patches		*F*-ratio	*P*-value
	IG_TF	EG_TF	EG_T0	IG_C	EG_C		
pH_{CaCl_2}	5.49 ± 0.06^{a}	5.62 ± 0.20^{a}	5.27 ± 0.06^{ab}	4.91 ± 0.07^{b}	5.06 ± 0.07^{b}	7.80	< 0.001
N_{tot} (mg/kg)	5066 ± 101	5041 ± 171	4886 ±1 87	4876.80 ± 190	5068.23 ± 255	0.27	0.897
P (mg/kg)	53.72 ± 7.37	41.40 ± 4.31	47.24 ± 6.78	51.36 ± 6.82	52.36 ± 7.15	0.56	0.693
K (mg/kg)	226.42 ± 38.23	192.12 ± 15.97	191.77 ± 14.63	156.47 ± 18.69	173.14 ± 18.96	1.49	0.228
Ca (mg/kg)	1910 ± 123	2016 ± 192	1830 ± 131	1470 ± 111	2036 ± 142	2.52	0.060
Mg (mg/kg)	178.46 ± 16.31	166.23 ± 22.70	152.38 ± 16.23	113.60 ± 12.52	159.93 ± 14.96	2.21	0.089
C_{org} (mg/kg)	49 838 ± 1047	53 800 ± 1528	52 563 ± 1955	48 655 ± 2466	54 892 ± 2736	1.66	0.181
C:N	9.84 ± 0.32^{c}	10.69 ± 0.32^{bc}	10.77 ± 0.32^{bc}	11.34 ± 0.26^{ab}	12.65 ± 0.61^{a}	11.54	< 0.001

Numbers represent average values of patches; ± values represent standard error of the mean. *F*-ratio – *F*-statistics for the test of a particular analysis; *P*-value – corresponding probability value. Significant differences ($P < 0.05$) between patches according to Tukey's post-hoc test are indicated by different letters in the row. Abbreviations for the type of patches see Table 1

https://doi.org/10.17221/177/2019-PSE

the frequency of defoliation, therefore some nutrients from dung were utilized for regrowth of the sward. In contrast to previous research, the presence of dung did not have any influence on the soil nutrient concentrations in any type of patches. Therefore we suppose that the non-utilized nutrients were either leached or volatilized, and thus soil nutrient enrichment was very low. The higher intensity of grazing can increase the utilization of nutrients from dung and can support higher forage production per area.

Acknowledgment

We would like to thank Crop Research Institute, Grassland Research Station Liberec employees for the support during the filed visits and laboratory analysis.

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Received on March 27, 2019
Accepted on June 25, 2019
Published online on July 14, 2019

Chapter 4

Restoration management of cattle resting place in mountain grassland

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Published as:

Kassahun T, Pavlů K, Pavlů V, Pavlů L, Novak J, Blažek P (2021) Restoration management of cattle resting place in mountain grassland. PLoS ONE 16(4): e0249445. https://doi.org/10.1371/journal.pone.0249445

PLOS ONE

RESEARCH ARTICLE

Restoration management of cattle resting place in mountain grassland

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OPEN ACCESS

Citation: Kassahun T, Pavlů K, Pavlů V, Pavlů L, Novak J, Blažek P (2021) Restoration management of cattle resting place in mountain grassland. PLoS ONE 16(4): e0249445. https://doi.org/10.1371/journal.pone.0249445

Editor: Tunira Bhadauria, Feroze Gandhi Degree College, INDIA

Received: September 30, 2020

Accepted: March 18, 2021

Published: April 1, 2021

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: This research was conducted under support from the Slovak Research and Development Agency (1/3453/06, https://www.apvv.sk/?lang=en); J.N. This paper was written under support from the Internal Grant Agency of Faculty of Environmental Sciences, Czech University of Life Sciences Prague, "Vertical and Horizontal sward structure of species rich upland grassland under long-term grazing management",

Abstract

This study investigated the effect of restoration management of a weed-infested area, previously used as cattle resting place, on herbage production and nutrient concentrations in the soil and herbage. The experiment was undertaken from 2004 to 2011 at the National Park of Nízké Tatry, Slovakia. Three treatments were applied: (i) cutting twice per year, (ii) herbicide application, followed after three weeks by reseeding with a mixture of vascular plant species and then cut twice per year, and (iii) unmanaged. Treatments had significant effect on biomass production and concentration of nutrients in the soil and in herbage. Nutrient concentrations in herbage and in soil declined progressively under the cutting treatments and reached optimum ranges for dairy cattle at the end of the experiment when herbage N was less than 15 g kg^{-1} and herbage P was 3.4 g kg^{-1}. There was also a strong positive relationship under the cutting treatments between soil nutrient concentrations and herbage nutrient concentrations for N, P, K, Mg and Ca. Although the cutting management as well as the combination of herbicide application with cutting management reduced nutrient concentrations in the soil and in herbage, the nutrient concentrations remained relatively high. We can conclude that restoration of grassland covered with weedy species like *Urtica dioica* and *Rumex obtusifolius*, with excessive levels of soil nutrients, cannot be achieved just by cutting and herbicide application.

Introduction

Grasslands are one of the most important components of the landscape in temperate regions of Europe [1]. Although the development of grasslands, and semi-natural grasslands in particular, is largely related to the history of agricultural management, their existence faces serious threats from either intensification of management or from land abandonment. These threats have increased especially in recent decades [2]. It is widely assumed that when grazing is stopped and abandonment proceeds, a natural succession would take place leading to restoration of the land to its climax state, which is typically dominated by perennials [3].

No. 20194211(https://www.fzp.czu.cz/cs/r-6897-veda-a-vyzkum/r-7266-interni-granty); T.K., K.P., V.P. and the Ministry of Agriculture of the Czech Republic (RO0418, http://eagri.cz/public/web/mze/); V.P., L.P. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Unfortunately, this does not happen often and instead it remains dominated by annual species [4] and invasive annual weeds. Persistence of many annual species in grassland is further supported by the increased rate of nutrient turnover, which is facilitated by the invasion of exotic annual species [5]. This challenge is exacerbated in high-altitude grasslands that were previously managed by regular grazing or as resting places for cattle, where they typically receive excessive nutrient returns from cattle excreta.

Restoration of botanical composition of semi-natural grasslands in these situations requires a reduction in the cover of weed species and improved performance of the perennial native species. This requires an integrated approach using multiple techniques, such as mechanical disturbance, fire, and in some cases the use of herbicides [6]. Among the various methods, the use of herbicide has been found to be an effective way to reduce or control weeds in grassland ecosystems, especially when mechanical control is expected to be too damaging [7]. Different types of herbicides are used, sometimes with formulations designed to target specific species such as *Rumex* spp., and others that are non-selective. Glyphosate is one of the most frequently used herbicides in the global market due to its effectiveness, relatively low cost, and its broad-spectrum application [8]. When the objective is to increase native species abundance and richness, broadcast spraying of herbicides is recommended [9,10]. Other studies recommend application of herbicide before the introduction of native species in order to open the sward and thereby increase opportunities for greater seedling density and survival.

Since its introduction in the 1970s, glyphosate remained popular among farmers across the world due to its broad-spectrum weed control capability [11]. During these periods, several countries in Central Europe such as Slovakia were struggling with the challenge of managing invasive weed species. Unfortunately, herbicide was widely used and glyphosate was the chosen chemical. Several studies have been conducted documenting the sever effects glyphosate based herbicide products and its wide spread presence in aquatic and terrestrial environments [12]. Among the main concern regarding glyphosate is its negative effect on non-target plant tissues and unintended areas through process like off target herbicide movement and root uptake [11]. Other consequences of glyphosate include reduction in soil dwelling earthworms reproduction capacity [13], bringing behavioral change in honey bees [14] and affecting the growth of aquatic bacteria and microalgae [15]. When application of herbicide is considered as unsuitable (e.g. due to off-site effects) cutting or mowing is considered [16,17]. Cutting especially has several attributes that can help control weeds. It can arrest flowering of weeds and thereby minimize the production of seeds and breaking their life cycle, leading to their eradication, and it can also increase tillering in some grasses and promote defoliation tolerant species [18–20].

Although the negative effects of non-selective herbicide application is well documented, very little is known about the effects of herbicide application combined with cutting, on changes in the nutrient content in herbage and soil, especially in mountain grasslands that are normally managed by grazing or used as a resting place. When control of invasive plant species is planned, intervention measures or control methods must be assessed not only in terms of their effectiveness in removing targeted species but also their impact on the ecosystem [21]. Herbicides like glyphosate are normally sprayed directly on to growing plants, and never applied intentionally on to the soil. Nevertheless, in open swards especially, there is a high chance that a significant portion may reach the soil surface during application. This technique was widely used in Slovakia, to eradicate invasive species. Against this background, a study was conducted in a mountain grassland area in Slovakia that is covered with weedy species (*Rumex obtusifolius* and *Urtica dioica*). In order to attempt to restore the grassland to its previous status, treatments that included a restoration measure of cutting and of herbicide (glyphosate) application combined with cutting, followed by reseeding with mixed grass species were applied. These treatments were selected based on discussion with administrators and managers of the study site (National Park of Nízké Tatry, Slovakia) and the existing practice of defoliation (cutting) and herbicide application, which was widely used in the country during the study period. However, this approach raised a number of critically important questions that justified the monitoring of the site for 8 years and which are reported in this paper. These questions are: does cutting management, herbicide application, or a combination of both followed by reseeding have an effect on (i) herbage productivity; (ii) nutrient concentrations in herbage and soil, and (iii) how fast are nutrients depleted from the soil.

Materials and methods

Study site and experiment design

This study was conducted with approval from the Ministry of Environment of the Slovak Republic.

In 2004, a randomized block experiment was set up at 1140 m a.s.l. in the National Park of Nízké Tatry (48˚51.22´N, 19˚14.57´E), Slovakia. At the study site, the mean annual precipitation and temperature were 800 mm and 8˚C respectively. The snow cover, which is higher than 10 mm, is 160 days per year. The soil type is classified as cambisol, and as the depth of the soil increases the lower the proportion of clay and silt fraction and the higher the proportion of sand fraction. The most dominant species recorded in the experiment plots were *U. dioica*, and *R. obtusifolius*. The total cover (%) of forbs, grasses, legumes and the mean value of the most abundant species in the experiment site under each treatment for the year 2004 (start of the experiment) and 2011 (end of the experiment) are shown in Table 1.

Table 1. Total cover (%) of forbs, grasses, legumes and the cover (%) of the most abundant species in each treatment.

	2004	2011		
Species	Treatment			
	Baseline	U	2CH	2C
Achillea millefolium	0±0.00	0±0.00	8±0.57	5±0.57
Alchemilla vulgaris	0±0.00	0±0.00	5.25±0.57	3.75±1.15
Agrostis capillaris	0±0.00	0±0.00	0.75±0.57	4.5±0.57
Dactylis glomerata	1±0.33	0±0.00	3±0.00	1±0.00
Festuca pratensis	0±0.00	0±0.00	6.25±1.0	1.5±0.57
Festuca rubra ssp. rubra	0±0.00	0±0.00	4.5±1.15	1.5±0.57
Myosotis sylvatica	4±0.53	4.25±0.00	0±0.00	0±0.00
Phleum pratense	0±0.00	0±0.00	10±1.00	0±0.00
Poa pratensis	0±0.00	0±0.00	7.5±0.57	0±0.00
Poa trivialis	4±1.41	3.75±0.57	0±0.57	13.25±1.00
Ranunculus repens	0±0.00	0±0.00	0.5±0.57	9.25±1.00
Rumex obtusifolius	76.5±1.20	76±0.57	0±0.00	3±1.00
Taraxacum officinale agg.	0±0.00	0±0.00	6.5±0.57	7±1.53
Trifolium repens	0±0.00	0±0.00	23±1.15	25.5±1.53
Trisetum flavescens	0±0.00	0±0.00	11.25±0.57	5±1.00
Urtica dioica	14.5±0.83	15±0.00	0±0.00	0±0.00
Total cover of grass	**5±1.27**	**4.75±0.57**	**43.75±2.64**	**27.25±3.78**
Total cover of legumes	**0±0.00**	**0±0.00**	**27.5±0.57**	**27±1.73**
Total cover of forbs	**95±1.30**	**95.25±0.57**	**28.75±1.53**	**34±3.61**

Numbers represent mean values in unmanaged (U), cutting twice per year (2C) and herbicide application, after three weeks reseeded with grass mixture and cut twice per year (2CH) for the year 2004 and 2011. ± Value indicate Standard deviation (S.D.).

https://doi.org/10.1371/journal.pone.0249445.t001

The experimental site was previously used for grazing and then for herding of heifers. However, during the decade before 2004, it was abandoned without any grazing or cutting management. The experiment was arranged in four randomized blocks each with the following treatments: (i) Unmanaged (U), (ii) Cutting twice per year (2C), and (iii) Herbicide application and, after three weeks, it was reseeded with 18 mixture of vascular plant species (list of species see Table 2) and subsequently cut twice per year (2CH). Glyphosate (active substance IPA 480 g.l.; Roundup; Monsanto) herbicide was applied on to the leaves of plants at 3 l ha^{-1} (0.30 ml agent + 20 ml water on 1 m^2) with a sprayer in the spring of 2004. Altogether 12 (three treatments x four blocks) plots were established for the experiment with each plot measuring 15 m^2.

Herbage biomass production and herbage chemical properties

The above ground dry matter (DM) biomass production for the whole vegetation season was determined in each of the years 2005–2011. It was calculated as the sum of sampled DM biomass (harvested in the spring and autumn for 2C and 2CH treatments). The harvested biomass in each treatment was measured in sub plots each of 6 x 2.5 m within each of the 15 m^2 experimental plots. In each treatment plot, the above ground biomass was cut 3 cm above the ground. In order to avoid any residual effect of herbage collection from previous years, the sampling for the U treatment was conducted from different sub plots outside the designated experimental plots in each year. To determine the DM content of biomass, and thus the DM yield, the harvested herbage samples were weighed fresh, and oven dried at 80˚C.

Concentrations of N, P, K, Mg and Ca were determined from the herbage samples collected in autumn for the DM biomass determinations. The samples were used for analysis, after digestion in aqua regia by ICP-OES. The crude fibre was determined using Weende analysis [22].

Table 2. List of vascular plant species that were reseeded after application of herbicide on the 2CH treatment (herbicide application, then after three weeks reseeded with grass mixture and cut twice per year).

Species	Proportion of the mixture (%)
Dactylis glomerata L.	25.00
Festuca pratensis Huds.	10.00
Phleum pratense L.	10.00
Poa pratensis L.	10.00
Festuca rubra L.	5.00
Trisetum flavescens (L.) P Beauv.	5.00
Trifolium repens L.	15.00
Trifolium pratense L.	3.00
Lotus corniculatus L.	3.00
Plantago lanceolata L.	2.00
Achillea millefolium L.	2.00
Carum carvi L.	2.00
Taraxacum officinale Weber	2.00
Alchemilla vulgaris L.	2.00
Daucus carota L.	1.00
Acetosa pratensis Mill.	1.00
Leucanthemum vulgare Lam.	1.00
Prunella vulgaris L.	1.00

https://doi.org/10.1371/journal.pone.0249445.t002

Soil chemical properties

Every autumn (in September) after the last round of cutting, soil samples (consisting of three sub samples) were randomly collected from depths of 0–10 cm and 10–20 cm of the soil profile using an auger, from each of the 15 m^2 treatment plots for the years 2004 to 2011. The soil samples were oven dried at 100 ^{o}C, ground in a mortar, and sieved to 2 mm after removal of biomass residues and living roots. Soil pH was determined in potassium chloride solutions. Plant-available P, K, Mg, Ca were extracted by Mehlich III reagent [23]. Total Nitrogen (N_{tot}) was determined using the Kjeldahl method and soil organic carbon (C_{org}) using the oxidimetric method according to Tiurin.

Statistical analysis

A general linear model (GLM) with treatment as fixed effects, replication as a random effect and year as continuous predictor was used to identify the effect of year, treatment and the year x treatment interaction, on nutrient concentrations in the herbage and in the soil for the whole experiment period. One-way ANOVA followed by Tukey HSD test was used to identify significant differences between treatments for chemical properties of soil and herbage for the last year of the experiment (2011). In order to control for false-discovery rate (FDR), we applied Benjamini-Hochberg's procedure [24]. All univariate analyses were performed using Statistica 13.1 [25].

To illustrate the influence of treatments on nutrient concentration of the soil and the herbage over the entire experiment period, a partial principal component analysis (pPCA) with replication as covariate was conducted. Canoco 5 was used to perform pPCA [26]. Moreover, to identify the relationship between plant available nutrients in the soil and the nutrient contents in the herbage a linear regression analysis was applied.

Results

Herbage biomass production

As anticipated, the data on DM biomass showed considerable annual variation especially during the early stages of the experiment. The response of biomass production to treatments resulted in statistically significant differences between U, 2C, and 2CH treatments. The GLM analysis showed that DM biomass was significantly affected by year and treatment ($P<0.001$) as well as the interaction of year x treatment ($P<0.001$) (Table 3). From 2005 to 2011, the mean annual values of herbage biomass production were as follows: 7.1 t ha^{-1} (U), 6.3 t ha^{-1} (2C) and 5.9 t ha^{-1} (2CH). Total DM biomass remained above 7 t ha^{-1} under the U treatment and remained stable during the entire experiment period, while under 2C treatment it slowly but continuously declined from approximately 7 to 6 t ha^{-1} (Fig 1). A large increase in DM biomass was observed under the 2CH treatment, from 2.5 to 6.5 t ha^{-1} at the beginning of the experiment, and it then stabilized at 6.3 t ha^{-1} (Fig 1). During the 7 years of biomass sampling, DM biomass production was significantly higher and stable under U, but after 2 years of the experiment, the DM under the cut treatments (2C and 2 CH) also became stable (Fig 1).

Herbage chemical properties

The GLM analysis revealed a significant effect of treatment on herbage nutrient concentrations of P, Mg and Ca, but not on crude fiber (CF), N and K. However, a significant effect of the year, and the interaction of year x treatment, was recorded for all nutrient concentrations except CF (Table 3; Fig 2). The results of one-way ANOVA showed that treatment had an effect on all herbage chemical properties except on CF (Table 4).

Table 3. Result of GLM analysis (year, treatment, year x treatment) of herbage and soil chemical properties for the whole experiment period.

		Year		Treatment		Year x Treatment	
		F-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Herbage	DM (%)	8.23	**0.005**	29.36	**<0.001**	17.88	**<0.001**
	Crude Fibre	6.50	**0.013**	2.92	0.060	2.63	0.078
	N	253.67	**<0.001**	0.24	0.781	64.73	**<0.001**
	P	326.79	**<0.001**	17.33	**<0.001**	80.33	**<0.001**
	K	292.26	**<0.001**	0.08	0.923	71.54	**<0.001**
	Mg	31.13	**<0.001**	21.48	**<0.001**	8.12	**<0.001**
	Ca	51.63	**<0.001**	3.59	**0.032**	12.40	**<0.001**
Soil							
	N_{tot}	178.29	**<0.001**	0.31	0.737	49.01	**<0.001**
	P	76.99	**<0.001**	4.59	**0.013**	19.19	**<0.001**
	K	171.17	**<0.001**	1.16	0.318	49.12	**<0.001**
	Mg	67.08	**<0.001**	0.22	0.805	18.12	**<0.001**
	Ca	27.28	**<0.001**	1.71	0.181	3.53	**0.034**
	C_{org}	10.96	**<0.001**	0.02	0.980	3.92	**0.023**
	C: N	204.17	**<0.001**	1.81	0.170	48.12	**<0.001**
	pH/KCl	15.51	**<0.001**	5.08	**0.008**	3.49	**0.034**

F represents the value derived from *F* statistics in GLM and *P* represents the resulting probability value. Significant results (after table-wise Benjamini-Hochberg's FDR correction) are highlighted in bold.

https://doi.org/10.1371/journal.pone.0249445.t003

The mean concentration of N in herbage dry matter ranged from 14.56 g kg^{-1} (2C) to 30.73 g kg^{-1} (U) and the mean concentration of P ranged from 3.39 g kg^{-1} (2CH) to 4.37 g kg^{-1} (U). Similarly, the lowest concentrations of Mg and K were under treatment 2CH and the highest under treatment U, and ranged from 1.67 g kg^{-1} to 2.59 g kg^{-1} and 19.94 g kg^{-1} to 37.12 g kg^{-1}, respectively. The mean concentration of Ca ranged from 2.24 g kg^{-1} (2CH) to 5.24 g kg^{-1} (U) (Table 4).

During the course of the experiment, significant amounts of nutrients were removed in harvested herbage under the cutting treatments. The removal of nutrients at the beginning of the experiment was much greater than in the last year of sampling. For instance, 135 kg ha^{-1} of N, 21.59 kg ha^{-1} of P and 171.31 kg ha^{-1} of K were removed under the 2C treatment at the start of the experiment. In contrast only 60.15 kg ha^{-1} of N, 14.09 kg ha^{-1} of P and 87 kg ha^{-1} of K were removed under 2C in the last year of the experiment (Table 5). Under the 2CH treatment the amount of nutrient concentrations removed in the first year was the lowest compared to the other sampling years. This is consistent with the amount of herbage biomass produced in the same period, which was also low as the treatment was reseeded with grass mixture during that period.

Soil chemical properties

Concentrations of N_{tot}, C_{org}, the plant available nutrients K, Mg and Ca, and the C: N in the soil were not significantly affected by treatments. However, year and the interaction of year x treatment, showed significant effects on all concentrations (Table 3; Fig 3). The one-way ANOVA result showed treatment had a significant effect on the soil chemical properties at the end of the experiment (Table 4). The mean concentrations of N, P, K, Mg and pH/KCL were lowest under the cut treatments (2C and 2CH) and the highest under U treatment, and ranged from 3007.50 mg kg^{-1} to 6825 mg kg^{-1}, 75.04 mg kg^{-1} to 400.01 mg kg^{-1}, 250.10 mg kg^{-1} to

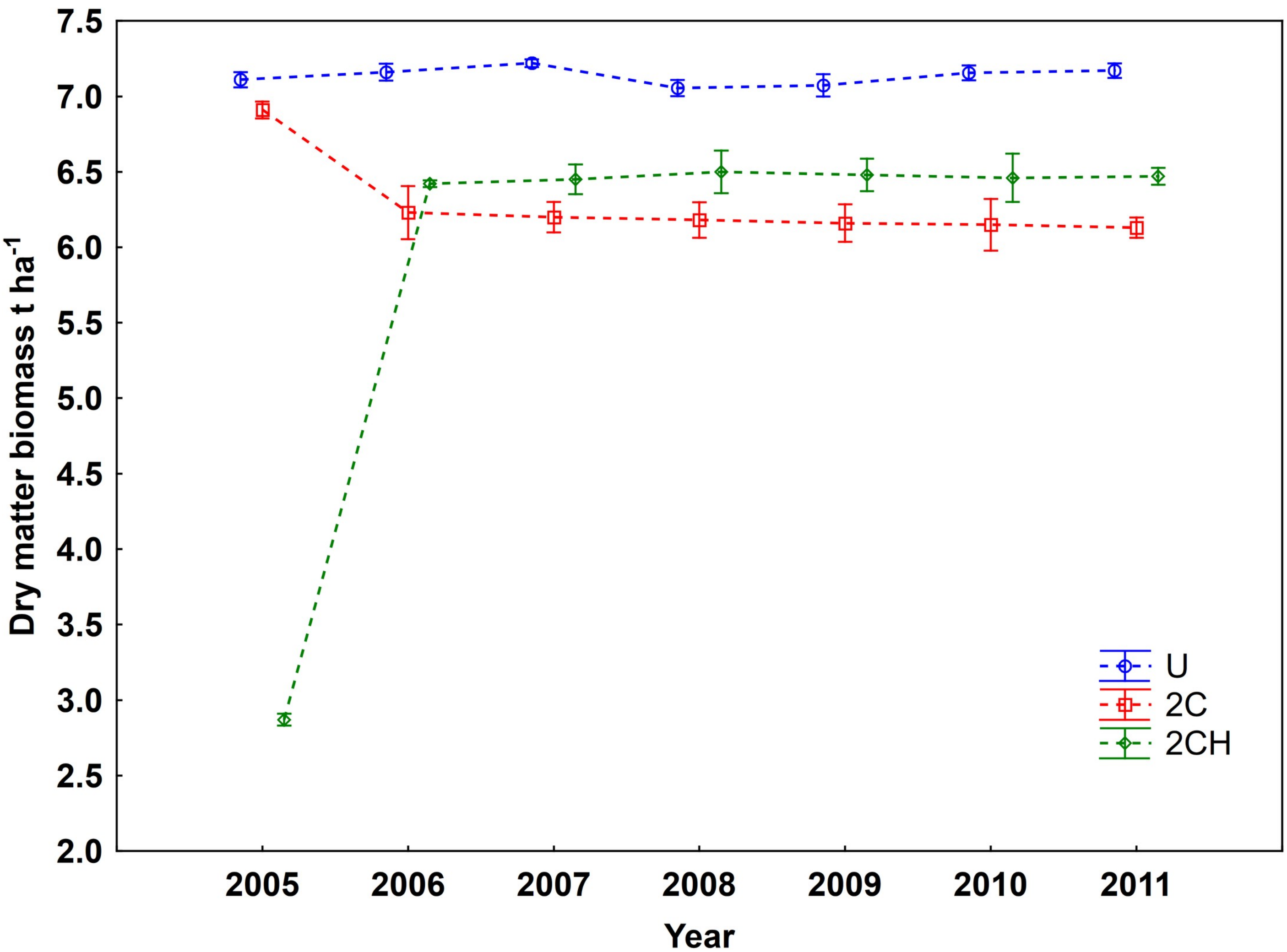

Fig 1. Dry matter biomass production in investigated treatments over the years 2005–2011. Error bars represent standard error of the mean (SE). For treatment abbreviation (U, 2C, 2CH) see Table 1.

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920.11 mg kg^{-1}, 197.50 mg kg^{-1} to 455.03 mg kg^{-1} and from 4.55 to 4.83 respectively. The C_{org} and the C:N ratio ranged from 50 220.11 (2C) to 60 810.01 (U) and 8.91 (U) to 16.71 (2C) respectively. The mean concentration of Ca ranged from 1455 mg kg^{-1} (2CH) to 2512 mg kg^{-1} (U) (Table 4).

Soil and herbage chemical properties

The pPCA analysis displayed the development and the decline of nutrient concentrations in the soil as well as in the herbage through the course of the experiment. The ordination showed nutrients under U treatment stable throughout the experiment period. In contrast, nutrient concentrations in the herbage and in the soil under the cutting treatments (2C and 2CH) declined starting from the second year, representing 64% of variation for the first axis. There were also small fluctuations in C:N and Ca in the soil as well as pH, representing about 10% of variation in the second axis (Fig 4).

In the cutting (2C and 2CH) treatments, the concentrations of N, P, K, Mg and Ca in the herbage increased with increasing concentrations of plant available N, P, K, Mg and Ca (Fig

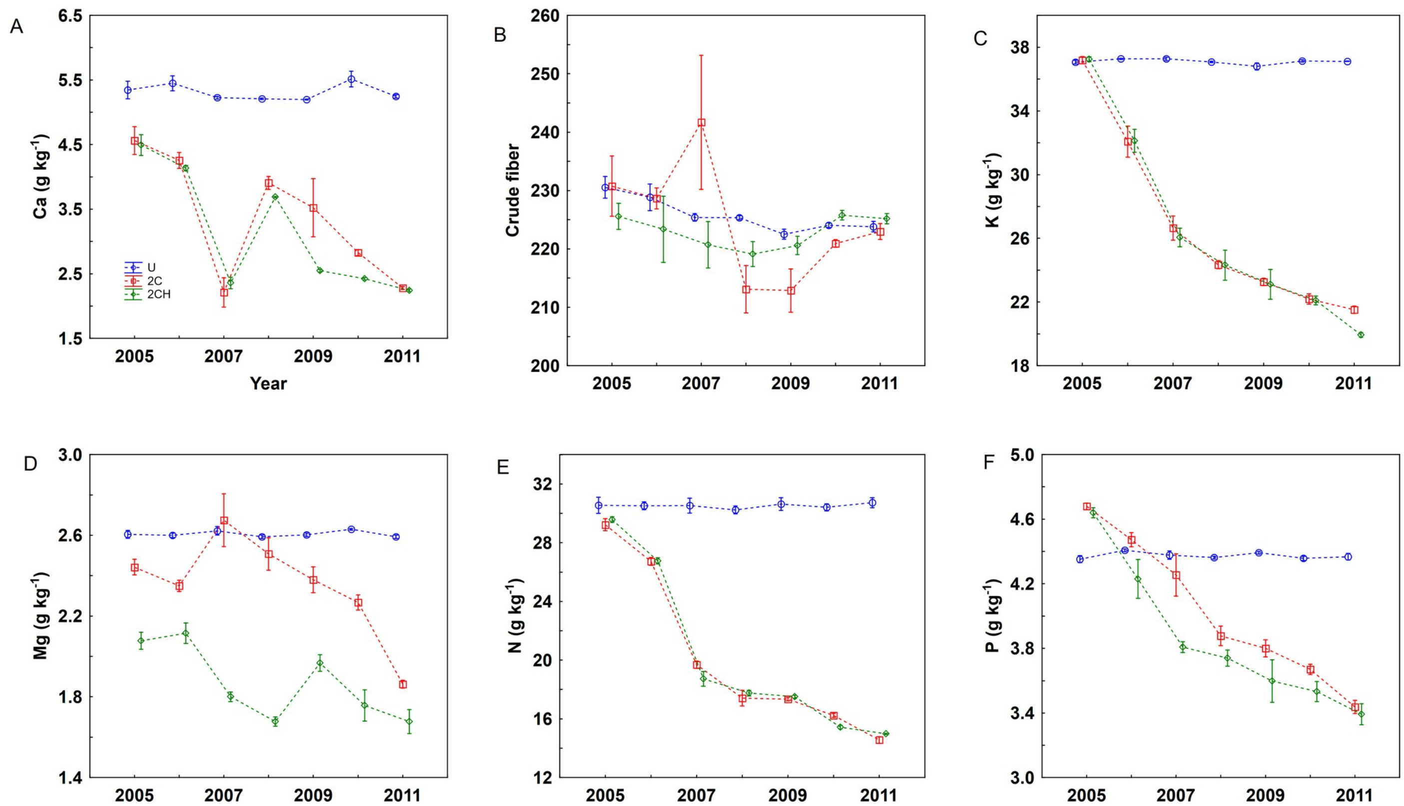

Fig 2. Concentration of Ca (A), Crude fiber (B), K (C), Mg (D), N (E) and P (F) in the herbage. Error bars represent standard error of the means (SE). For treatment abbreviation (U, 2C, 2CH) see Table 1.

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5). Under U treatment, the concentrations of Ca, K and N in the herbage was negatively related to the concentrations of plant available Ca, K and N (Fig 5A, 5C and 5E). In contrast, the concentrations of P and Mg in the U treatment were related positively, and similar to the cutting treatments (Fig 5B and 5D).

Discussion

Herbage biomass production

Based on the results from the studied site, we could classify the site as a productive grassland with herbage productivity ranging from 6 to 7.4 t ha^{-1} per year, which is very high for Central European conditions that normally exhibit only 2 to 4 t ha^{-1} per year [27]. Even though we observed a decline in nutrients (discussed later) resulting from the removal of biomass from cutting, the site still produced a high amount of herbage dry matter for Central European conditions. This may indicate a high nutrient reserve within the soil. The variation in the DM biomass production observed during the early period of the experiment could be attributed to climatic conditions such as temperature and precipitation distribution during the vegetation season, as well as the species composition, management applied and altitude [28,29]. Such variability in biomass production is expected and similar results have been reported in other long-term studies in Central Europe [30–32]. One major outcome from this study is that biomass production did not increase either in response to the cutting or to the combination of cutting and herbicide application. Rather it continued to slowly decline and it stabilized throughout the experiment period under the cutting treatments (2C and 2CH). The sharp rise

Table 4. Mean soil and herbage characteristics and mean dry matter biomass under the different treatments in 2011.

Characteristics	U	2C	2CH	*F*- ratio	*P*- value
Herbage nutrient					
CF g kg^{-1}	223.82±0.89	222.99±1.35	225.18±0.86	1.11	0.38
N g kg^{-1}	30.73±0.34 a	14.56±0.22 b	14.97±0.04 b	1501.01	**<0.001**
P g kg^{-1}	4.37±0.021 a	3.44±0.04 b	3.39±0.07 b	143.44	**<0.001**
K g kg^{-1}	37.12±0.02 a	21.51±0.23 b	19.94±0.16 c	3458.48	**<0.001**
Mg g kg^{-1}	2.59±0.01 a	1.86±0.02 b	1.67±0.06 c	181.49	**<0.001**
Ca g kg^{-1}	5.24±0.03 a	2.28±0.02 b	2.24±0.20 b	5647.75	**<0.001**
Soil Chemical Properties					
N_{tot} mg kg^{-1}	6825.01±128.41 a	3007.50±170.41 c	4075.11±155.91 b	166.63	**<0.001**
P mg kg^{-1}	400.01±7.07 a	75.04±2.88 b	135.00±26.29 b	119.62	**<0.001**
K mg kg^{-1}	920.11±1.66 a	267.50±12.50 b	250.10±18.25 b	893.58	**<0.001**
Mg mg kg^{-1}	455.03±17.08 a	197.50±7.50 b	222.51±19.31 b	83.92	**<0.001**
Ca mg kg^{-1}	2512.50±26.57 a	1455.01±79.74 b	2115.11±215.27 a	16.02	**<0.001**
C_{org}	60810.01±1057.88 a	50220.11±2616.81 b	66047.51±1573.98 a	18.67	**<0.001**
C: N	8.91±0.04 b	16.71±0.11 a	16.23±0.24 a	784.46	**<0.001**
pH/KCl	4.83±0.01 a	4.59±0.03 b	4.55±0.03 b	30.21	**<0.001**

F-ratio = *F*-statistics for the test of a particular analysis, *P*-value = corresponding probability value, d.f = (2, 9) in all tests. The numbers reflect the average of four replicates, ± standard error of the mean (SE). Significant results (after table-wise Benjamini-Hochberg's FDR correction) were highlighted in bold. Significant differences between treatments in Tukey test are indicated by different lower-case letters (alphabetic order represents decreasing values of means, i.e. a represents the largest mean). For treatment abbreviation (U, 2C, 2CH) see Table 1.

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in biomass production at the early stage of the experiment under 2CH treatment is most likely due to the effect of reseeding, which was done at the start of the experiment. Furthermore, the continued decline of N_{tot} and of plant available P and K in the soil (discussed later) also

Table 5. Amount of nutrients removed in the harvested biomass for the years 2005 to 2011.

Year	Treatment	Nutrients				
		N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Mg (kg ha^{-1})	Ca (kg ha^{-1})
2005	2C	135.97	21.59	171.31	11.251	21.30
	2CH	56.67	8.93	71.27	3.97	8.47
2006	2C	110.89	18.511	133.35	9.75	17.46
	2CH	114.83	17.92	137.47	9.05	17.82
2007	2C	82.13	17.654	112.02	11.05	9.49
	2CH	81.65	16.34	110.25	7.73	10.18
2008	2C	71.79	16.02	100.26	10.34	16.08
	2CH	77.30	16.29	105.53	7.26	15.98
2009	2C	71.13	15.57	95.49	9.77	14.48
	2CH	75.92	15.66	100.03	8.50	11.06
2010	2C	66.56	15.02	91.00	9.29	11.57
	2CH	66.58	15.24	95.17	7.58	10.48
2011	2C	60.15	14.09	87.89	7.61	9.28
	2CH	64.59	14.71	86.01	7.24	9.71
Total	2C	598.65	118.48	789.58	69.08	99.68
	2CH	537.59	105.12	707.53	51.36	83.70

Numbers represent average of four replicates. For treatment abbreviation (2C and 2CH) see Table 1.

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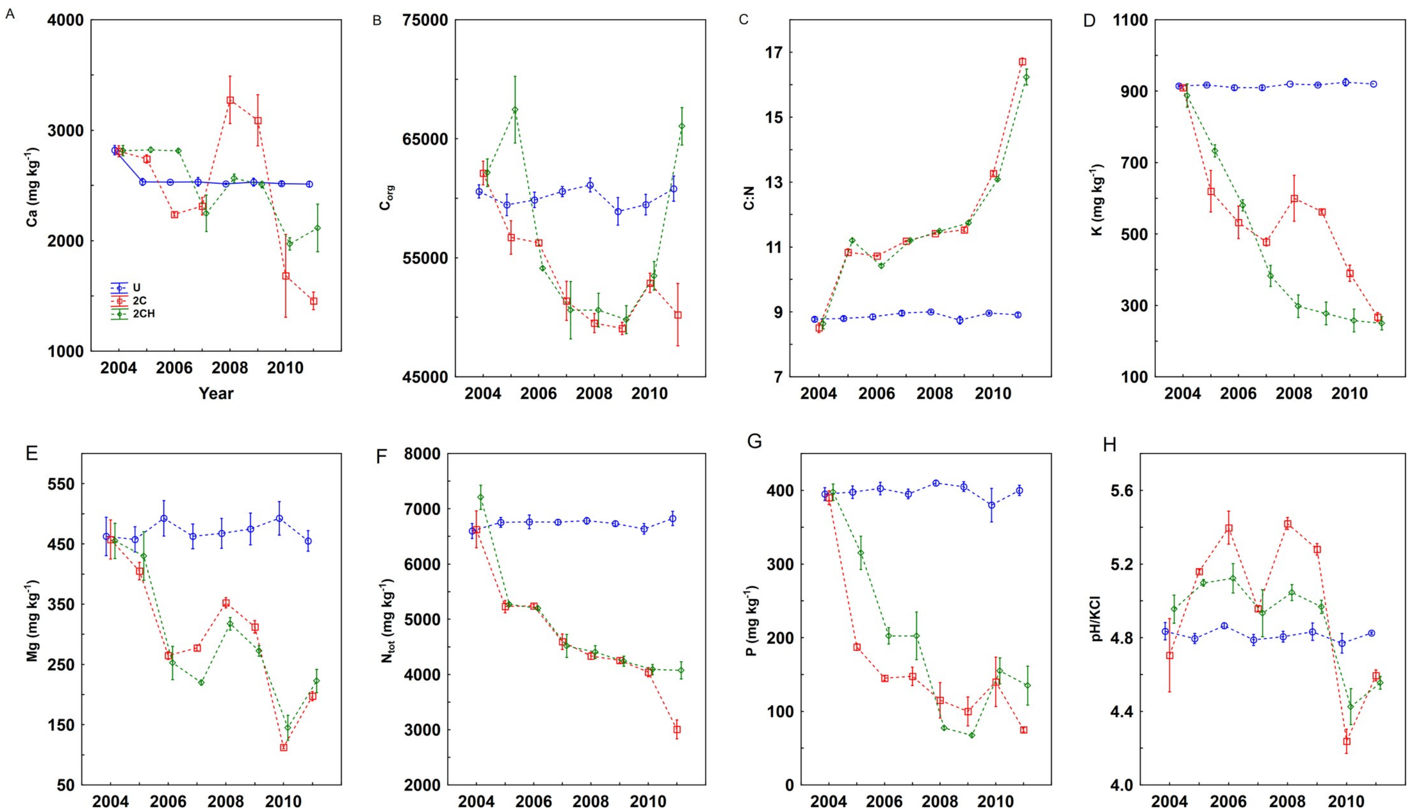

Fig 3. Concentrations of Ca (A), C_{org} (B), C: N (C), K (D), Mg (E), Total N (F), P (G) and pH/KCl (H), in the soil (0–10 cm). Error bars represent standard error of the means (SE). For treatment abbreviation (U, 2C, 2CH) see Table 1.

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showed similar patterns of decline under the 2C and 2CH treatments. This could be one of the reasons for the continuous decline in biomass production under the cutting treatments. However, the decline in biomass production under cutting management over the duration of the experiment were not huge. This may indicate a relatively high content of $N_{tot,}$ and of plant available P and K in the soil, especially at the start of the experiment.

Herbage chemical properties

The concentration of P in the herbage declined and reached 3.39 g kg^{-1} under the 2CH treatment at the end of the experiment, whereas at the beginning of the experiment there was a very high concentration of P of around 4.7 g kg^{-1}, indicating that biomass growth was not limited by P [33] A relatively high herbage P concentration recorded in the early periods of the experiment could be explained by the high presence of weedy *U. dioica*, in the harvested biomass, which is typically characterized by high concentrations of P [34]. The high concentration of P recorded even under the U treatment is quite remarkable when compared to the low concentration (less than 2 g kg^{-1}) recorded in low productive semi-natural grasslands [35,36]. Similarly, the high concentrations of K, N and Ca in the herbage, especially during the early periods of the experiment, in all treatments (though much more and stable under U), but declining under 2C and 2CH, could also be attributed to the dominant presence of *U. dioica* and *R. obtusifolius*, in the harvested biomass as these weed species are considered to have high concentrations of P, N and Ca [34,37–39]. The high nutrient concentrations recorded under the unmanaged treatments is very much connected to the high production of *U. dioica*

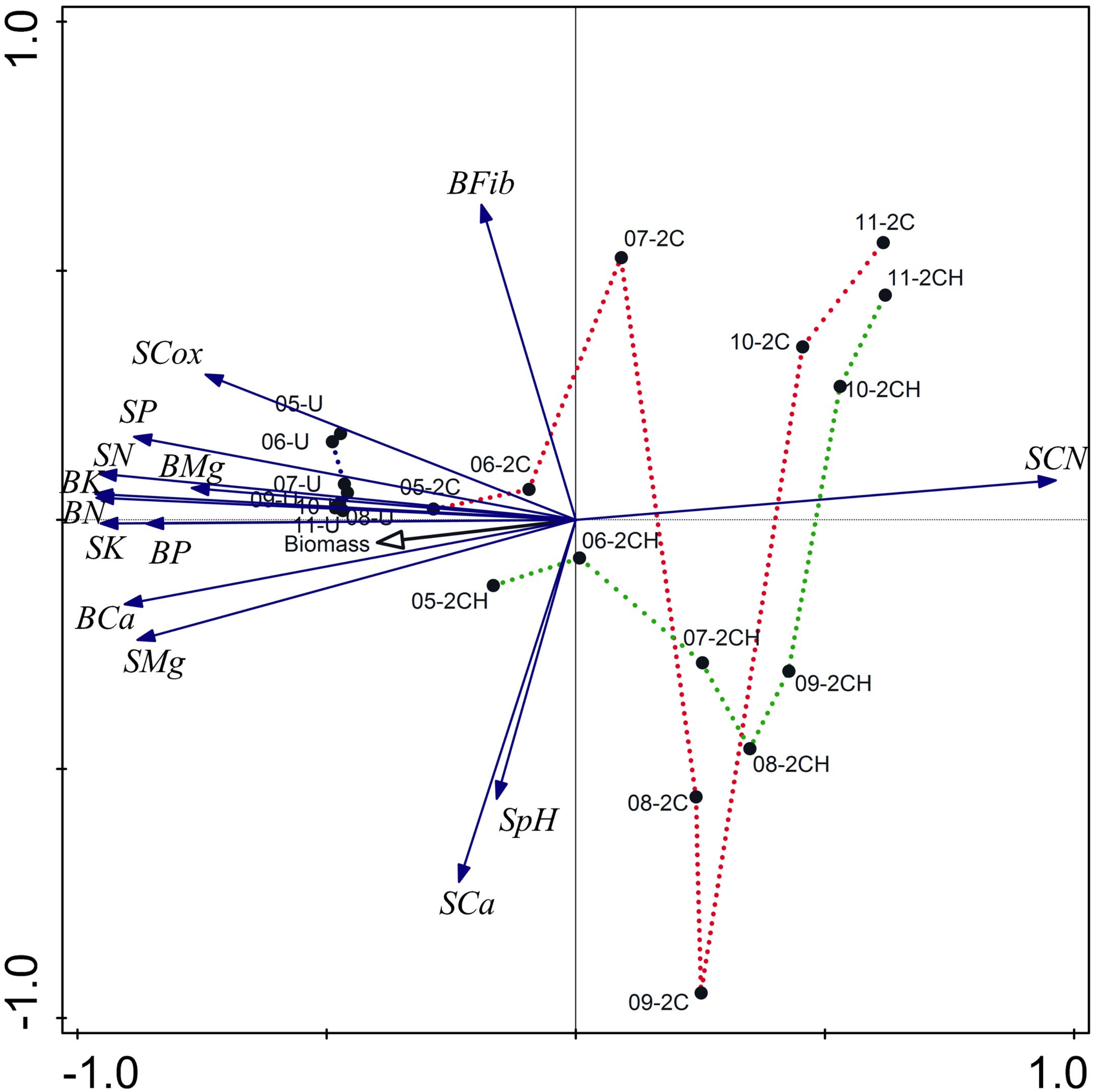

Fig 4. Principal component analysis (pPCA) of the nutrient concentrations in the herbage and in the soil indicating the influence of treatment and its development over the years from 2005 to 2011. The first and the second axis explain 64% and 10%, respectively. Labels include nutrient names and abbreviations: B—herbage nutrient, S—soil nutrient, Fib–crude fibre. Sample labels include treatment abbreviations (see Table 1) and year of sampling.

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compared with other grassland species. Hence, a higher nutrient concentration is recorded on the above ground biomass under unmanaged treatments throughout the experiment period [40]. On the other hand, the cutting (2C and 2CH) treatments had lower nutrients, which may be explained by the consistent and continuous removal of nutrients that occurs under cutting (Table 5).

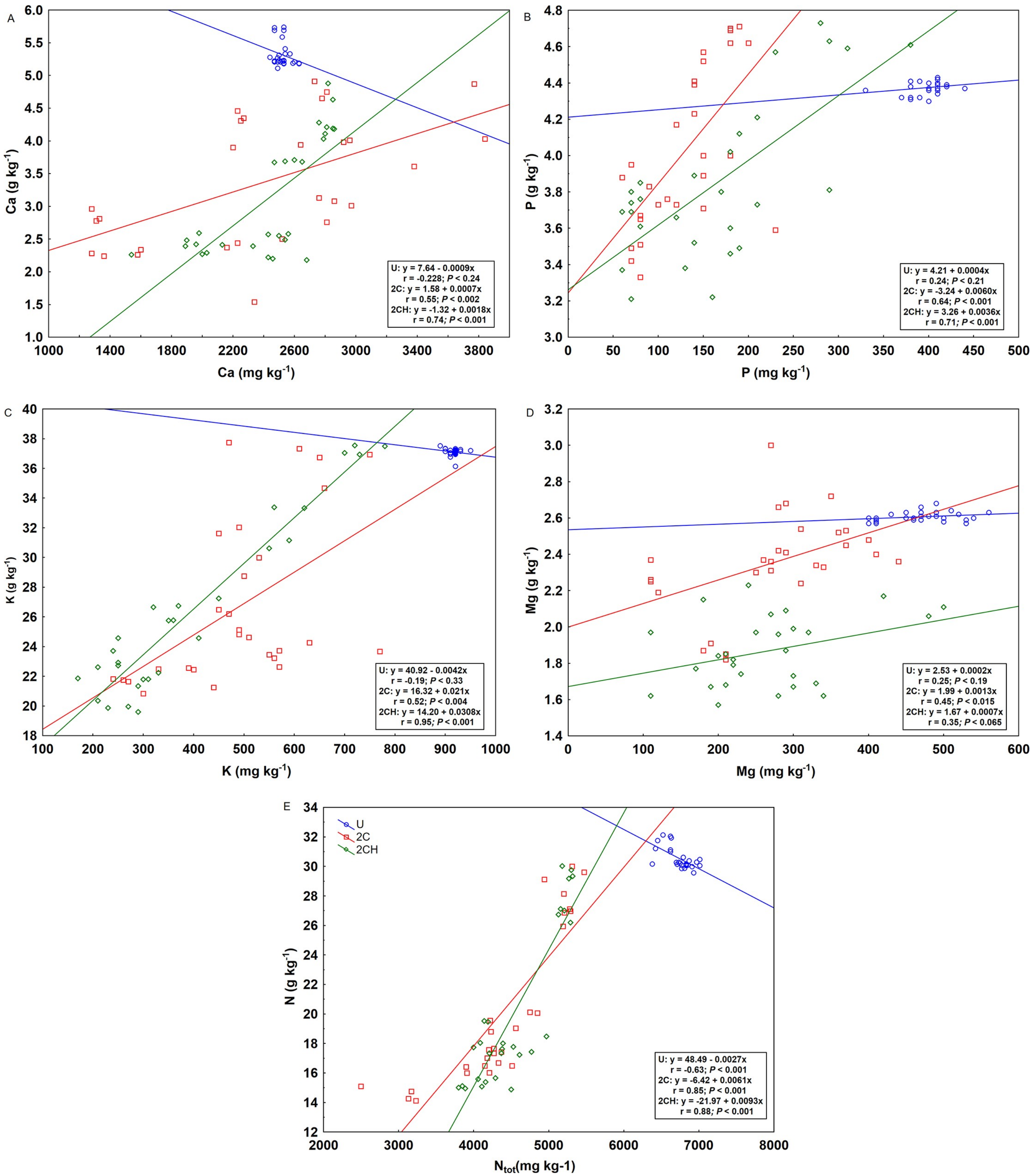

Fig 5. Relationship between concentrations of calcium (A), phosphorus (B), potassium (C), magnesium (D) and nitrogen (E) in the herbage and in the soil. For treatment abbreviation (U, 2C, 2CH) see Table 1.

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At the start of the experiment the relative high proportion of forbs, which were mostly represented by *U. dioica*, and *R. obtusifolius* in the unmanaged treatment (Table 1) are largely responsible for the high concentrations of nutrients in the herbage. It is common for certain plant functional groups to dominate a grassland after cessation of grazing, and the functional groups are dominated by species that are best suited to the given habitat [41]. In contrast, after the introduction of management (2C and 2CH), it was possible to see that in the final year of the experiment (2011) a significant increase in the cover of graminoids (Table 1) which have relatively lower mineral concentrations than forbs [42,43]. This shift from forbs to graminoids could explain the decline in herbage nutrient concentrations in the 2C and 2CH treatments. According to [44], the optimal concentrations of P and N in the herbage for dairy cattle ranges from 2.3 to 3.7 g kg^{-1} and 19.2 to 25.6 g kg^{-1} respectively. In this study, the optimal values or ranges under the cutting management were reached relatively rapidly in the last years of the experiment.

Soil chemical properties

Similar to the changes in nutrient concentrations in herbage, the major plant available nutrients N, P, K and Mg in soil on the experiment site showed a decline over the duration of the study under the cutting treatments (2C and 2CH). Although the amount of nutrients that are removed via harvested biomass each year is relatively small [45], it is well documented that cutting with biomass removal over a sustained period can result in nutrient depletion from the soil in the absence of any compensatory fertilizer application [46,47]. The decline for all plant available nutrients in the 0–10 cm soil layer was very similar to the decline recorded for all plant available nutrients in the 10–20 cm soil layers (S1 Fig). For instance, the decline in concentration of P is consistent with a reported decline in concentration of plant available P in a long-term cutting management without application of P and K fertilizer [48]. Similarly, plant available K concentration was expected to decrease under the cutting treatments, as this has been reported in other studies [48,49]. It is generally possible to remove K from the soil quickly by cutting and removing herbage, but similar rapid removal of P is less likely [50]. This result also indicates a positive relationship between the concentrations of herbage P and K and plant available concentrations of P and K (discussed later), which was also confirmed in another study in the Czech Republic [40]. Not surprisingly, the nutrient concentrations in the soil under the U treatment remained largely stable throughout the experiment period. This could be explained by the absence of management and thus no removal of herbage, which would otherwise have led to removal of nutrients similar to that of the plots with cutting treatments.

The removal of Ca and Mg in the soil under the cutting treatments was relatively small. This might be explained by the limited duration of the experiment, which was conducted for only 8 years, as significant removal of such nutrients is likely to require a long-term period [46,48,51]. Concerning the use of the herbicide glyphosate, it contains C, N, and P and these are essential nutrients for soil microorganisms, and the microorganisms acquire C and N by decomposing plant residues and other organic material added to the soil. The ratio of C:N in glyphosate is 3:1 (considered as low) and this may definitely have an immediate impact on soil microbial activity [52]. In our study the C:N ratio under the 2CH treatment showed increases every year. This may indicate that glyphosate application made a contribution to the increased rate of C and N mineralization [53] on the experiment site.

Soil and herbage chemical properties

Despite the variation in the different axes, the patterns illustrated by the pPCA largely overlapped with the GLM results and, after two years of the experiment, concentrations of most nutrients in the soil, as well as in the herbage, declined sharply except under the unmanaged plots. Even though we can see decline in the nutrient concentrations, they remain high in terms of requirements for grassland species in all treatments. This is perhaps because the area was previously used over a long period (since the 15th century firstly as resting place for sheep and then for heifer) as a resting place for heifers, which would have resulted in excessive amounts of nutrient deposition through urine and faeces on the site. Furthermore, the sharp decline in nutrient concentrations at the early stage of the experiment, which has not been commonly observed in other experiments, can be explained by the high initial amounts of available nutrients in the area as well as the dominance of some nutrient-rich species like *U. dioica* and *R. obtusifolius*.

The nutrient concentration analyses of P, K, N, Mg and Ca in the herbage and in the soil revealed that the cutting management with biomass removal had an effect on nutrient concentrations in both the soil and in herbage. This could be one of the reasons for the strong positive correlation shown (2C and 2CH) between the herbage and plant available concentrations of P, K, N and Ca. This finding is consistent with the conclusions of previous work [40,50,54], that found P and K showing strong relationships between the soil and herbage concentrations. However, the positive relationship between total soil N content and the concentration of N in the herbage under the cutting management in the current study was contrary to the findings of [50] that showed a negative relationship indicating high total N content in the soil, which means poor soil quality and slow mineralization. The current study was conducted on a site that was used previously as a resting place for cattle, unlike the other studies such as [50], which was a cutting experiment without cattle. Due to the presence of cattle and the site being used as a resting place, high amounts of nutrients through deposition of dung and urine on the site are to be expected. According to [55] the amount of nutrients supplied from dung on an individual patch are 40–60 g N/m^2, 14–20 g P/m^2, 16–25 g K/m^2, 40–60 g Ca/m^2 and 10–14 g Mg/m^2. Hence, dung deposition has a significant effect on the chemical status of the soil and thus presents a potential source of available nutrients for plants [56,57]. Furthermore, urine is another source of nutrient especially N, which occurs primarily as a hydrolyzed urea, and is easily plant-available after deposition [58] and enables increased plant biomass N uptake and biomass productivity [59,60].

Conclusions

1. The introduction of cutting management as well as a combination of cutting with herbicide application and reseeding had effects on herbage production and nutrient concentration in the herbage as well as in the soil.
2. The optimum range of nutrient concentrations in the forage (N and P) which is suitable for dairy cattle were reached within 8 years with low frequency of cutting management.
3. Even though the decline of nutrients from the soil associated with biomass removal was relatively high and fast compared with that of other long-term studies in central Europe, the study still showed that high amounts of nutrients remained. If the management applied on the experiment site were to be stopped or interrupted, we would expect that the weeds (*U. dioica and R. obtusifolius*) would emerge and become dominant once again. Therefore, removal of nutrients as well as eradication or suppression of *U. dioica and R. obtusifolius* with cutting management alone for some years will not be sufficient when the soil contains excess amounts of key nutrients.

4. Finally, considering the result from this experiment and other similar studies, we can see treatment with herbicide (glyphosate) application combined with cutting (2CH) did not demonstrate significant difference in removing nutrient from the soil/herbage compared to the nature friendly cut treatment (2C). We conclude restoration measures in national parks or other protected areas are better off without the application of destructive and non-selective herbicide as a potential measure against invasive weed species.

Supporting information

S1 Fig. Concentration of Ca (A), Cox (B), C:N (C), K (D), Mg (E), Total N (F), P (G) and pH/KCl (H), in the soil (10–20 cm). Error bars represent standard error of the means (SE). For treatment abbreviation (U, 2C, 2CH) see Table 1.
(DOCX)

S1 Table. Botanical composition of semi-natural grassland species in the vicinity of the experiment.
(DOCX)

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Writing – original draft: Teowdroes Kassahun, Klára Pavlů.

Writing – review & editing: Teowdroes Kassahun, Vilem Pavlů, Lenka Pavlů.

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Chapter 5

The effect of first defoliation and previous management intensity on forage quality of a semi-natural species-rich grassland

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Published as:

Pavlů K, **Kassahun T**, Pavlů VV, Pavlů L, Blažek P, Homolka P (2021) The effects of first defoliation and previous management intensity on forage quality of a semi-natural species-rich grassland. PLoS ONE 16(3): e0248804.

https://doi.org/10.1371/journal.pone.0248804

PLOS ONE

RESEARCH ARTICLE

The effects of first defoliation and previous management intensity on forage quality of a semi-natural species-rich grassland

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OPEN ACCESS

Citation: Pavlů K, Kassahun T, Pavlů VV, Pavlů L, Blažek P, Homolka P (2021) The effects of first defoliation and previous management intensity on forage quality of a semi-natural species-rich grassland. PLoS ONE 16(3): e0248804. https://doi.org/10.1371/journal.pone.0248804

Editor: Tanvir Shahzad, Government College University Faisalabad, PAKISTAN

Received: November 13, 2020

Accepted: March 8, 2021

Published: March 30, 2021

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: The experiment was maintained under support of the Ministry of Agriculture of the Czech Republic Project No. MZe RO0418 (VP, LP). The laboratory analyses were financed by the Ministry of Agriculture of the Czech Republic, Project No. MZe RO0718 (KP, PH). This paper was finalized under support by the Internal Grant Agency – Faculty of Environmental Sciences, Czech

Abstract

Semi-natural grasslands occupy large parts of the European landscape but little information exists about seasonal variations in their nutritive value during the growing season. This paper presents results of novel data showing the effect of 13 years of previous contrasting management intensities on herbage nutritional value in relation to different dates of first defoliation (by grazing or haymaking). The treatments were: extensive management and intensive management from previous years (1998–2011). Both treatments were cut in June followed by intensive/extensive grazing for the rest of the grazing season (July–October). To evaluate forage quality in the first defoliation date, biomass sampling was performed in the year 2012 for 23 weeks from May to mid-October, and in 2013 for seven weeks from May to mid-June. Sampling was performed from plots that were not under management during the sampling year. Previous extensive management was associated with significantly reduced forage quality for in vitro organic matter digestibility (IVOMD), crude protein, neutral detergent fibre, acid detergent fibre and reduced divalent cations (Ca, Mg) and Na during the first seven weeks of the grazing season and the forage was suitable only for beef cattle. Due to low forage IVOMD, the forage is suitable only for cattle maintenance or for low quality hay when the start of grazing was postponed from seven weeks of vegetative growth to 13 weeks, regardless of the previous intensity. Herbage harvested after 13 weeks of the grazing season was of very low quality and was unsuitable as a forage for cattle when it was the only source of feed. Agri-environmental payments are necessary to help agricultural utilisation to maintain semi-natural grasslands by compensating for deterioration of forage quality, not only for the postponement of the first defoliation (either as cutting or grazing) after mid-June, but also when extensive management is required.

University of Life Sciences Prague, Project No. 20194211 (KP, TK). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Permanent grasslands comprise about 35% of the total utilized agriculture area in the EU-28 countries of Europe [1, 2]. They provide not only forage for livestock, but also support other ecosystem services including carbon sequestration, and provision of landscapes and habitat [3]. Until the mid-twentieth century permanent grasslands were one of the most important feed sources for ruminant nutrition. Intensification of grassland managements (amelioration, reseeding with high productive mixtures, fertilization) and introduction of intensive milk production based on maize silage and concentrate mixtures, has resulted in semi-natural grasslands losing their main role of supplying feed for ruminants [4]. Nowadays, large areas of the semi-natural low-production grasslands in Europe that are characterised by rich floristic composition are managed under various types of agri-environmental schemes. These schemes frequently involve a reduction of management intensity and delaying the first cut or early season grazing in order to allow flowering of target species or to protect ground nesting birds. The result is the reduction of forage quality, especially digestibility of organic matter, in comparison with values from intensively managed grassland. In EU reduced forage quality is compensated by the different payment schemes to farmers that are under agri-environmental schemes [5].

Forage quality and biomass yield are the most important factors that affect decisions about the date of harvest of grassland. Achieving high forage quality together with high herbage production has been an important goal in grassland research in the context of intensive grassland management [6]. Therefore, there is much information available concerning the utilisation of high-production grasslands, particularly sown swards. On the other hand, there is considerably less information about forage quality and production of semi-natural species-rich grasslands, although such information is necessary for determination of appropriate management of grassland managed under agri-environmental measures [7]. Further, there have been few studies of changes in forage quality in relation to ageing of swards during the vegetation season [8–11]. Generally, fibre contents (acid detergent fibre (ADF) and neutral detergent fibre (NDF)) show a progressive increase but in vitro organic matter digestibility (IVOMD), nitrogen and phosphorus concentrations ('dilution effect') generally decrease with ageing of the forage during the vegetation season [6, 11–13]. Forage in the early part of the growing season (or in new regrowth) usually has high digestibility values but low herbage yields; in contrast, with increasing maturity and net accumulation, biomass yields increase but there is also an increase in cell wall content and a decline in digestibility [6]. Therefore, for livestock farmers utilising semi-natural grassland, there are important questions concerning the most suitable time to start the grazing season or to apply the first cut, if grazed or mown herbage is to support the nutritional and mineral requirements of cattle. The suitability of the time of grazing or mowing is affected not only by herbage maturation but also by the type of vegetation, weather conditions and grassland management [14].

Where grassland is managed for conservation objectives within an agri-environmental programme, continual sampling of the grassland herbage during the vegetation season is necessary to determine the optimum range of dates for forage harvesting or grazing periods. However, very few such studies have been done [13]. Several studies have evaluated the forage quality of semi-natural low-production grasslands [10, 11, 13, 15], but these have not dealt with forage maturation during the vegetation season in relation to management intensity.

Semi-natural grasslands are an important part of European grasslands, and the *Arrhenaterion* alliance [16] with *Agrostis capillaris* and *Festuca rubra* dominance is one of the most widespread in Central Europe. However, not much is known about the nutritional properties of this grassland type in relation to the period of the vegetation season and management intensity. Within this context we aimed to answer the following questions: i) what is the impact of previous different grazing intensity types on dry matter standing biomass (DMSB), digestibility (IVOMD), concentrations of crude protein (CP), fibres (NDF, ADF), and macro-elements during the grazing season? ii) when is the appropriate period to introduce grazing or cutting of forage in order to meet cattle nutrition requirements?

Materials and methods

Study site

The study was conducted at 'Oldřichov Grazing Experiment' located in the Jizerské hory Mountains in the northern part of the Czech Republic, in the village Oldřichov v Hájích, 10 km to the north of the city Liberec (50˚50.34′N, 15˚05.36′E; 420 m a.s.l.). This long-term experiment was established in 1998 [for details see 17]. We selected two treatments for this study where hay cutting (in June) was followed by aftermath intensive or extensive grazing.

The site has 30-year mean annual precipitation of 805 mm and a mean annual temperature of 7.2˚C. Table 1 summarises the monthly rainfall and mean monthly temperature for the site (Liberec Meteorological Station). The bedrock is granite and medium deep brown soil (cambisol) with the following characteristics: pH ($CaCl_2$) = 5.45, P = 64 mg kg^{-1}, K = 95 mg kg^{-1} and Mg = 92 mg kg^{-1}. There are about 24 vascular plant species *per* square metre, and the dominant species of the sward are *Agrostis capillaris*, *Festuca rubra* agg., *Trifolium repens*, and *Taraxacum officinale*. Since 1998 the mean cover of dominant vascular plant species was recorded by visual percentage estimation every year in spring before the first management application in all treatments of Oldřichov Grazing Experiment [for details see 17]. Table 2 shows this information for the years 1998 (base line), 2003, 2008, 2012 and 2013. The experimental area has been continuously stocked by young heifers (initial live weights of 150 to 250 kg), since 1998 from June (after cut) until mid or late October, however, the first week of May is the common period for starting the grazing season in this region. In the years 2002–2015 the mean total dry matter biomass production in the study area under intensive and extensive grazing ranged from 2.4 to 5.0 t ha^{-1} and from 2.3 to 4.7 t ha^{-1} respectively [18].

Table 1. Monthly precipitation (mm) and mean monthly temperature (°C) recorded in the years 2012 and 2013.

	Precipitation (mm)			Temperature (°C)		
Month/Year	2012	2013	1998–2013	2012	2013	1998–2013
January	134.9	99.2	72.8	-0.6	-2.3	-1.3
February	78.7	53.2	60.2	-5.4	-1.7	-0.5
March	34.6	35.8	63.6	4.8	-1.5	2.7
April	39.3	39.5	40.4	8.2	7.8	8.5
May	37.0	133.2	74.5	14.3	12	13.1
June	64.1	201.9	85.0	15.9	15.5	15.9
July	151.1	125.6	116.9	17.7	18.6	17.6
August	139.4	64.6	113.2	17.2	17.2	17.0
September	35.7	94.7	63.8	13.1	11.6	12.9
October	33.4	57.1	58.9	7.5	10.1	8.4
November	75.0	65.9	64.0	5.3	4.3	3.9
December	48.7	40.1	64.6	-0.9	2.4	-0.4
Total Sum/Mean	**871.9**	**1010.8**	**877.8**	**8.1**	**7.8**	**8.1**

Values are compared with the 16-year mean 1998–2013 (Liberec meteorological station).

https://doi.org/10.1371/journal.pone.0248804.t001

Table 2. Mean botanical composition (%) of the most abundant vascular plant species.

Treatment	EG					IG				
Species/Year	1998	2003	2008	2012	2013	1998	2003	2008	2012	2013
Aegopodium podagraria	14	4	14	8	9	16	0	0	0	0
Agrostis capillaris	0	9	7	11	12	0	16	12	21	21
Alchemilla sp.	10	8	7	8	9	5	2	2	2	2
Alopecurus pratensis	28	3	4	8	9	22	3	4	1	1
Festuca rubra agg.	8	8	10	13	20	22	11	13	15	15
Galium album	15	8	10	5	5	6	0	1	1	0
Hypericium maculatum	1	2	5	7	9	5	0	0	0	0
Poa trivialis	2	3	6	3	3	2	3	14	16	18
Ranunculus repens	3	1	1	1	1	2	5	1	2	3
Rumex acetosa	1	3	5	3	2	2	1	3	4	4
Taraxacum spp.	2	26	14	13	12	2	22	29	22	32
Trifolium repens	0	13	3	1	1	0	33	24	18	9
Veronica chamaedrys	13	3	3	3	4	4	1	2	4	7
Veronica serpyllifolia	0	0	0	0	0	0	1	0	1	0

Numbers represent mean for the years 1998, 2003, 2008, 2012 and 2013 under extensive (EG) and intensive (IG) treatment.

https://doi.org/10.1371/journal.pone.0248804.t002

Experimental design

The experiment was established in two randomised blocks in the year 1998. Herbage sampling from two contrasting treatments were chosen: i) cutting in June followed by extensive grazing (EG) for the rest of the growing season, in which the stocking rate was adjusted to achieve a mean target sward surface height of more than 10 cm, and ii) cutting in June followed by intensive grazing (IG) for the rest of the growing season, in which the stocking rate was adjusted to achieve a mean target sward surface height of less than 5 cm throughout the grazing season. Both treatments were replicated twice in four plots. Each plot was approximately 0.35 ha.

Data collection and laboratory analyses

The sampling area, a strip about 20 m x 4 m in each plot, was fenced with electric wire in 2012 and 2013 to protect the sward from grazing animals from the start of grazing season to the end of sampling period of each study year. Each year, the sampling area was situated on the opposite side of the plot. It allowed us to collect grassland biomass during maturation period which was affected by the different management intensity in the previous years (S1 Fig). Six randomly selected herbage biomass samples within 50 x 50 cm quadrats were cut by electric clippers once a week. To avoid repeated sampling from the same places, the sampling areas from where samples had been taken were marked with coloured sticks.

In 2012 the herbage biomass samples were collected from each paddock once a week from 2 May to 3 October (23 weeks of sampling x 2 treatments x 2 blocks x 6 samples; i.e. 552 samples in total) to determine forage quality throughout the whole grazing season. Concentrations of N, P, K, Na, Ca and Mg were determined from the 552 herbage samples collected. For analyses of IVOMD and fibres (ADF and NDF), samples were bulked to three *per* paddock. Since the main development on the forage quality was revealed during the first six weeks of sampling in the year 2012 (S2 and S3 Figs), we reduced the sampling from 23 weeks to seven weeks (early part of the grazing season) for the next grazing season in 2013.

In 2013 the herbage biomass samples were collected from each paddock once a week from 2 May to 13 June (7 weeks of sampling x 2 treatments x 2 blocks x 6 samples; i.e. 168 samples in total). Concentrations of N, P, K, Na, Ca and Mg were determined from the 168 herbage samples collected. For analyses of IVOMD and fibres (ADF, NDF) samples were bulked to three *per* paddock.

The fresh herbage biomass samples were weighed then oven dried (48 h at 60˚C) to determine DMSB. Finally, samples were weighed and the dry herbage biomass was recalculated on a *per* ha basis, then milled and passed through a 1mm sieve. The concentration of N was determined by the Kjeldahl method [19] and then multiplied by 6.25 to obtain CP content. The concentrations of P, K, Na, Ca and Mg were determined by ICP-OES after digestion in *aqua regia* in an accredited laboratory of the Crop Research Institute in Chomutov. The NDF and ADF concentrations were specified according to the protocol described by [20] and [21] using the Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), analysed at the Institute of Animal Sciences in Prague. Digestibility (IVOMD) was determined by the Ankon Daisy incubator (ANKOM Technology) modification of enzymatic in vitro digestion method [22, 23] in the Institute of Animal Sciences in Prague.

The herbage samples chemically analysed for IVOMD, ADF and NDF collected in the year 2012 were further analysed by NIRS (FOSS NIRSystems 6500; NIRSystems, Inc., Silver Spring, USA) and calibration equations for IVOMD, ADF and NDF were calculated. The herbage samples collected in the year 2013 were analysed by the FOSS NIRSystems 6500 only.

The experimental land is not a part of any protected area and Crop Research Institute, Prague is the owner, therefore no specific permissions were required for this location. Further, we confirm that the field study did not involve any endangered or protected species.

Data analysis

To obtain information about seasonal development of forage quality, data for the whole grazing season were collected in the year 2012 and are presented in the (S2 and S3 Figs). Based on the most important changes in forage quality in the year 2012, the first seven weeks period of sampling was chosen as a sampling period in the year 2013. Therefore, data from the first seven weeks of the grazing seasons of both 2012 and 2013 were statistically analysed.

A general linear model (GLM) with week (seven weeks as a continuous predictor) and treatment as fixed effects, with block and year as a random effects were used to analyse the effect of treatment, week and their interactions on DMSB, organic components (CP, IVOMD, ADF, NDF) and minerals (P, K, Ca, Mg, Na). Minerals data were log-transformed to meet GLM assumptions requirements. The effects were considered significant at the $P < 0.05$ level and Benjamini-Hochberg's procedure was applied to control for false-discovery rate (FDR) [24]. All GLM analyses were performed in Statistica 13.1 [25].

Results

Dry matter standing biomass production

The DMSB was significantly influenced only by week (Table 3). In the early part of the grazing season DMSB had similar development till the sixth week in both treatments (Fig 1A); after that there was a tendency of divergence between the treatments with higher DMSB under the EG treatment. The highest mean value of DMSB in the EG treatment was recorded in the twentieth week (5.9 t ha^{-1}) and in the IG treatment in the twenty-second week (5.3 t ha^{-1}). From the eighteenth week to the end of the grazing season there was no development of DMSB under either treatment (S2a Fig).

Table 3. Results of GLM for DMSB, IVOMD, CP, ADF, NDF, P, K, Ca, Mg, Na, K/(Ca+Mg).

Characteristics	Effect	*Df*	*F*-ratio	*P*-value
DMSB	Treatment	326	0.36	0.549
	Week		638.24	**<0.001**
	Treatment x Week		3.21	0.074
Organic components				
IVOMD	Treatment	144	50.07	**<0.001**
	Week		217.53	**<0.001**
	Treatment x Week		3.96	0.048
CP	Treatment	309	33.29	**<0.001**
	Week		1156.61	**<0.001**
	Treatment x Week		4.10	0.044
ADF	Treatment	144	43.93	**<0.001**
	Week		93.73	**<0.001**
	Treatment x Week		2.41	0.123
NDF	Treatment	144	30.86	**<0.001**
	Week		87.41	**<0.001**
	Treatment x Week		5.36	**0.022**
Minerals				
P	Treatment	309	5.72	**0.017**
	Week		214.39	**<0.001**
	Treatment x Week		0.50	0.481
K	Treatment	309	0.02	0.884
	Week		61.71	**<0.001**
	Treatment x Week		0.04	0.845
Ca	Treatment	309	36.39	**<0.001**
	Week		7.56	**0.006**
	Treatment x Week		7.46	**0.007**
Mg	Treatment	309	60.57	**<0.001**
	Week		8.92	**0.003**
	Treatment x Week		8.75	**0.003**
Na	Treatment	309	32.95	**<0.001**
	Week		1.50	0.221
	Treatment x Week		5.34	**0.021**
K/(Ca+Mg)	Treatment	309	13.62	**<0.001**
	Week		55.88	**<0.001**
	Treatment x Week		3.06	0.081

Abbreviations: GLM—general linear model, DMSB—dry matter standing biomass, IVOMD—in vitro organic matter digestibility, CP—crude protein, ADF—acid detergent fiber, NDF—neutral detergent fiber. *Df* represents degrees of freedom, *F* represents the value derived from *F* statistics in GLM and *P* represents the resulting probability value. Results are summarized by denominator degrees of freedom *Df* (numerator *Df* was 1 in all tests). Significant results (after table-wise Benjamini-Hochberg's FDR correction) are highlighted in bold.

https://doi.org/10.1371/journal.pone.0248804.t003

Organic components

The concentrations of IVOMD, CP, ADF and NDF were significantly affected by treatment and week. The concentration of NDF was significantly also influenced by treatment x week interaction (Table 3). During the early part of the grazing season a sharp decline in IVOMD was recorded in both treatments (Fig 1B). The mean values of IVOMD were significantly higher in the IG than in the EG treatment, and ranged from 64.5 to 82.5% in the IG treatment

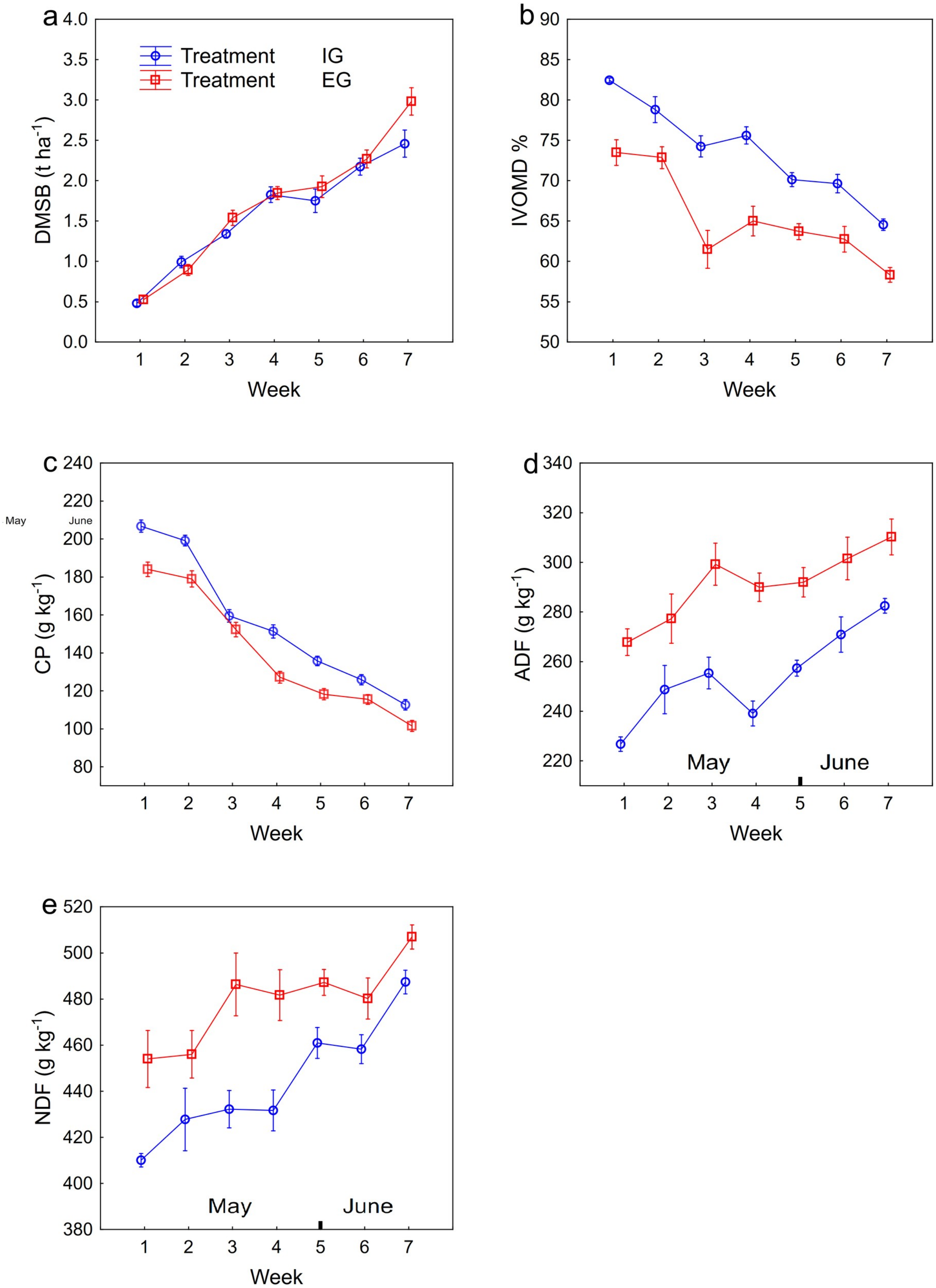

Fig 1. Mean dry matter standing biomass and organic components under extensive (EG) and intensive (IG) management. X-axis refers to the first seven weeks of grazing season in the years 2012 and 2013. Error bars represent standard error of the mean. For abbreviations see Table 3.

https://doi.org/10.1371/journal.pone.0248804.g001

and from 58.3 to 73.5% in the EG treatment. From the eighth week till the end of the grazing season a moderate decline was recorded with the mean values in the range 43–55% in both treatments (S2b Fig).

Concentrations of CP and fibres (ADF, NDF) showed opposite development trends over the whole period of the grazing season (Fig 1C–1E; S2C, S2D and S2E Fig). In the early part of the grazing season CP concentration was significantly higher in the IG treatment than in the EG treatment, and mean values ranged from 101.5 to 184.0 g kg^{-1} for the EG treatment and from 112.6 to 206.8 g kg^{-1} for the IG treatment (Fig 1C). In the eighth week the mean values of CP concentration were about 100 g kg^{-1} in both treatments and they oscillated around this value till the end of the grazing season (S2c Fig). Fibre concentrations (ADF, NDF) were higher in the EG treatment in comparison with the IG treatment during the early part of grazing season. For ADF concentration the mean values ranged from 226.8 to 282.5 g kg^{-1} for the IG treatment and from 267.8 to 310.2 g kg^{-1} for the EG treatment. For NDF concentration the mean values ranged from 410.1 to 487.4 g kg^{-1} for the IG treatment and from 454.0 to 506.1 g kg^{-1} for the EG treatment in this period (Fig 1D and 1E). After the seventh week ADF and NDF concentrations were higher than 300 and 500 g kg^{-1}, in both treatments respectively, (S2d and S2e Fig) though with no significant trend.

Mineral nutrients

The concentrations of Mg and Ca were significantly influenced by treatment, week and interaction of week x treatment. The concentration of P and the K/(Ca +Mg) ratio were both significantly influenced by treatment and week. Concentration of Na was significantly influenced by treatment and interaction of treatment x week, and concentration of K was significantly influenced only by week (Table 3).

The sharp decrease of P concentration in the herbage was recorded from the second to the seventh week for both treatments (Fig 2A) with the highest mean values of 3.5 g kg^{-1} in the second week in both treatments. From the eighth week the mean values were maintained at almost the same level for both treatments and their range was approximately between 1.9 to 2.5 g kg^{-1} till the end of the grazing season (S3a Fig).

In the early part of the grazing season the K concentration reached it highest peak in the second week under EG treatment and in the third week under IG treatment. There was then a decline in K concentration up to the seventh week in the both treatments with mean values ranging from 14.2 down to 9.6 g kg^{-1} in the IG treatment and from 15.2 to 9.9 g kg^{-1} in the EG treatment (Fig 2B). This declining trend was maintained for the rest of the grazing season (S3b Fig) in both treatments, with mean values ranging from 12.3 down to 8.3 g kg^{-1}.

Concentrations of both cations Ca and Mg in the herbage were significantly higher in the IG than in the EG treatment in the early part of the grazing season (Fig 2C and 2D); nevertheless, no developmental trend was recorded in any treatment during this period. The mean values of Ca concentration in the herbage ranged from 4.7 to 6.3 g kg^{-1} for the EG treatment and from 6.7 to 7.3 g kg^{-1} for the IG treatment. The mean values of Mg concentration in the herbage ranged from 1.3 to 1.9 g kg^{-1} for the EG treatment and from 2.2 to 2.5 g kg^{-1} for the IG treatment in this period. From the ninth week onwards the herbage Ca concentration in the EG treatment tended to be higher than in the IG treatment, whereas Mg concentration was similar in both treatments for the remainder of the season (S3c and S3d Fig).

In the early part of grazing season Na concentration in the herbage was significantly higher in the IG than in the EG treatment; the mean values ranged from 0.2 to 0.7 g kg^{-1} for the EG treatment and from 0.7 to 1.1 g kg^{-1} for the IG treatment (Fig 2E). The concentration of Na in the herbage decreased during the whole of the grazing season in both treatments (S3e Fig).

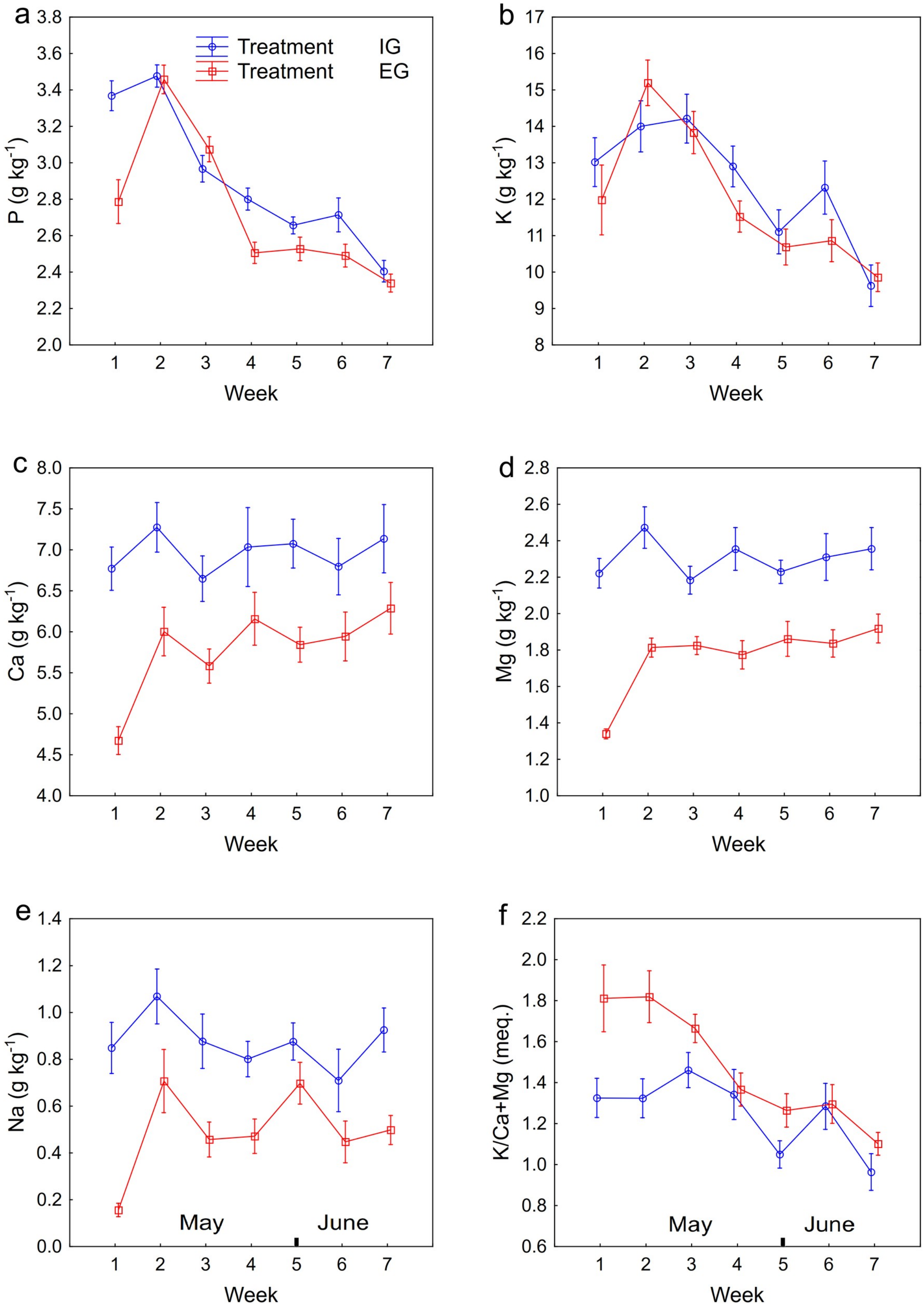

Fig 2. Mean concentration of minerals and K/(Ca+Mg) ratio under extensive (EG) and intensive (IG) management. X-axis refers to the first seven weeks of grazing season in the years 2012 and 2013. Error bars represent standard error of the mean.

https://doi.org/10.1371/journal.pone.0248804.g002

In the early part of the grazing season the K/(Ca+Mg) ratio (meq.) showed a slow decline in both treatments and this ratio was significantly higher in the EG than in the IG treatment (Fig 2F). The mean values of the K/(Ca+Mg) ratio ranged from 1.0 to 1.5 for the IG treatment and from 1.1 to 1.8 for the EG treatment in this period. From the eighth week throughout the rest of the grazing season the mean values for the K/(Ca+Mg) ratio were predominantly higher in the IG than in the EG treatment (S3f); however, no development was observed in this period.

Discussion

The timing of grazing activities and the grazing intensity are generally considered to be the key factors that affect both the quality and quantity of pasture forage [13, 15, 26, 27]. The stage of maturity of harvested herbage is affected by the date of harvesting and this greatly influences the overall forage quality, because of the increasing proportion of cell wall components during the growth of most grassland species [8, 11, 28].

During the early part of the grazing season rapid changes in forage quality and DMSB were found in our experiment. These occurred in both management intensities; nevertheless, the previous grazing intensity had a significant effect on value of many qualitative components of forage in this period. Of particular note was that parameters of forage quality in the EG treatment in the first week of the grazing season were negatively affected by the presence of overwintered herbage from the previous vegetation season.

Dry matter standing biomass production

The DMSB development reflected typical biomass growth at the study site [18] and it was not affected by treatment during the early part of the grazing season. From the seventh week the value of DMSB started to increase under the EG treatment, although total biomass production was higher under the IG treatment in the plots that previously had been defoliated regularly [18]. It seems that the taller vegetation that developed under extensive management could provide higher DMSB than the short vegetation under the IG treatment [17].

Organic components

Values of IVOMD and CP concentrations showed similar patterns over the course of the grazing season. In both treatments there was a sharp decline from the early part of the grazing season, as young forage in vegetative state has higher digestibility values and contains higher concentrations of N compared with more mature forage [13, 29, 30]. A gradual decrease of IVOMD as the sward herbage increases in maturity is usually linked to increasing accumulation of structural carbohydrates and lignification [6, 31] and this is also associated with a reduction in plant N content and therefore of CP. The optimal value of IVOMD required in forage for dairy cows is higher than 67% [6] but for beef cattle a lower threshold of at least 60% may be assumed [32]. A maintenance value of IVOMD in forage for cattle is around 50% [33].

In our experiment the optimum level of IVOMD required in forage for dairy cows was fulfilled during the first six weeks of the grazing season in the IG treatment but only during the first two weeks in the EG treatment. It means that the digestibility of forage is affected not only by the intensity of grazing during the recording period, as also shown in several studies previously [8, 34–37], but also that the grazing intensity applied during previous years can play an important additional role. In both the EG and IG treatments the value of IVOMD was suitable for feeding beef cattle during the whole early part of the grazing season, as beef cattle do not require forage to be of the high digestibility as that required by dairy cows [32]. In the period from the seventh week to the end of the grazing season 2012 the value of IVOMD seemed not to be affected by the previous grazing intensity, and maintenance values of IVOMD for feeding cattle were sufficient until the 13th week of the grazing season under both treatments. Similar IVOMD development is typical for upland European grasslands [e.g. 13, 38]. However, the herbage harvested after 13 weeks in the year 2012 was of very low quality and was not usable as the only source for feed for cattle, although such herbage may be used for combustion [11].

Higher proportion of legumes or *Taraxacum* species in the sward of the IG treatment could contribute to higher CP concentration in the herbage especially during the early part of the grazing season. These plant species usually have higher CP concentrations than occur in grasses [e.g. 39–41]. The concentrations of CP were appropriate for the requirements of dairy cows (>160 g kg^{-1}) [42] only for the first two weeks in both treatments. However, the low amounts of DMSB do not permit the economical utilisation of herbage biomass in this period. After a sharp decline during the first seven weeks the CP concentrations in the forage were about 100 g kg^{-1} regardless of treatment, a level which still met the requirements for beef cattle (80 g kg^{-1}) [42].

In both the EG and IG treatments forage quality in terms of NDF concentration was not suitable for dairy cows at all, the acceptable threshold being about 300–400 g kg^{-1} [43, 44]. The relatively high NDF concentration in the forage means that it is useable only for beef cattle [32]. Except for the first week in the IG treatment, the concentrations of ADF in forage of both treatments were so high as to be considered not acceptable for dairy cows, as recommended thresholds for dairy cows are about 190–240 g kg^{-1} [43, 44]. After the first seven weeks of the vegetation season in the year 2012 both NDF and ADF concentrations in the herbage increased and remained suitable only as forage for beef cattle [32].

Mineral nutrients

The concentrations of minerals in the herbage are mainly affected by the nutrient concentration in the soil [45], and also by phenophases and representation of individual agro-botanical groups in grassland during the vegetation season [10]. Other factors, such as shading intensity, soil moisture and pH, may also affect mineral concentrations in the herbage biomass [45]. During the grazing season a significant decline of P, K and Na concentrations occurred, most likely due to the 'dilution effect' described by [12], in which during the maturation the herbage biomass increases whereas mineral concentration declines [10, 46]. Dairy cows have greater nutritional requirements for P, K, Ca, Mg and Na minerals than beef cattle and sheep, mainly due to the needs of lactation [30].

In both the EG and IG treatments dietary concentration of P in herbage met the requirements of productive animals (2.4–4.0 g kg^{-1}, [30]) only during the first six weeks. After sharp decline in the first seven weeks of grazing season P concentration was relative stable in the rest of grazing season; nevertheless, they were mostly below recommended threshold [30].

Potassium was the only mineral that exceeded the recommended range for cattle nutrition (5–9 g kg^{-1}, [30]) during almost the whole grazing season in both treatments. Especially in the spring, K concentration in the biomass was high, but during the course of the vegetation season it decreased gradually, a finding also described by [47]. The physiological requirements of K for animals tend to be significantly lower than is usually present in herbage [30, 48]. However, due to high Ca and Mg concentrations in the herbage in our experiment the grass tetany ratio K/(Ca+Mg) in meq. of 2.5 [49, 50] was never exceeded.

The concentration of Ca in the IG treatment in the early part of grazing season was sufficiently high to meet nutritional requirements for dairy cows (4–6.0 g kg^{-1}, [30]). It was probably caused by higher proportions of legumes and *Taraxacum* species in the IG treatment as these species contain high concentrations of Ca [30, 48, 51–54]. In later periods the relative proportions of legumes and *Taraxacum* species decreased with increased growth of grasses

(*Agrostis capillaris*, *Festuca rubra* agg., *Poa trivialis*), which have generally lower mineral concentrations than forbs [55]; together with the 'dilution effect' this resulted in a decline in Ca concentration with maturation of the sward. In this period Ca concentration in the IG treatment was suitable only for low productive milking cows (threshold 3.0 g kg^{-1}) and beef cattle (threshold 2.9 g kg^{-1}) [30].

In the EG treatment the concentration of Ca, with no trend, mostly met the requirements for dairy cows during the whole grazing season. Its value was lower than in the IG treatment in the early part of grazing season only. Further, in the EG treatment in the late part of grazing season several tall forbs (*Aegopodium podagraria*, *Galium mollugo* agg., *Hypericum maculatum*), which would likely have had higher concentrations of Ca than grasses [55], increased their proportion in the sward at the expense of the grasses (unpublished observation). Thus, higher Ca concentration in the herbage in the EG treatment than in the IG treatment in the late part of grazing season could be caused by seasonal development of plant species composition, as described also by [10].

The concentration of Mg in the herbage fulfilled the requirements for dairy cows (at least 2.0 g kg^{-1}) only in the early part of the grazing season in the IG treatment. During the later period the herbage was mostly suitable only for beef cattle (1.6 g kg^{-1}) in both treatments [30].

The requirements for Na by dairy cows (2.0 g kg^{-1}) as well as beef cattle (1.0 g kg^{-1}) usually exceed the Na concentration present in herbage [30]. In our experiment concentration of Na in the forage was not sufficient for the requirements of either dairy cows (2.0 g kg^{-1}) or beef cattle (1.0 g kg^{-1}) [30] in both treatments during the whole grazing season in the year 2012. In general, however, it is usually possible to deal with mineral imbalances by supplying livestock with free-choice mineral supplements [48, 56].

Conclusion

The previous extensive management had a carry-over effect which significantly reduced the quality of organic components (IVOMD, ADF, NDF, CP), divalent cations (Ca, Mg) and Na in herbage of *Agrostis capillaris* and *Festuca rubra* dominated grassland during the first seven weeks of the spring grazing season. Due to the high concentration of fibres (ADF, NDF) the forage was suitable only for beef cattle even during the first seven weeks of the grazing season. Besides Na and K, the concentrations of other tested minerals were in the range recommended for cattle feeding and were also affected by species composition of the sward. Herbage mineral concentrations declined over the course of the sward maturation. When the beginning of grazing or hay-making was postponed from the 7th to 13th week of the grazing season the forage was sufficient only for cattle maintenance (based on IVOMD) in both extensive and intensive treatments. Herbage harvested after 13 weeks had very low quality and was not suitable for use as the only source for cattle feeding.

Thus agri-environmental payments are necessary to compensate for deterioration of forage quality if the utilisation of semi-natural grassland is restricted for environmental reasons, and this will apply not only for the postponing of the first defoliation (either as cutting or grazing) to after mid-June, but also when extensive management is required.

Supporting information

S1 Fig. The design of the experiment.
(TIFF)

S2 Fig. Mean dry matter standing biomass and organic components under extensive (EG) and intensive (IG) management. Axis X refers to the whole grazing season (23 weeks) in the

year 2012. Error bars represent standard error of the mean. For abbreviations see Table 3.
(TIF)

S3 Fig. Mean concentration of minerals and K/(Ca+Mg) ratio under extensive (EG) and intensive (IG) management. Axis X refers to the whole grazing season (23 weeks) in the year 2012. Error bars represent standard error of the mean.
(TIF)

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Writing – review & editing: Klára Pavlů, Vilém V. Pavlů, Lenka Pavlů, Petr Homolka.

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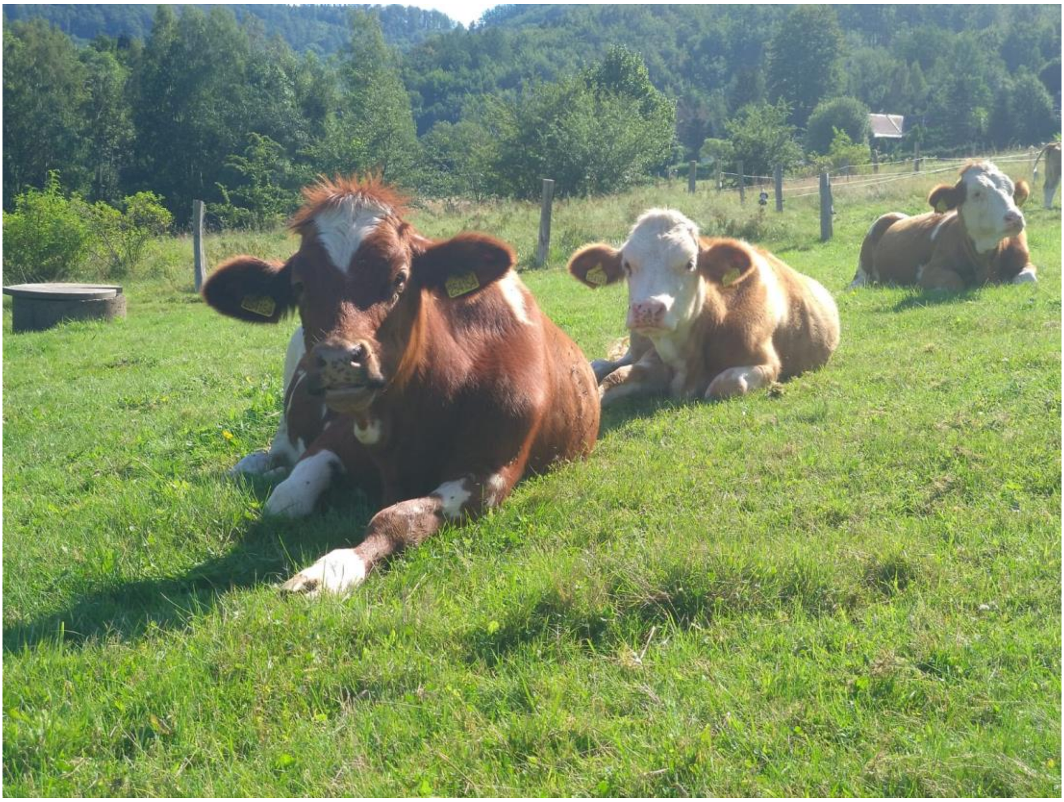

Chapter 6

Effects of long-term grazing on dry matter biomass production and heifers performance

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Published as:

Pavlů V, Pavlů L, Gaisler J, **Kassahun T**, Hejcman M, Pavlů K. (2018). Effect of long-term grazing on dry matter biomass production and heifer performance. Grassland Science in Europe. 23, 413-415.

Effect of long-term grazing on dry matter biomass production and heifer performance

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Abstract

Stocking intensity is one of the main factors affecting grazing productivity. The effects of different grazing intensities on herbage production and live-weight gains of heifers were studied in an upland area in the northern part of the Czech Republic over 20 years (1998-2017). The sward was maintained at a target height of 5 and 10 cm under intensive (IG) and extensive (EG) grazing, respectively. Total biomass production in the grazing season was found to be higher under IG than under EG treatment. Heifers grazing the EG treatment had higher average daily weight gain in comparison to heifers grazing in IG. The particular year and month of vegetation season had the highest effect on seasonal daily weight gain of heifers, but there was no significant difference between breeds. Seasonal live-weight output per hectare under IG was approximately 1.5 times higher than EG treatment. However, if state subsidies are included, EG can be more profitable under the current Czech conditions than IG and satisfies both farmer and nature conservation objectives.

Keywords: grassland, cattle, herbage, live-weight gain

Introduction

In temperate grasslands, grazing intensities and animal preference have influence on the floristic composition and heterogeneity of vegetation, resulting in patchy structure of swards. Changes in agricultural management, such as intensive dairy production, has resulted in only a proportion of grassland being used while a vast area has been abandoned. The situation is exacerbated in more remote areas such as mountainous areas that have low productivity, where semi-natural grassland is common (Isselstein *et al.,* 2005). Extensification in terms of avoiding or minimising intensive application of fertilisers, as well as change in the frequency and timing of defoliation, can be beneficial. The main aim of this 20 year study in the Czech uplands was to investigate how intensive and extensive grazing affects forage yields and live-weight gains of heifers.

Materials and methods

The study was carried out at a 20 year long grazing experimental site (Oldřichov Grazing Experiment) located in the Jizera Mountains in the northern Czech Republic; in Oldřichov v Hájích village (420 m a.s.l.; average annual precipitation 803 mm; mean annual temperature 7.2 °C: Liberec meteorological station). Since 1998, the experimental site has been continuously stocked with young heifers each year from May to October /November. The experimental site was established in two completely randomised blocks. One block was formed using two paddocks with different grazing treatments and each experiment paddock was approximately 0.35 ha. Two treatments are applied: (1) extensive grazing (EG), where the stocking rate was adjusted to achieve a mean target sward surface height greater than 10 cm, and (2) intensive grazing (IG), in which the stocking rate was adjusted to achieve a mean target sward surface height of less than 5 cm. Further, stocking rate was changed throughout the grazing season by increasing or decreasing the area available for grazing by moving fences with a set number of stock per plot for IG or EG. The sward height in IG was maintained by four or five heifers and for EG by two or three heifers per paddock. The weight of heifers at the beginning of the experiment ranged from 150 - 250 kg with different types of breeds during the years 1998 - 2017. There was supplementary feeding of hay in the first 14 days of the grazing season. Data for dry matter (DM) production was collected every three weeks, from four movable cages 1 m × 1 m in size which were installed in each treatment paddock throughout the grazing seasons 2002 - 2017. Subsequently, the samples were weighted and dried for 48 h at 85 °C for DM yield. During each grazing season 1998 - 2017 (May - September), the heifers were weighed individually each month. Data were analysed using repeated measures of ANOVA to evaluate the effect of grazing on forage production and live weight gain of heifers during the growing seasons.

Results and discussion

There was a significant effect of treatment ($P < 0.001$), month ($P < 0.001$), year ($P < 0.001$), month and treatment interaction ($P = 0.020$) on biomass production but there was no effect of year and treatment interaction. Total biomass production in the grazing season was found to be higher under IG than EG and varied between 2.4 and 5.0 DM t ha^{-1} year^{-1} under IG, and between 2.3 and 4.7 DM t ha^{-1} year^{-1} under EG (Figure 1a). After the spring peak in May, the biomass production decreased regardless of the treatment during the vegetation season. Double peak (spring and summer) curves of biomass growth during the growing season were identified nine times in the 16 year experiment which makes it very unique compared to the more commonly found single peak curve in the spring in Czech uplands. The overall biomass production in both treatments consistently fluctuated from year to year and these fluctuations in biomass could be attributed to fluctuations in climatic parameters such as temperature and precipitation (Craine *et al.*, 2012).

There was a significant effect of treatment ($P < 0.001$), month ($P < 0.001$), year ($P < 0.001$), month and treatment interaction ($P = 0.041$), but no effect of year and treatment interaction on daily live-weight gain of heifers. The seasonal development of both treatments, with a peak in June, was similar and could be attributed to heifer's adaptation to pasture forage. Although the forage quality was higher in IG than EG treatment (Kassahun *et al.*, 2018), daily live-weight gain of heifers was higher under EG (803 g) than IG (703 g) treatment (Figure 1b). This could be due to the selective grazing of heifers assigned to the EG treatment, obtaining forage which reflected their need regardless of the quality. Is it also possible that EG treatment heifers could select forage of higher quality and their diet may not have differed that much compared to IG. A relatively higher year to year variability of daily live-weight gain of heifers from 424 to750 g under IG and from 620 to1020 g under EG was caused by: (1) different forage production and quality (Pavlů *et al.*, 2006); (2) selective grazing (Ludvíková *et al.*, 2015); and (3) grazing heifers of different live weight and ability to digest fresh forage at the beginning of the grazing season (Doležal and Gregoriadesova, 1996). The mean stocking rates over the grazing seasons were about 600 kg ha^{-1} for EG

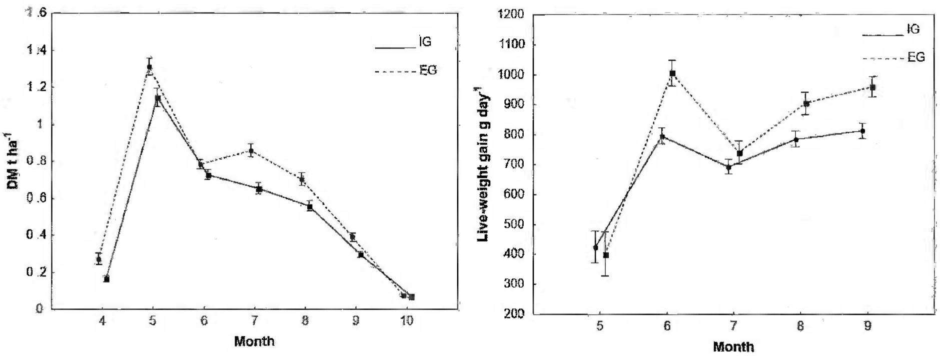

Figure 1. Seasonal development of a) on the left: biomass DM production (t ha^{-1}) in IG and EG treatment; b) on the right: live-weight gain of heifers (g day^{-1}) in IG and EG treatment. Numbers refer to the month of the year: 4-April, 5-May, 6-June, 7-July, 8-August, 9-September, 10-October. Standard errors are indicated by the vertical lines.

and about 1000 kg ha^{-1} for the IG treatment. As a result, although the stocking rate was almost double the total live output of heifers per hectare in the IG, treatment was about one and a half times higher than EG.

Conclusion

Considering the number of herbivores in the Czech Republic, findings suggest that EG is a better landscape management that can fulfil the livestock needs and mitigate temporary or permanent abandonment of grasslands.

Acknowledgement

This research was conducted under the support of the MACR (RO0417) and the paper was finalised under the support of Interreg VA SN/CZ (100264999 – DiverGrass).

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Chapter 7

7.1 General Discussion

7.1.1. Semi-natural grassland management

Several studies indicate the need to protect and preserve semi-natural grasslands, as they are key habitats in maintaining biodiversity in agricultural landscapes (Duelli and Obrist, 2003). However, the last 100 years have brought tremendous change across the agricultural regions of Europe, and with it, a change in grassland utilization have occurred. Due to decline in grassland diversity, the overall biological diversity is under threat and becoming a major conservation problem. According to Hejcman et al. (2008) and Isselstein at al. (2005), the main reason for declining diversity is the abandonment of large areas of grasslands. Because of a change in agricultural management (largely intensive milk husbandry) only small portion of grasslands are in use for forage production. This problem is more acute in areas that are less accessible such as mountain areas where majority of semi-natural grasslands are located. Numerous studies (Bakker, 1989; Hansson and Fogelfors, 2000; Pykala, 2003) confirm a decline in plant species richness following abandonment of semi-natural grasslands. Abandonment also have an effect on the soil properties, due to increased accumulation of dead plant material which affects the decomposition process (Tappeiner and Cernusca, 1995).

It is well understood that resources that are necessary to preserve semi-natural grasslands and their numerous endangered species (Gärdenfors, 2000) are very scarce. Some of the main defoliation management systems (described in **Chapter 1**) that are typical for managing semi-natural grasslands are grazing, cutting and in some cases mulching. We can define defoliation as “removing plant shoots by cutting or grazing, and can be described by several features such as intensity, interval between events, timing according to season or plant growth stage and by its spatial heterogeneity” (Sollenberger et al., 2012). Of course, the different management options that are available for grassland managers are selected and introduced based on the objective and the intended results. Against this background, this PhD thesis analysed a long-term data collected from a semi-natural grassland located in the Jizera Mountains, northern Czech Republic and from the National Park of Nízké Tatry, Slovakia. In both areas, different contrasting managements are applied in order to reach specified objectives. In general, the thesis work can be divided in to 5 major sections analysing: 1) how cutting and grazing intensities affect the vertical distribution of different functional groups; 2) the effect of dung on sward height patches under different grazing intensities on nutrient concentrations in soil and herbage; 3) evaluate the restoration measures (cutting and herbicide application) of typical mountain grassland infested with expansive weedy species; 4) identify the correct period/time to introduce management in order to reach the critical nutritional requirements of cattle; and 5) analyse the effect of 20 year grazing intensities on biomass productivity and heifers performance. Thus, the aim was to offer a unique overview how different grassland management techniques (**Chapter 1**) under long-term observation influence different sward parameters (biomass production, nutrient concentration in the herbage and in the soil, vertical distribution and sward height patches) in semi-natural grassland. Several biomass and soil samples over a long period of time were collected for the analysis. Thus, the thesis benefits from long-term experiment data that are critical to understand the process in soil, vegetation and microorganisms that are long-term in relation to any change in management (Lemaire, 2007).

7.1.2. Contrasting effects of grazing and cutting management on herbage biomass

It is well known that defoliation from grazing animal affect herbage biomass (Bilotta et al., 2007). Two scenarios could occur. Firstly, Grazing may have no effect on herbage biomass (Hart et al., 1988) as the plants compensate for tissue removal by grazing (Langlands and Bennett, 1973); secondly grazing could increase herbage biomass (Cluzeau et al., 1992) when overcompensation by the plants occur for the tissues removed

(McNaughton, 1983). Of course, these different responses by the herbage depends on several factors such as stocking rate and other grazing management practices which influence the frequency and severity of herbage removal (Dowling et al., 2006).

For instance, the herbage biomass production was slowly but continuously declining throughout the experiment period under cutting management system (**Chapter 4, Fig 1**). Although the biomass was declining every year, we can classify it as highly productive grassland, as the mean biomass production in all treatments throughout the experiment period was above 3 t ha^{-1} per year which is the lower limit for high productive grassland in Central Europe (Hejcman et al., 2010). The decline in biomass could be attributed to the continuous decline in plant available nutrients (N, P and K) due to cutting and removal of herbage biomass (**Chapter 4, Table 5**). This finding with a declining trend of biomass production sharply contrasts with other defoliation management strategies. For example, the biomass production under grazing management, especially intensive grazing provided a higher biomass throughout the grazing season. Specifically, the total biomass production under intensive grazing (2.4 to 5.0 dry matter t ha^{-1}) compared to extensive grazing (2.3 to 4.7 dry matter t ha^{-1}) was higher (**Chapter 6, Fig 1a**). Similar result was reported by Kassahun et al., 2016 and Pavlů et al., 2006a. This could be explained by the grazing behaviour of the heifers, as they prefer to graze low younger biomass, hence the plants remain in vegetative stage unlike in the extensive grazing were heifers have choice between young and mature plants. However, if we consider the development of biomass growth especially in our study site 1(for site description see **Chapter 1**), dry matter standing biomass increased under extensive grazing (after seven weeks from the start of the grazing season), although the overall total biomass production remained higher under intensive grazing plots (Kassahun et al., 2016 and **Chapter 6**) that remained defoliated regularly (**Chapter 5**).

Generally, grazing reduces the chance for plants to have a number of stems and tissues that can reach mature stage, hence the age of tissues is typically lower than those found under no grazing (Schönbach et al., 2012). Furthermore, the possibility of nutrient return from the livestock in the form of dung and urine that are easily and readily available for plant growth (Risser and Parton, 1982) could be the reason why the biomass production under grazing management does not show a declining trend similar to the cutting management (**Chapter 4**). Intensive grazing supports frequent defoliation of swards; hence, nutrients from dungs are utilized for regrowth of the sward (**Chapter 3**) leading to a higher herbage production per area.

Although biomass production under intensive grazing was higher in comparison to extensive grazing, the year-to-year variability in biomass production (be it in cutting or grazing management) was consistent (**Chapter 6**). This finding is in line with other studies from Central Europe (Honsova´ et al., 2007; Hrevusova´ et al., 2009; Hejcman et al., 2012; Pavlů et al., 2006a and Kassahun et al., 2016) for cut grassland and pasture. The year-to-year variability in biomass production (**Chapter 4 and Chapter 6**) could be because of difference in precipitation amount and distribution throughout the vegetation season as well as temperature affecting the mineralization of soil organic matter and supply of nutrient (Hejcman et al., 2010).

It is expected that biomass production reaches its peak during the vegetation season in the summer when temperature and precipitation are optimal. There is a positive relationship between peak biomass production and precipitation (Wu et al., 2011). In continental scale, precipitation is considered as the most important driver of grassland productivity (Knapp and Smith, 2001; Huston and Wolverton, 2009). Productivity of the grasslands (pastures) in the study area (**site 1**) is mostly limited from end of April to end of November. Hence supporting production of quality forage production during the grazing season and preparation for winter storage is vital for dairy or farm productivity.

What is important is to understand precipitation, temperature as well as grazing interact to change or affect the herbage quality (Walter et al., 2012) and quantity (Klein et al., 2007). The data from the long-term grazing experiment in "Oldřichov Grazing Experiment" (**study site 1**) showed peak biomass production in the spring under both intensive and extensive grazing. What is more unique was second peak that was recorded during the summer (**Chapter 6**). The double peak in biomass production observed in our experiment suggests that the early conclusion of only a single peak (Orr et al., 1998 and Velich, 1991) in spring in Czech uplands is not necessarily true.

7.1.3. Defoliation management and grassland plant species

The promotion of grazing or long-term exclusion/abandonment typically leads to a change in dominance of certain plant functional groups, eventually affecting the proportion of unpalatable forbs (**Chapter 4**) and palatable grasses (Zhao et al., 2020). When abandonment (due to termination of grazing or mowing) prevails the challenge observed in semi-natural grassland is the dominant presence of tall grass and herb species, while in some cases even trees and bush covers (Bakker, 1989). The lack of frequent defoliation may cause a decline in the regional grassland plant species pool due to local extinction of defoliation dependent species (Pykala et al., 2005).

In some cases, abandonment leads to a natural succession, which is typically dominated by perennials (George et al., 1992). However, very often these places are mainly dominated by annual (Rietkerk and Koppel, 1997) and expansive weedy species. This challenge is exactly what we have tried to analyse in **Chapter 4**, where a typical upland semi-natural grassland that used to be a resting place for cattle has ended up being infested with expansive weedy species of *Urtica dioica* and *Rumex obtusifolius* after the site (for site 2 description see **Chapter 1**) was fully abandoned. The response of the abandoned grassland to the introduction of management was revealed in different ways. According to Doležal et al. (2018) different cutting frequencies change the composition of plant community and reduces competitive interaction, hence supporting the coexistence of subordinate and dominant species. In certain conditions introduction of management (such as cutting) brings a shift in dominance of certain functional groups like shift from forbs to graminoids (**Chapter 4**). At the start of the experiment in our study site, forbs, which were largely represented by *Urtica dioica* and *Rumex obtusifolius*, dominated the site. This could be explained by forbs strong competitive ability and lack of tolerance to disturbances such as cutting (Pavlů et al., 2011). The higher presence of legumes and graminoids in the cut treatment compared with the unmanaged treatment is consistent with other studies (Hansson and Fogelfors, 2000; Ryser et al., 1995; Wahlman and Milber, 2002). One reason for this could be the better light condition and opportunity to colonize gaps in cut treatments (Pavlů et al., 2011; Zhao et al., 2020). The unmanaged plot in our experiment over 8-years highlighted the importance of defoliation as a determining factor affecting community diversity (Piqueray et al., 2019). The shift from forbs towards graminoids after management introduction is similar to Pavlů et al. (2011), which has also affected the herbage mineral concentration (discussed later) in our study as forbs generally have higher mineral concentration than graminoids (Pirhofer-walzl et al., 2011; Liebish et al., 2013).

Numerous studies have shown different ways to assess the effect of grazing or abandonment, and functional group analysis seems to be the better approach that can overcome the constraints of an individual species approach (Diaz et al., 2001). Some studies (such as: McIntyre and Lavorel, 2001, Jauffret and Lavorel, 2003) suggest different species share some traits which enables them to respond similarly to disturbances. Furthermore, each functional group has a specific role in the ecosystem, any change in their relative proportion could affect the function and state of the ecosystem (Naeem, 1998). Therefore, understanding the effect of different defoliation management in different vertical layers of the sward (**Chapter 2**) is better understood and analysed using functional groups.

Grazing and cutting management are the most widely practiced management system in Central Europe (**Chapter 1**). Grazing systems, intensity and grazing species, greatly influence sward structure as well as the density of the sward (Tainton et al., 1996). It is well understood that grazing modifies the species composition, vertical structure, plant traits and several other characteristics of grassland (McIntyre and Lavorel, 2001). Several contradictory results have been reported highlighting reduced, unaffected or even increased diversity (due to grazing) or even a shift in plant functional group (Kurtz et al., 2018). According to Pucheta et al. (1992), grazing is expected to increase the abundance of graminoids (**Chapter 2**), which is mainly due to the frequent removal of biomass (graminoids) through grazing, leading to stimulation of sward regrowth from the available light reaching the sward base (Deregibus et al., 1985) as well as the high ability to colonise gaps by tillering (Margareta and Hakan, 2000). A very old modelling work by Huston, (1979) concludes a system exposed to a constant condition develops "system-inherent" features. The high dominance or presence of graminoids especially in continuous grazing (such as intensive grazing, **Chapter 2**) treatment, in comparison to other treatments, could imply such system trait (Margareta and Hakan, 2000).

Other functional groups such as forbs are characterized by its variable traits (Naeem, 1998). This variable trait could explain the presence of forbs species in grazed areas (such as extensively grazed) and the high probability of having grazing resistant species within the group (Bermejo et al., 2012; **Chapter 2**). Interestingly forbs (such as *Taraxacum* spp) which are commonly absent in unmanaged plots (similar to legumes-*Trifolium repens* which has low ability to compete for light) due to adverse effect of reduced light at lower depth/layer are surprisingly present in a higher proportion in the upper layer of undefoliated plots (**Chapter 2**). This might be due to forbs being a heterogeneous group with a wide range of morphological traits, that does not show a uniform response to different disturbances (Sternberg et al., 2000) and in some cases lack of disturbance coupled with other factors such as excess nutrient in the soil creates opportunities for forbs (largely invasive weeds like *Urtica dioica* and *Rumex obtusifolius)* to dominate undefoliated plots (**Chapter 4).**

According to Hoogendoorn and Holmes, (1992) swards that are not frequently grazed reveal increased stem and dead material content, which is mainly explained by the greater age of the plant tissues (Korte et al., 1984). As growing herbage gradually reaches maturity, a greater proportion of green matter will be found in the upper layer (> 3 cm) and dead biomass accumulates at the bottom layer (< 3 cm) (**Chapter 2**). This is mainly because reduced penetration of light at the lower layer typically leads to tiller death (Ong, 1978) which ultimately results in increased proportion of dead biomass (Tuñon et al., 2013). This raises a critical issue how experiment conduct their biomass sampling for forage quality as well as productivity analysis. Previous studies by Mayne et al (1987) and Michell and Fulkerson (1987) reported increased herbage biomass at the bottom layer of the sward in the later part of the grazing season, mainly due to steam and dead material accumulation. Hence, sampling biomass below 3 cm, which is normally full of stem and dead material and typically left ungrazed by heifers could result in misleading result of forage quality and productivity (**Chapter 2**).

7.1.4. Nutrient concentration in the herbage and soil

Grazing activity greatly influence biomass production and species composition of grassland ecosystems (Bakker et al., 2004; Olff et al., 1999). Spatial heterogeneity in grasslands are created by grazing animals and maintained through selective grazing, trampling and return of nutrients in spatially heterogeneous manner (Adler et al., 2001). Under less intensive or low stocking rate, animals prefer to graze areas that were previously defoliated during the current season as the herbage found in the previously defoliated areas are likely to be less mature (**Chapter 5**), which makes it easy for digestion compared to herbage found in not previously grazed areas (Cid and Brizuela, 1998). It is known that frequent grazing normally reduces the annual dry matter production, it also keeps the plant into active growing rather than tall and maturing phase, which ultimately means improved forage with better nutritive value (Bruinenberg et al., 2002). This cycle continues with those areas that are frequently grazed remain short and in vegetative growth due to frequent defoliation, while adjacent areas remain ungrazed or see little defoliation. This of course leads to what we call "patch grazing" (Adler et al., 2001) resulting in mosaic of tall and short patches (Tonn et al., 2019; **Chapter 3**) where contrasting levels of grazing intensity exist on micro scale.

Generally, cattle avoid tall stem herbage where the sward is difficult to graze (De Vries and Daleboudt, 1994) as well as areas that are contaminated by dung (MacDiarmid and Watkin, 1972a). Hence, dung deposition coupled with trampling and grazing, can be considered as the main factor that can explain vegetation structure (Kohler et al., 2004). Grazing animals affect the flow of nutrient in grasslands by stimulating their turnover. More than 60% of nutrients and minerals ingested by livestock is returned back to the soil in the form of dung and urine, with only small portion used by the animals (Haynes and Williams, 1993). Of course, the minerals and nutrients found in the dung and urine are much more easily available for plants than those found in the soil (McNaughton et al., 1988). Several studies have been conducted about the effect of patches on botanical composition especially on patches created by dung. However, a critical question remains unanswered about the soil and herbage nutrient concentrations under these tall patches that are created due to selective grazing and avoidance of grazing because of dung deposition (**Chapter 3**).

It is well understood that nutrient consumed by grazers are returned or recycled back in the form excreta and are a significant input to the production system. Due to the input of these nutrients, soil fertility and increased forage nutrition are observed (Haynes and Williams, 1993). Previous studies (Williams and Haynes, 1995; Aarons et al., 2004) reported increase in phosphorous (P) and potassium (K) in soil beneath dung pads. Similarly, a higher concentration of available soil nitrogen (N_{tot}), P and exchangeable K was detected up to 15 cm around the dung pat (Deenen and Middelkoop, 1992). In contrast, our study (**Chapter 3**) showed plant available nutrients P, K, Ca and Mg as well as concentration of C_{org}, N_{tot}, not affected by the type of patches (i.e patch created due to dung and patch created due to selective grazing without dung). It is not always the case that nutrients in the soil will increase just because we have nutrient released or lost from dung. Nutrients released from dung could automatically be used by plants under the dung as soon as they are released (Dickinson and Craig, 1990). The low soil nutrient enrichment from dung in **Chapter 3**, unlike other studies could be due to differences among types of grassland ecosystems, grazing management, soil type, differences in plant species, and environmental factors. The finding suggests, there might have been a significant downward or lateral movement of nutrients in the soil (Dickinson and Craig, 1990).

Numerous studies report dung deposition having serious implication on soil chemical status. For instance, a large portion of N from the dung is lost by NH_3 volatilization or due to leaching (MacDiarmid and Watkin, 1972b; **Chapter 3**), but it is still true that dung deposition is a potential source of available nutrient for plants (Aarons et al., 2004). Hence, the nutrient content in the herbage indicates the nutrient supply to the plant that ultimately affects the nutritive value of herbage (Whitehead, 2000). What is also important to understand is the patch type be it due to dung or selective grazing, available soil nutrient under the different patches (tall or short) could be very different due to differences in nutrient cycling (Güsewell et al., 2005). Comparing herbage nutrient under different patch type we found the highest concentration of herbage nutrient (N, P,

K) in patches under intensive grazing with dung (**Chapter 3**). This implies nutrients were released under this patch and dung was the main driver. Furthermore, intensity of grazing influenced the utilization of the nutrients released from the dung, as intensity of grazing increases frequency of defoliation increases, which ultimately means more nutrient, needed and used for regrowth. In contrast, patches (both patch with dung and without dung, see Table 2, **Chapter 3**) under extensive grazing had no effect on herbage nutrient concentration as well as on dry matter standing biomass, implying significant loss of nutrient from dung due to leaching or volatilization. This agrees with Cameron et al. (2013), who reported dung or urine deposition in spring or autumn can increase leaching below active root zone leading to lowered availability of nutrients especially N for patches and this issue is more acute if excreta deposition is followed by precipitation.

Unlike grazed grasslands that are typically influenced by a number of factors such as trampling, nutrient addition via faeces and urine, and selective defoliations by grazers, (Rook et al., 2004) grasslands managed by cutting respond differently. Studies show that defoliation management with cutting without the application of fertilizer significantly decreases plant available N, P and K relatively faster (**Chapter 4)** than under grazing management (Hejcman et al., 2010). This is mainly because: (1) large part of the nutrient (60 to 90%) ingested forage under grazing are returned to the system via excreta (Kayser and Isselstein, 2005); (2) young and leafy biomass is typically known to have a higher concentration of N, P and K compared to old biomass or mature biomass (Pontes et al., 2007; **Chapter 5**). Hence, frequent defoliation will help to keep the plants in vegetative stage, which means higher nutrient concentrations that can be removed with cutting.

When the objective is to create a desirable grassland community, regular cutting or grazing management is necessary (Hansson and Fogelfors, 2000). However, when the challenge is to restore a species rich grassland to its previous status, especially those that have been heavily fertilized then the task is difficult and long process. The procedure typically involves reducing the amount of nutrient available in the soil (Pavlů et al. 2012). A number of studies have shown several years of harvesting plant biomass without adding fertilizer leads to nutrient depletion from the soil (Oelmann et al., 2009; Perring et al., 2009; **Chapter 4**). Even though nutrients cycles would be progressively affected with different cutting regimes (Giese et al., 2013), the results are not always straightforward due to different site conditions. In **Chapter 4,** we saw a decline in nutrient concentration in the herbage and the soil under the cutting treatments. However, the declines were not enough to fully say cutting management has removed sufficient nutrients from the soil, which can help for the restoration of the site (see **Chapter 1** for site description). One reason is the site being used for long time (since the 15th century) as a cattle-resting place, hence huge amount of dung and urine deposition in the site from the start. Additionally, the dominant presence of *Urtica dioica* and *Rumex obtusifolius* that have high concentration of P, N and Ca (Taylor, 2009; Baeten et al., 2011) may also be another reason especially at the early stage when the site was not under any management. Furthermore, the heavy presence of *Rumex obtusifolius* is a big problem as it is one of the five most widely distributed (non- cultivated) plant species in the world. It is a major concern and affects the dry matter yield and significantly reduces the nutritive value of herbage (Hejduk and Dolezal, 2004). Interestingly combination of cutting with herbicide such as glyphosate, was expected to demonstrate a much better result (in the herbage/soil nutrient analysis) compared to the cutting measure applied (**Chapter 4).** Although herbicide application coupled with cutting removed almost entirely the weedy species after 3 or 4 years the result was no so much different with the cutting alone measure which showed almost similar result at the end of the experiment. Hence, careful considerations need to be taken especially when restorations of protected areas are involved. The nature friendly cutting management could serve good result than using destructive and non-selective herbicides.

Even though nutrient depletions are reported by frequent cutting, a multi-year studies are vital to fully understand the effect of cutting on total N, P, Ca and Mg in the soil (Pavlů et al., 2013). Especially at sites which are heavily covered by weeds such as *Urtica dioica* in unmanaged condition is indicative of a site with good soil nutrient supply and water (Prach, 2008). Therefore, understanding the site fully and its botanical composition is important, since it will have an impact on the herbage production potential as well as the nutritive value of herbage (Frame, 1991). For instance, a rapid decline of K from the soil with cutting has been generally reported but similar rapid removal should not be expected for P (**Chapter 4**), this is mainly because few years of cutting management will not affect soil P (Pavlů et al., 2013). Likewise, plant available nutrients of Ca and Mg removal from the soil was small (**Chapter 4**). This again shows the need for long-term applications of cutting management since removal of such nutrient requires long-term period (Hansson et al., 2000; Pavlů et al., 2011).

It has been extensively studied by several researchers about the importance of temperate semi-natural grasslands for biodiversity conservation (Pärt and Söderström 1999; Öckinger et al., 2006; Wilson et al., 2012). Unfortunately, deterioration in some part of Europe (e.g Western Europe) has caused strong negative impact on many species. Hence one measure that has been followed well is the Agri-environmental schemes (AES). It is in simplest form subsidies for management of semi natural grasslands (Berg et al., 2019). Historically, semi-natural grasslands are managed either by cutting or grazing (**Chapter 1**). Hence, AES are designed and implemented to support the conservation of organisms that are dependent on cutting or grazing and low chemical inputs in grasslands (Wissman et al., 2013; Caruso et al., 2015). For instance, framers must agree to fully apply the guidelines of AES regarding grazing which instructs minimal grazing intensity to achieve short swards, avoid accumulation of litter and shrub encroachment (Berg et al., 2019). Of course, this kind of decision has consequences for farmers as it leads to reduction of forage quality

Defoliation management in a semi-natural grassland and its effect on several sward parameters especially on forage quantity as well as quality is important for decision making and choosing appropriate management methods. However, important questions remain unanswered with respect to finding the best time for starting grazing or first cut that can meet the nutritional and mineral requirements of cattle (**Chapter 5**), especially for those grasslands that are manged under AES. And this question is critical for framers as well as nature conservation agencies who are interested in maintaining high forage quality, but have to reduce management intensity and delay early grazing as well as delay the first cut in order to allow flowering of target species and nesting birds (Lakner et al., 2020). It is a well-known fact that harvesting date of herbage and grazing activities coupled with grazing intensities have implication on forage quantity as well as quality, due to increase in cell wall components during the growing period (Tallowin and Jefferson, 1999; Pavlů et al., 2006b; **Chapter 5**). We have seen in our study that forage quality was continuously declining as the growing season proceeds and the forage was suitable for dairy cows only in the first seven weeks. After that the forage was able to support beef cattle as the acid detergent fibre (ADF) and neutral detergent fibre (NDF) kept increasing. Similarly, mineral concentrations (P, K, Ca, Mg and N) that are essential especially for dairy cows that have high nutritional requirements are also affected as the herbage matures along the growing season while the mineral concentrations decline. This study highlighted the decline in overall quality of forage when cutting or grazing is planned after the first seven weeks entailing potential decline in productivity of dairy cows. Hence, to guarantee participation of farmers in conservation of semi natural grasslands via AES, continuation of financial compensation is critical for declining forage quality when the first cutting or grazing is postponed after mid-June or if the interest is to promote extensive grazing.

7.2. Management implications

Overall the aim of this thesis work was to provide insight into effects of different management methods on sward parameters and provide potential recommendations for semi-natural grasslands based on the results from the included papers. Given the evidence of a growing population and constant pressure of ensuring food security, emphasises the need of utilizing available resources wisely. The thesis highlighted the importance of semi-natural grasslands, especially those that are located in upland areas which are typically considered as marginal and abandoned, having huge value given the right management. By showing the value of this marginal areas using different sward parameters (biomass production, forage quality, nutrient in soil, etc) important information were generated that could be useful for decision making be it for land owners or for AES. The previously abounded grasslands used for this thesis work showed that grazing as well as cutting management in the different sites can provide high biomass production and good forage quality which can contribute to heifer performance.

According to **Chapter 2** result, grazing intensity had significant effect on the total biomass as well as on the vertical distribution of different functional groups. Importantly the presence of high dead biomass in the lower bottom in contrast to the high living biomass in the upper layer calls for rethinking our methodological approach when sampling biomass from grasslands for productivity or forage quality analysis. This adjustment will minimize the possibilities of inflating or reporting incorrect results by avoiding the bottom layer which is normally ungrazed by heifers and filled with dead biomass.

Sward structure in grassland is not only affected by grazing intensities (**Chapter 2**) but also by other grazing related factors such as trampling and dung deposition. The result from **Chapter 3** indicates patches under different intensities were affected differently by the presence of dung. The key driver for N, P and K concentrations in the herbage in our study was the presence of faeces under intensive grazing. Interestingly, dung had no significant effect on soil nutrient concentration which was in contrast to other similar studies. This highlights the need for conservation aims or management to consider the site condition as the effects may differ for different grassland species as well as grassland characteristics.

The result from **Chapter 4** suggests a need for further research and flexibility, even though the management applied (cutting twice per year and cutting twice per year coupled with herbicide application) showed encouraging results. But the excessive presence of soil nutrients and abandonment of the grassland for several years led to the dominance of weedy species. Hence, additional management that can supress or eradicate this weedy species will be necessary. Among the recommended methods goat grazing and sheep grazing showed encouraging result in other study areas (Hejcman et al., 2014; Zaller, 2006). But the applicability of such recommendations must be carefully studied as the site is part of a national park. But leaving the site without any management will definitely lead to the reclamation of the land by weedy species and the excess nutrient in the soil supports it.

It is a well-known fact that grasslands main function is to provide forage for livestock. But due to their importance beyond forage production, many grasslands especially, semi-natural grasslands are protected for instance via AES. But such conservation aims or approaches involve reduction of management intensity such as delaying first cut or early season grazing. Finding the balance between the aim of nature conservation and interest from framers is critical. Therefore, **Chapter 5** result show up to the first seven weeks of the vegetation season the forage quality is suitable for cattle even as the only source of feed, but after that the forage quality is very low and it is only suitable for low productive cows and beef cattle. This result stresses the need to maintain the AES payments to compensate farmers for potential loss of high-quality forage that can affect heifers performance. This approach will protect the semi-natural grasslands and give framers the incentive to keep up with less intensive management despite the potential decrease in forage quality. Similarly, **Chapter 6** result indicates extensive management can meet cattle requirements and at the same time help in landscape management by decreasing the chance of temporary abandonment of grasslands. If states subsidies are also included for instance in Czech conditions extensive grazing can be profitable for private farmers as well as meeting nature conservation objectives.

7.3. Conclusion

The published papers included in this thesis suggest that the different defoliation management methods have different effects on the sward parameters of semi-natural grassland. One of the most important factors that influenced the sward parameters is grazing and its intensity. Compared to the traditional cutting management, grazing management seems to offer a higher biomass production, influences the nutrient cycle of the grassland via dung and urine return to the system as well as influencing the species composition of the grassland in the long run. In contrast the cutting management, played important role especially in upland areas that are typically neglected of management and under threat of encroachment by shrubs or dominance of weedy species. In our study area we highlighted the significant effect of cutting in restoring heavily infested (weedy and expansive species) grassland. This management method showed good result in removing excess nutrient from the system. But the extreme presence of soil nutrient meant further management is required. Hence choosing the appropriate method for specific sites, must consider the previous management history of the site, the existing condition, the future plan or objective and the cost implication for management.

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Curriculum vitae

&

List of publications

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Education and Academic Titles

Date	Institution	Degree
09/2016 – Present	Czech University of Life Sciences, Prague, Czech Republic	Ph.D. (in progress)
09/2014 – 06/2016	Czech University of Life Sciences, Prague, Czech Republic	Ing. in Nature Conservation
09/2003 – 07/2006	Mekelle University, Mekelle, Ethiopia	B.Sc. in Natural Resource Economics and Management

Training and other experience

01/01/2018 -30/06/2018 & 18/04/2017 -30/09/2017 ERASMUS+ Program Internship: Support to Project Management, public relations, environmental education and fundraising
NABU e.V. Headquarters Berlin, Africa Department, Germany

28/08/2017–02/09/2017 Summer School on Bioeconomy, Warsaw University of Life Sciences, Warsaw, Poland

11/01/2016–26/01/2016 Certificate on Forest Governance Assessment and Monitoring, World Bank Group

04/12/2011–09/12/2011 Project Management Training, Vrje University Amsterdam & MDF

11/08/2006 - 18/08/2006 Participatory Rural Appraisal (PRA), Heinrich Böll Foundation

Working experience

01/03/2021-Present	Project Assistant and Researcher **Crop Research Institute, Liberec, Czech Republic**
03/09/2016–02/28/2021	Researcher and support to Project Management **Czech University of Life Science Prague (CULS), Czech Republic**
19/09/2018-31/12/2018	Consultant for Project Development **NABU e.V. Headquarters Berlin, Africa Department, Germany**
01/01/2018-30/06/2018	Support to Project Management and Dissemination **NABU e.V. Headquarters Berlin, Africa Department, Germany**
09/04/2012–01/09/2014	Officer for Natural Resources and Forest **NABU e.V., Project Office Bahir Dar, Ethiopia**
01/05/2010–31/03/2012	Project Officer for Climate Change Adaptation and Mitigation **Horn of Africa Regional Environmental Centre (HoA-REC), Addis Ababa, Ethiopia**
01/02/2009–28/02/2010	Field Research Expert and Adviser for Participatory Methods **Deutscher Entwicklungsdienst (DED)/ GIZ Programm "Sustainable Use of Natural Resource", Climate Change Department, Addis Ababa, Ethiopia**
1/10/2006-29/12/2008	Research Assistant and Organisation of Field Research **Bonn University, Geographic Development Research**

Grants

IGA FŽP 20194211: Vertical and Horizontal sward structure of species rich upland grassland under long-term grazing management, main researcher

EU, Interreg SN/CZ r.n. 100264999: Sustainable grassland management for supporting biodiversity, assistant researcher

EU, Interreg CZ/PL r.n. CZ. 114.120/0.016_026/0001092: Grassland biomass as a renewable resource of energy-Biodiversity-Biomass-Biogas, assistant researcher

Language Skills

Language*	Understanding	Speaking	Writing	Certificate
Amharic*	C2	C2	C2	-
English	C2	C1	C1	IELTS (7.5)
German	B1	B1	A2	Goethe Institute Sprachart Berlin
Czech	A1	A1	A1	-

* mother tongue underlined

List of author’s publication during Ph.D. study period

<u>Publications (Web of Science and Scopus)</u>

<u>Teowdroes Kassahun</u>, Klára Pavlů, Vilém V. Pavlů, Lenka Pavlů, Petr Blažek. (2021). Effect of 15-year sward management on vertical distribution of plant functional groups in a semi-natural grassland. *Applied Vegetation Science*, 2021;24:e12568. https://doi.org/10.1111/avsc.12568

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Klára Pavlů, **<u>Teowdroes Kassahun</u>**, Vilém V. Pavlů, Lenka Pavlů, Petr Blažek, Petr Homolka. (2021). The effects of first defoliation and previous management intensity on forage quality of a semi-natural species-rich grassland. *PLos ONE.* 16(3): e0248804. https://doi.org/10.1371/journal.pone.0248804

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Chukwudi Nwaogu, **<u>Teowdroes Kassahun</u>**, Eneche P.U.S. (2020). Climate Change Induced Soil Compaction: Evaluating the Adaptation Measures to Enhance Maize Yields in a Tropical Humid Acidic Soil, Nigeria. In: Leal Filho W., Nagy G., Borga M., Chávez Muñoz P., Magnuszewski A. (eds) Climate Change, Hazards and Adaptation Options. *Climate Change Management*. Springer, Cham. https://doi.org/10.1007/978-3-030-37425-9_36

Klára Pavlů, **<u>Teowdroes Kassahun</u>**, Chukwudi Nwaogu, Lenka Pavlů, Jan Gaisler, Petr Homolka, Vilém Pavlů. (2019). Effect of grazing intensity and dung on herbage and soil nutrients. *Plant, Soil and Environment*, 65, (7), 343-348. https://doi.org/10.17221/177/2019-PSE

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Other peer reviewed publications

Tadesse Woldemariam Gole, Svane Bender, Rolf D. Sprung, Solomon Kebede, **Teowdroes Kassahun**, Alemayehu Negussie, Kerya Yasin, Motuma Tafa. (2020). Sustainability at the centres of Origin: Lessons from UNESCO Biosphere Reserves in Ethiopia. In. Maureen G. Reed, Martin F. Price (eds). UNESCO Biosphere Reserves Supporting Bio cultural Diversity, Sustainability and Society. Routledge, London pp 164 - 175. DOI: 10.4324/9780429428746-13

Jan Titěra, Henning Haase, **Teowdroes Kassahun**, Chukwudi Nwaogu, Klára Pavlů, Matthias Kändler, et al. (2018). Divergrass – a cross border project to promote sustainable management of grasslands. ACC Journal, 24 (1), 61 – 80.

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