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DYNAMIC OF NUTRIENTS IN GRASSLAND BIOMASS UNDER DIFFERENT MANAGEMENT PRACTICES

(Dynamika obsahu živin v biomase travního porostu při různém způsobu obhospodařování)

(Ph.D. Dissertation Thesis)

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AUTHOR'S STATEMENT/DECLARATION

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Abbreviations:

DMSB - Dry Matter Standing Biomass

IVOMD - In Vitro Organic Matter Digestibility

CP - Crude Protein

CF - Crude fibre

SH - Sward Height

DM - Dry Matter

NDF - Neutral detergent fibre

ADF - Neutral detergent fibre

WSC - Water Soluble Carbohydrates

N_{tot} - Total Nitrogen in the soil

C_{org} - Organic carbon in the soil

BRE - Brignant Restoration Experiment (UK)

OFQE - Oldřichov Forage Quality Experiment (Czechia)

OPE - Oldřichov Patch Experiment (Czechia)

RPE - Resting Place Experiment (Slovakia)

Contents

CHAPTER 1

General introduction

1.1 Permanent grassland

Permanent grasslands are a major attribute of land use in Europe and comprise about 35% of the total utilized agriculture area in the EU-28 countries of Europe (Dudek et al., 2020; Smit et al., 2008). They comprise both semi-natural (man-made) and natural grasslands. Natural grasslands originate in areas, where there aren't suitable conditions for the creation of a forest, therefore they do not have to be maintained by men. Forest formation is mostly limited by temperature, mechanical damage, altitude and lack of rainfall (Pavlů et al., 2019b; Vera, 2000).

Permanent grasslands are complex of plant communities, which are characterized by large variety of botanical, morphological, physio-chemical and anatomical composition. These grasslands consist of grasses, forbs and legumes. Permanent grasslands have an important role in protecting and creating of environment such as water retention and erosion protection (Frame, 1992). They also support other ecosystem services including provision of landscapes and habitat, carbon sequestration, they provide the forage for livestock and further secure great source of plant and animal diversity (Le Clec'h et al., 2019).

Majority European grasslands are man-made (Hejcman et al., 2013) and regular human intervention based on defoliation is necessary for their maintenance (Frame, 1992; Novák, 2008a). Grasslands are divided into pastures and meadows according to the method of management, pastures are maintained by grazing and meadows are maintained by cutting (Gibson, 2009).

Pastures were originated in Central Europe after the last glaciation, when steppe vegetation areas were grazed by large herbivores (European bison, wild horse, wild cattle), which defended the formation of the forest. Their impact was very significant until the beginning of agriculture (during Neolithic period, 5300–4300 BC). Livestock farming (cows, goats, sheep, horses and pigs) was based on grazing, which kept or expanded existing the landscape in forestless until the early Iron Age (750–500 BC) (Hejcman et al., 2013; Vera, 2000).

Meadows originated much later than pastures and their origin is related with the invention of the main tool for harvesting of meadow stands-scythe, which first appeared around 500 BC. Their look was different from today's scythes, they were short tools and biomass had to be harvested a bit higher above the ground (Hejcman et al., 2013).

Botanical composition, sward density, type of defoliation, nutrient cycle, presence of faeces and disturbance are the major differences between pasture and meadow. Pasture are usually characterized by creeping and short species, plants with a ground leaf rosette and with defence mechanisms against the grazing animals. Additionally, the sward in the pasture has high density. Aboveground biomass is removed selectively on the pastures during the grazing season, whereas on meadows non-selectively and at one time. About 80-90% of the nutrients are usually returned back to the soil in the form of faeces and urine (uneven distribution) to the pastures and on the contrary biomass from meadows is removed after cutting and if fertilizers aren't applied, nutrients are gradually depleted from the soil (Pavlů et al., 2019b). The presence of faeces on the pastures, which grazing cattle avoid (MacDiarmid and Watkin, 1972), leads to local eutrophication and the formation of a mosaic structure. Further, regular soil disturbance by animals (trampling) creates conditions for germination and subsequent growth of seedlings on the pastures (Pavlů et al., 2019b).

1.2 Plant species composition of grassland

Grasslands are formed by the different herbaceous plant species, which reflect soil-climatic conditions and management. Generally, plant species can be divided into these main functional (agro-botanical) groups: grasses, legumes and other forbs (Frame, 1992; Gibson, 2009; Whitehead, 2000).

1.2.1 Grasses

Grasses belong to the *Poaceae* family (Kaplan et al., 2019), which consists of different species with different characteristics. They usually create a dense sward, which resists livestock grazing very well (Pavlů et al., 2006c; Regál and Krajčovič, 1963). Further, roots of the grass sward significantly increase resistance of soil to water erosion (Kollárová et al., 2007) and in general, grasses produce more root biomass than legumes and other forbs (Whitehead, 2000).

Forage of grasses has the highest quality (the highest level of nutrients and digestibility) before and at the time of ear emergence, but during and after the flowering concentration of nutrients and digestibility decreases rapidly. This is due to increasing concentration of lignin, fiber and silicic acid, therefore, it is recommended to do the first cut or beginning of grazing before the flowering (Frame, 1992; Whitehead, 2000).

1.2.2 Legumes

Legumes belong to *Fabaceae* family (Kaplan et al., 2019) and they are primarily perennial plants. Symbiosis with tuberous bacteria is their very significant property, that enriches the soil with nitrogen up to 500 kg ha^{-1} N $year^{-1}$, but more common is about 80–100 kg ha^{-1} N $year^{-1}$ (Whitehead, 2000). They are demanding of light, therefore dense vegetation is not suitable for them and their maximum yield is usually achieved from the second to third year after sowing. They are modest to the temperature, but some of them are more sensitive to hard frost and harsh climatic conditions (Frame, 1992; Regál and Krajčovič, 1963).
Legumes are characterized by high mineral concentrations (Mg, Ca, P, K), lower concentration of fiber and high concentration of crude protein (Hakl et al., 2015; Kollárová et al., 2007). Growing legumes are more sensitive to soil acidity or low concentrations of K and P than grasses (Frame, 1992).

Legumes have higher digestibility than grasses due to slightly incrusted and soft epidermis (Frame, 1992), so they are very important component of feed for livestock in fresh forage and also in hay (Ivanič, 1984). However, feeding only young clover, especially *Trifolium hybridum, T. pratense* and *T. repens*, is very dangerous, because it can cause bloating of animals. Legumes are evaluated as the best forage in terms of yield and quality, but greater average losses during conservation of forage are a major shortage of legumes (Frame, 1992; Regál and Krajčovič, 1963).

1.2.3 Other forbs

Generally, forbs have good digestibility (in the optimum harvest time) and good mineral concentrations. During extensive use of grassland (with low amount of harvest without fertilization), forbs are able to quickly adapt to changing trophic regime of soil (Kollárová et al., 2007). Forbs have a higher mineral content than grasses, drought resistance and acceptability to stock (Frame, 1992; Pirhofer-Walzl et al., 2011).

1.3 Main methods of grassland management

Biomass production has been the main goal of grassland management for centuries and forage quality has been reflection of the available mechanization and management (pasture and hay production) throughout history (Vera, 2000). Most grasslands were intensified in the second half of the last century and only a little of low-production grasslands remained. The intensification of grasslands was achieved by i) application of fertilizers - more frequent defoliation, ii) earlier cutting - silage production needs younger vegetation than hay production, iii) replacement of permanent grasslands by temporary grasslands with species of high productivity (Isselstein et al., 2005).

1.3.1 Grazing

Grazing is the oldest way of grassland management, which resulted in creation of pastures. Short vegetation adapted to trampling and regular defoliation is typical for them. In Central European conditions a pasture sward is characterized by high proportion of short grasses together with rosette and creeping forbs, especially *T. repens* (Frame, 1992; Hejduk and Gaisler, 2006; Mládek and Hejcman, 2006).

The choice of grazing system depends on the climatic conditions, possibilities of pasture fencing, numbers and species of animals and their experience, botanical composition, areas of the grassland and soil properties (Pavlů, 2001). Pastures can be divided in to temporary or permanent, extensive or intensive (Novák, 2008b). Their intensity and the timing of grazing activities are important factors, that affect quality and quantity of grazing forage (Henkin et al., 2011; Koidou et al., 2019; Ma et al., 2014; Pavlů et al., 2006b). It means, that the determination of the convenient term for grazing should be suggested by several factors: plants, animals, site, management and economic factors (Vallentine, 2001).

Cattle are grazing generalists, it means they aren't very selective during grazing (Rook and Tallowin, 2003), but they avoid areas with tall-stem herbage, that are difficult to graze (DeVries and Daleboudt, 1994) and also areas with excrements (MacDiarmid and Watkin, 1972). Grazing by large herbivores causes a soil disturbance, alters concentration of nutrients in the patches after defecation and urine, which leads to changes in grassland structure and composition (Gibson, 2009; Ludvíková et al., 2014; Rook et al., 2004). For pastures, which are only grazed, it is advisable to cut the ungrazed patches after the first and second grazing (Nágl and Rais, 1961).

Different grazing intensity changes productivity of grasslands and their botanical composition and thus affects mineral contents in the herbage (Vermeire et al., 2008). Grasslands are the main source of minerals for grazing herbivores, which are necessary for maintaining livestock health, growth and reproduction (Jones and Tracy, 2013).

There are different preferences among grazers, goats and cattle prefer grasses, whereas sheep prefer mainly forbs. Leaves are part of the forage which has the highest-quality, so they are preferred by animals, if they have a choice (Gibson, 2009).

Different digestibility of single plant species or their parts is a reason of selective grazing of animals. Forages with higher yield and lower digestibility are more suitable for less demanding animals (Matches and Burns, 1985).

1.3.2 Cutting

Cutting with biomass removal is an alternative mechanical defoliation (Frame, 1992), which is a common management used in many grasslands in Europe. It keeps out woody plants, reduces forbs, encourages growth of grasses, maintains the required sward heights (Gibson, 2009). It is also used for hay and silage production, the optimum stage of phenology at harvest time is necessary for their quality, cutting at late maturity causes lower quality (Frame, 1992). Cutting is carried out using different tools: small mechanization, manual mowing by scythe or self-propelled and tractor mowers; in which above-ground biomass is separated from the stubble at a height of 3–10 cm (Hejduk and Gaisler, 2006). More frequent cutting generally supports short species, especially species with ground rosettes of leaves or species with creeping growth (Pavlů et al., 2019b). Height and frequency of cutting depends on the specific site condition and goals of management.

Cutting was primarily used for obtaining feed for livestock, later it began to be used for optimization of vegetation structures and species composition (Pavlů et al., 2019b). Optimum time and number of cutting are chosen according to optimal sward maturity it means compromise between quality and quantity of forage, the type of vegetation, habitat, soil conditions, altitude and climate (Frame, 1992). However, there are two main target groups of stakeholders. Farmers which are focused on the good forage quality and biomass production and on the other hand nature conservationists, who are focused on maintaining (or increasing) species diversity (Pavlů et al., 2019b).

1.3.3 Combination of grazing and cutting

Grasslands can also be managed by combination of cutting and grazing (Pavlů et al., 2021; Van Diggelen and Marrs, 2003). Cutting is non-selective removal of above-ground biomass, while grazing influences sward by selectivity of grazing, stocking rate, nutrient enrichment and trampling (Pavlů et al., 2019a; Stewart and Pullin, 2008; WallisDeVries, 1998). This combination is generally recommended for management supporting species richness (Krahulec et al., 2001).

1.3.4 Fertilization

The type of soil acidity affects the need for fertilization and nutrient availability for plants (Pavlů et al., 2006a). Fertilization can be minimized or omitted, if permanent grasslands are managed by grazing, because nutrients are returned (80–90%) into the soil in the form of solid and liquid faeces (Královec, 2001).

Fertilization is important especially in grasslands with long-term cutting, where a large loss of soil mineral nutrients, which can be up to tens of kg per ha during the year (Pavlů et al., 2016), is detected. It is very important to set up amount and type of fertilizer, date and method of fertilization. Breach of these principles usually leads to undesirable change in the species composition of grassland and leaching of nutrients (Hejduk and Gaisler, 2006). Organic fertilizers are more suitable for grassland fertilization than mineral ones from the nature conservation point of view, because they have the large surface, improve the soil structure and bind the nutrients, which are released gradually and thus prevent their leaching (Pavlů et al., 2019b).

The NPK fertilizers usually increase yield and the sward height, which creates unsuitable lighting conditions for short plant species and thus reduces their competitiveness. Therefore, short species react negatively to fertilization and as the sward usually comprises nearly half of short species, their decline significantly reduces grassland diversity (Titěra et al., 2020).

1.3.5 Mulching

The lack of livestock and the use of grassland biomass in Central Europe changed management of semi-natural grasslands and previously intensified upland grasslands. Mulching has been used as a low-cost management since the 1990s in the Czech Republic as alternative to grazing or conventional cutting (Gaisler et al., 2019). Above-ground biomass is mechanically separated from the stubble, crushed and evenly spread back on the stubble (Hejduk and Gaisler, 2006), to avoid the unwanted changes in botanical composition of grasslands, that are not currently used for agriculture (Gaisler et al., 2019). Mulching should not theoretically deplete available soil nutrients from the upper soil layer, unlike cutting, where biomass is removed. The nutrients released during the decomposition of organic matter should be reused by plants. Long-term use of mulching isn't suitable as substitute for standard grassland management, because it negatively affects the structure of vegetation, biodiversity of plant species and animals in grassland (Pavlů et al., 2016; Pavlů et al., 2019b).

1.3.5 Seeding

Seeding of high productive cultivars of legumes and grass species is used to improve quality and production of biomass (Hejduk and Gaisler, 2006), especially in case if the sward is sparse or composed from poor quality plants (Frame, 1992; Nágl and Rais, 1961). Cultivars for sowing are usually selected according to the optimum temperature for germination, growth conditions, emergence and early growth. Deep soils are better for seeding as they have smaller fluctuations in daily temperatures and more available water (Pearson and Ison, 1997). Seeds on the soil surface have worse conditions, because they can absorb water only through the part, which is in contact with the soil surface, so large seeds are at disadvantage in comparison with small ones (McWilliam and Dowling, 1970).

1.4 Quantitative parameters of grassland

The amount of harvested biomass depends on the soil moisture, fertility, intensity of grassland management (Pavlů et al., 2019b), type of management (Hejcman et al., 2010; Klimeš and Klimešová, 2002) and ecological conditions of the habitat. The range of biomass yield is generally between 5-12 t DM ha $^{-1}$ year $^{-1}$ in European grasslands (Kollárová et al., 2007; Wilkins, 2002) and the growth peak of the grassland is at the turn of May and June. The year-on-year variability of yields can be very different, due to various weather fluctuations, especially in the distribution and total precipitation (Kassahun et al., 2016).

1.5 Qualitative parameters of grassland

Forage quality and biomass yield are the most important factors that are affected by the date of harvest (Frame, 1992), type of vegetation, grassland management, weather conditions (Schaub et al., 2020), phenophases and representation of individual agro-botanical groups in grassland during the vegetation season (Mládek et al., 2011).
Qualitative parameters are changing with ageing of swards during the vegetation season (Boob et al., 2019; Fiems et al., 2004; Mládek et al., 2011; Tallowin and Jefferson, 1999). Forage usually has high digestibility values but low herbage yields in the early part of the growing season. Later, biomass yield increases with increasing maturity and net biomass accumulation, which is connected with increase in cell wall content and decline in digestibility (Frame, 1992). Forage from single species have different properties. For instance, legumes are generally of higher quality than grasses, but the digestibility of legumes and cool-season grasses is very similar (Colins and Fritz, 2003).

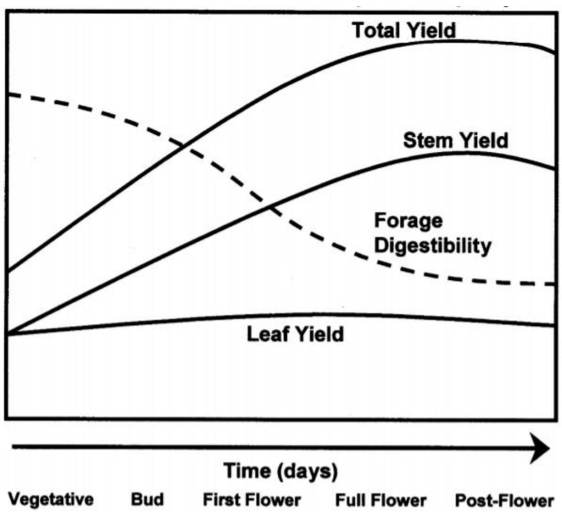

Fig.1. Relationship between yield and quality (http://www.ostrich.org.uk)

Generally, fibre contents show a progressive increase but in vitro organic matter digestibility decrease with ageing of the forage during the vegetation season (Figs. 1,2) (Boob et al., 2019; Frame, 1992; Koidou et al., 2019) Similar decrease with ageing of the forage also occurs in case of P and K concentrations (Duru and Ducrocq, 1997).

The general knowledge about nutrient concentrations in the forage helps to predict their deficiency and suggest supplements according to their needs (Vallentine, 2001). Leached or depleted nutrients from the soil can be returned back by organic or mineral fertilizers (Novák, 2008b). Forage quality of grasslands is also affected by the secondary compounds such as allelochemicals and anti-herbivore defences, in which many of them are toxic and affect nutritive value and digestibility of forage (Gibson, 2009). These structural anti-quality factors have a negative effect on herbivore ingestive abilities by reducing chewing rate, chewing efficiency, bite rate and bite mass and also by reducing the digestibility of other nutrients (Laca et al., 2001). They can also affect herbivore physiology, that causes stress or toxicity. The terpenoids, phenolics and nitrogen compounds are most important ones (Gibson, 2009).

Therefore, there are important questions concerning the most suitable time to start the grazing season or to apply the first cut in relation to the nutritional and mineral requirements of cattle. Suitable time for grazing or mowing is affected not only by herbage maturation but also by the type of vegetation, weather conditions and grassland management (Schaub et al., 2020).

Animals generally prefer young plants with actively growing leaves and stems than older mature plants. If young forage is not available, the animals prefer the forage in the order of: green leaves, green stems, dry leaves, and dry stems (Wallace, 1984).

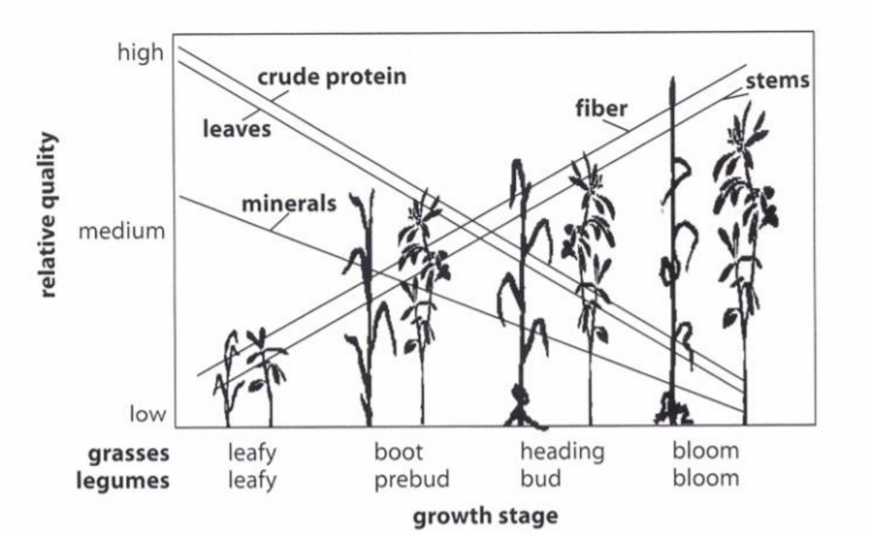

Fig. 2. Effect of plant maturity on forage quality (Ball et al., 2001)

1.5.1 Mineral nutrients in the herbage

The contents of minerals in the plants depend on several factors, which include plant genotype, stage of maturity, climate, soil properties (e.g. soil moisture, content of elements in the soil and their availability), intensity of shading (Míka, 1997; Schaffers, 2002; Suttle, 2010).

Mineral elements are divided into macro and micro-elements according to their amounts in the herbage. Macro-elements include nitrogen (N), potassium (K), phosphor (P), magnesium (Mg), calcium (Ca), sodium (Na), sulphur (S) and chlorine (Cl); while micro-elements include manganese (Mn), zinc (Zn), copper (Cu) iron (Fe), and selenium (Se) (Frame, 1992; Novák, 2008b; Whitehead, 2000).

Changes in the soil pH have significant influence on content of micro-elements in the herbage, on the contrary they have only slight influence on content of macro-elements. For example, contents of Se and Mo in the forage increase with growth of pH in the soil, conversely contents of Mn and Co in the forage decrease with slight increase of pH in the soil (Míka, 1997). Herbage in the early part of the growing season usually contains enough nutrients, but as soon as these plants begin to mature, the level of nutrient concentrations decreases (Vallentine, 2001). This is due to the 'dilution effect' described by Duru and

Ducrocq (1997), in which during the maturation stage the herbage biomass increases whereas mineral concentration declines (Míka, 1997; Mládek et al., 2011).

Herbs and legumes have higher concentration of minerals, especially K, Ca, Mg than grasses (Míka, 1997; Pirhofer-Walzl et al., 2011). *Dactylis glomerata* is connected with the highest and *Phleum pratense* with the lowest mineral concentration in the frame of grasses in temperate grassland. Content of Na is very variable unlike contents of Ca, K, N, P, where variability is relatively low (Míka, 1997).

Nitrogen is considered to be the most important element in plant nutrition, nonetheless, its high content in the soil causes greater grass growth at the expense of forbs, particularly legumes (Frame, 1992). High plant-available concentrations of nitrogen and phosphorus in the soil promote especially growth of tall grasses, while high concentration of nitrogen together with low phosphorus concentration supports the growth of short sedges and short grasses. Excessive amount of phosphorus reduces the species richness of grassland (especially in combination with nitrogen), although plants uptake only a small amount of phosphorus from the soil. Higher concentrations of potassium do not affect the number of species as negatively as phosphorus (Janssens et al., 1998). Common application of potassium and phosphorus (or each alone) without nitrogen application supports the occurrence of forbs, primarily legumes (Hejcman et al., 2007; Titěra et al., 2020).

When availability of some minerals decreases so much, that the level of nutrients in body of animal falls below threshold values, characteristic symptoms of disease may occur (Familton, 1990). Amount and ratio of nutrients are very important for adequate performance, which varied for different animals' categories (Pozdíšek et al., 2002). Intake of elements, which are in deficiency as well as in excess, is undesirable in animal nutrition (Míka, 1997; Suttle, 2010). Animal mineral deficiency is result of complex series of interactions involving cycle of soil-plant-animal (Familton, 1990). Lack of elements for animals is connected with those, which plants need in a small amount (Se, Co, I) and on the contrary surplus of elements occurs when plants need them in a bigger amount (K). Cattle can adapt to low intake of Na in the long term, but excessive intake has not positive effect on the performance (Suttle, 2010).

The need of minerals for animals depends on species, breed, ability of the animal to utilize minerals and on physiological state (e.g. pregnancy, lactation, growth). Some domestic animals are especially sensitive to mineral deficiency (Familton, 1990). The knowledge of available nutrients in the forage can help to predict their deficiency and suggest supplementation according to the needs of animals. Further, the growth phase of plants (phenology) also significantly influences nutritional levels in the forage (Vallentine, 2001).

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 (Whitehead, 2000). The forage for livestock must be variable according to their requirements, which are different in various stages of production (Vallentine, 2001). Minerals that are in a shortage and do not meet the requirements for animals must be added by mineral licks (Novák, 2008b; Whitehead, 2000).

1.5.2 Organic components in the herbage

Organic components usually comprise crude protein, fat, fiber and nitrogen-free extract (Zeman et al., 2006). Digestible nutrients we call those, which are not excluded by faeces. They do not have to be only nutrients that have been resorbed in the gastrointestinal tract, but also nutrients that have been converted by microbial digestion in the stomach of ruminants, e.g. gas that is excreted from the organism through belching (Zeman et al., 2006). Increasing proportion of structural carbohydrates and lignification in herbage maturity causes gradual decrease of digestibility (Frame, 1992; Rymer, 2000). Young herbage cell wall has high digestibility up to 90%, but it declines during aging to 30% due to increasing lignification (Buxton, Russell and Wedin, 1987).

The concentration of the cell walls increases with increasing temperature, because high temperatures accelerate growth, flowering and maturation, that causes an increasing lignification and decreasing digestibility, because of reducing concentrations of soluble carbohydrate (Deinum and Dirven, 1976; Gibson, 2009), which might be supported by cloudy weather or shading (Pearson and Ison, 1997).

Amount of digestible matter allows us to evaluate the nutritional value of feed. (Koukolová et al., 2010). The optimal value of digestibility required in the forage for dairy cows is higher than 67% (Frame, 1992), for beef cattle at least 60% (NRC, 2000) and maintenance value in the forage for cattle is around 50% (ARC, 1980).

Carbohydrate complex (fiber) is one of the most important components of the forage. Carbohydrates are divided into the non-structural (sugars, starches, neutral detergent soluble fibers), which are stored in the cell protoplasm and on structural (crude fibers - hemicellulose, cellulose and lignin), which are stored in the cell walls (Urban et al., 1997; Van Saun and Koukal, 2003).

Crude fiber can be divided into neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin. The NDF consists from hemicellulose, cellulose and lignin, after removing the hemicellulose from NDF remains a part that is composed of cellulose and lignin, which is called acid detergent fiber (ADF) and after removing the cellulose remains only indigestible lignin and silica (Horrocks and Vallentine, 1999).

Hemicellulose and cellulose are partially digestible for ruminants due to their microbial populations within digestive system, which break down these components by fermentation (Vallentine, 2001). Plant cell walls form a large part of the forage (40 to 80%), but they are less digestible (Hatfield, 1989; Van Soest, 1982) and structures, which are comprised of lignin and some mineral undigested residues, remain after digestion (Pearson and Ison, 1997). Indigestible lignin inhibits availability of hemicellulose and cellulose for microbial digestion (Horrocks and Vallentine, 1999). Hemicellulose (Fig.3), which is fixed into the cell wall and protected by layer of lignin, has variable and lower digestibility as well as lignin. Digestibility of hemicellulose itself is 70%, whereas of lignin is less than 3% in plant material (Minson, 1982)

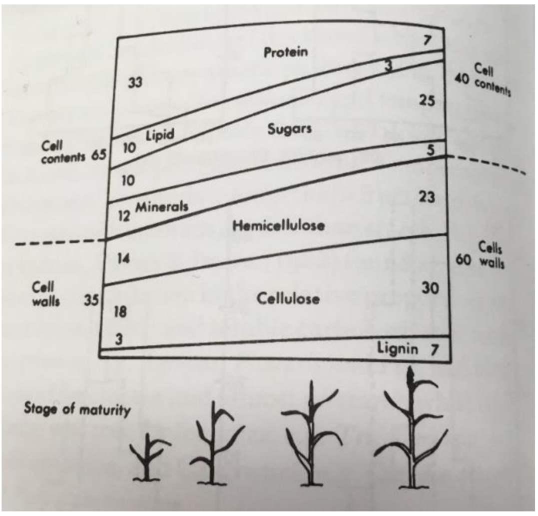

Fig.3. Scheme of changes in the chemical composition of grasses as they age (Osbourne, 1980).

Generally, the optimal concentration of fiber in the diet of ruminant animals provides a mechanical saturation, promotes intestinal peristalsis and motility of rumen, limits intake and digestibility of feed (Zeman et al., 2006). It prolongs time the forage stays in the rumen (Thornton and Minson, 1973) and simultaneously reduces intake forage by animals regardless of other chemical compositions (Hogan, Kenny and Weston, 1987). Lignification is generally evaluated as a major factor, that limits digestibility of plant cell walls, though the current composition of the cell wall, based on polysaccharides (sugars), may affect the extent and rate of digestion by rumen microorganisms, regardless of lignin (Buxton, Russell and Wedin, 1987). Herbage inserts lignin (non-carbohydrate material) into the cell walls with advancing aging, this complex supplies the plant rigidity and tensile strength, thereby reducing the quality parameters of the forage (Horrocks and Vallentine, 1999).

1.6 Soil characteristics

Content of specific minerals in the soil is determined by bedrock properties (Familton, 1990). Further, nutrients also come to the soil by groundwater from the parent rock, waste and decomposition by organic fytomass (Novák, 2008b). Decomposition is the breakdown of organic substances into inorganic ones that are available for intake by plants and up to 90% of primary production in grasslands comes through a decomposition process (Úlehlová, 1992).

The concentration of the ions at the root/soil interface is affected by the amount of available nutrients in the soil, the transport of specific ions through the soil and the buffering capacity of the soil (Pearson and Ison, 1997).

Mineral concentrations in the herbage are indicators of nutrient supply in the soil, and decrease in nutrition reserves in the soil reduces the nutrient concentrations in the herbage, and this affects quality and yield of biomass (Novák, 2008b; Pavlů et al., 2021).

Plant available nutrients in the soil are only in a small amount from the total amount of nutrients present in the soil (nitrogen 5%, potassium 1–2%, phosphorus 0.01%) and these nutrients are mainly in inorganic form (Ashman and Puri, 2002).

Nitrogen is available for plants in inorganic form as soluble NH_4^+ or NO_3^- (Schimel and Bennett, 2004) and activity of soil microorganisms is necessary during nitrogen mineralization (Gibson, 2009). Phosphorus is available for plants in soluble phosphate ions as $H_2PO_4^-$ and HPO_4^{2-} in the soil surface, its intake depends on root distribution. Phosphorus removal by leaching from the soil can be difficult, because it is very stable and poorly mobile in soil solution (Gibson, 2009; Pavlů et al., 2019b). Plants uptake potassium, calcium and magnesium in the form of K^+, Ca^{2+} and Mg^{2+} (Whitehead, 2000). In general concentration of potassium is relatively high in clay soils and low in sandy soils, and unlike phosphorus, potassium is leached more easily (Pavlů et al., 2019b).

1.7 Grassland and Agri-environmental schemes

The intensification of agriculture began in the second half of the 20th century, when sown meadows with higher yields and good forage quality began to be preferred and less productive species-rich meadows were omitted. Nature conservation authorities and active farmers have very different ideas concerning quality of grasslands. Nature conservation favours management, which maintains or increases species diversity and protects endangered plant and animal species, while farmers prefer management focused on higher yields of biomass with high quality of forage for livestock (Isselstein et al., 2005). In recent years, nature conservationists tried to motivate and involve farmers into the nature conservation trough agri-environmental schemes that support species diversity (Pavlů et al., 2019b). These schemes frequently involve a reduction of management intensity and delaying of the first cut or beginning of grazing season in order to allow flowering and seed creation of target species or to protect ground nesting birds (Lakner et al., 2020). However, these extensive managements, in comparison with intensive ones, lead to the reduction of forage quality, especially digestibility of organic matter (Tallowin and Jefferson, 1999). In EU reduced forage quality is compensated by the different payment to farmers that are under agri-environmental schemes (Lakner et al., 2020).

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CHAPTER 2

Main goals and questions

2.1 The objective of the thesis

The objective of the thesis is to answer the following questions:

2.1.1 Finding nutritional properties of semi-natural grasslands with *Agrostis capillaris* and *Festuca rubra* dominance in relation to the period of the vegetation season and management intensity *(Chapter 3)*.

a) What is the impact of previous different grazing intensity on dry matter standing biomass, digestibility, concentrations of crude protein, fibres (ADF, NDF) and macro-elements during the grazing season?

b) When is the appropriate period to introduce grazing or cutting of forage in order to meet cattle nutrition requirements?

2.1.2 Determining 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 *(Chapter 4).*

a) 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?

b) Does the presence of dung on the surface beneath tall sward-height patches affect dry matter standing biomass, dry matter content, dead biomass, and nutrient concentrations in the herbage?

c) Is there any relationship between soil nutrient concentrations and herbage nutrient concentrations under the tall sward-height patches?

2.1.3 Investigating 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 *(Chapter 5).*

a) 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?

b) How fast are nutrients depleted from the soil?

2.1.4 Comparing the different restoration management by sheep grazing, with and without lime application; hay cutting only, with and without lime application; and hay cutting followed by aftermath grazing, with and without lime application and their influence on forage quality and herbage soil relationships on improved upland grassland *(Chapter 6)*.

a) What are the effects of long-term restoration managements and previous liming on forage quality?

b) What are the relations between herbage and soil chemical properties, species richness and soil chemical properties?

CHAPTER 3

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

Klára Pavlů, Teowdroes Kassahun, Vilém V. Pavlů, Lenka Pavlů, Petr Blažek and Petr Homolka

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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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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.

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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).

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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.

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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.

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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

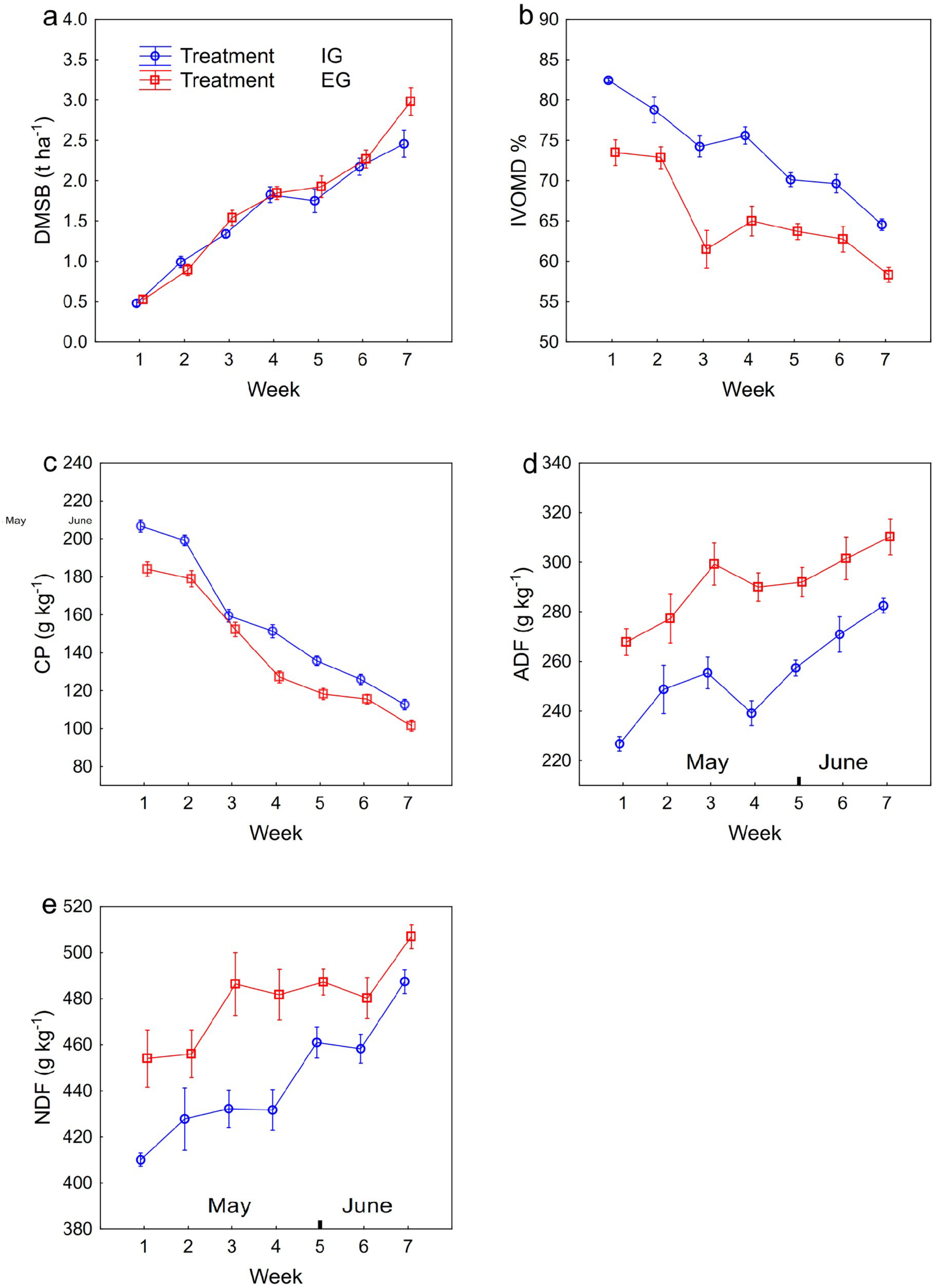

Fig 1. Mean dry matter standing biomass and organic components under extensive (EG) and intensive (IG) management. X-axis refers to the first seven weeks of grazing season in the years 2012 and 2013. Error bars represent standard error of the mean. For abbreviations see Table 3.

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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).

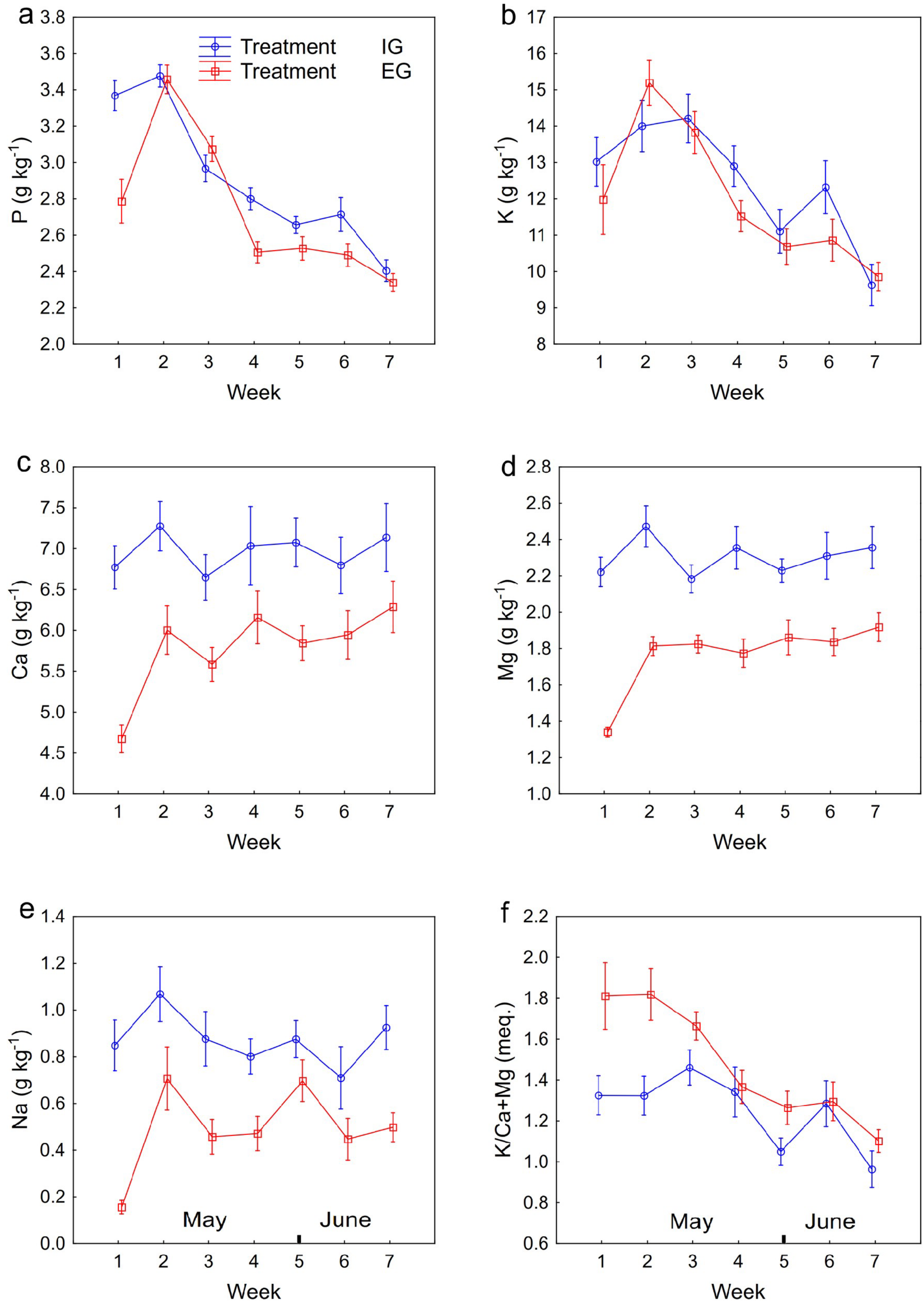

Fig 2. Mean concentration of minerals and K/(Ca+Mg) ratio under extensive (EG) and intensive (IG) management. X-axis refers to the first seven weeks of grazing season in the years 2012 and 2013. Error bars represent standard error of the mean.

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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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CHAPTER 4

Effect of grazing intensity and dung on herbage and soil nutrients

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Effect of grazing intensity and dung on herbage and soil nutrients

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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).

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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.

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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.

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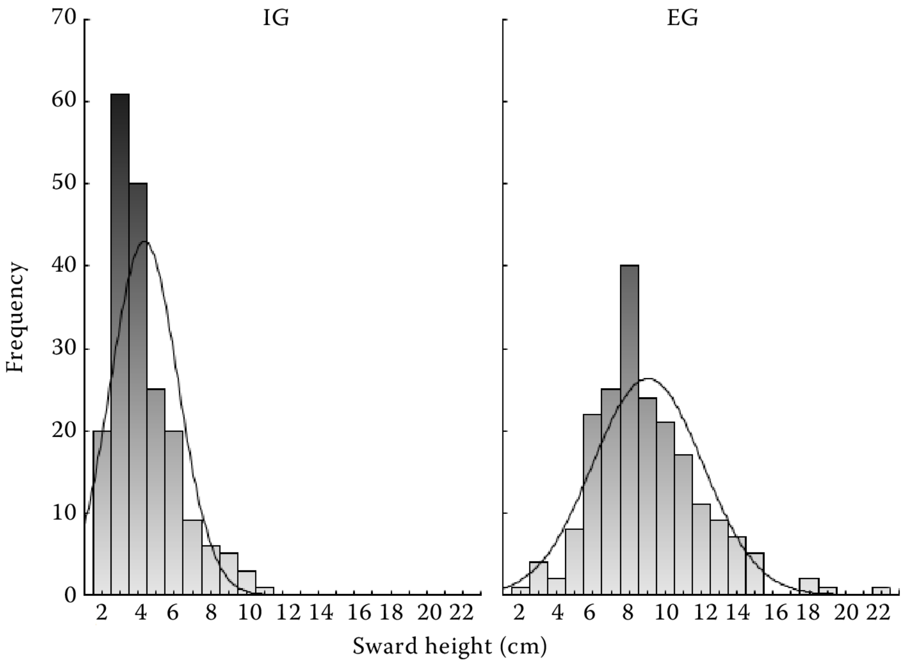

Figure 1. Frequency of distribution showing sward height variation in intensive grazing (IG) and extensive grazing (EG) treatments

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

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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

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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.

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CHAPTER 5

Restoration management of cattle resting place in mountain grassland

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RESEARCH ARTICLE

Restoration management of cattle resting place in mountain grassland

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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].

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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.).

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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

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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 °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.

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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

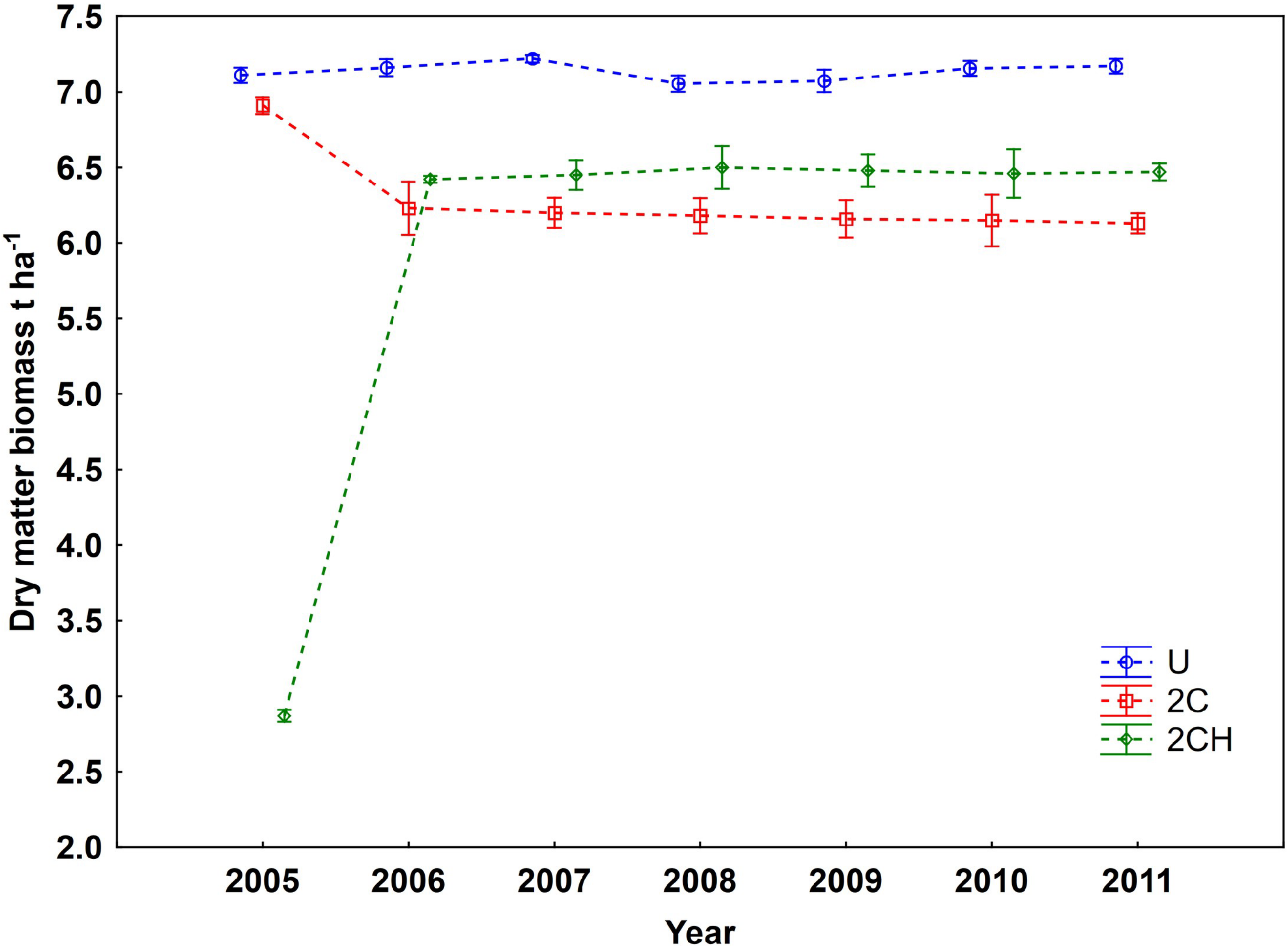

Fig 1. Dry matter biomass production in investigated treatments over the years 2005–2011. Error bars represent standard error of the mean (SE). For treatment abbreviation (U, 2C, 2CH) see Table 1.

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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

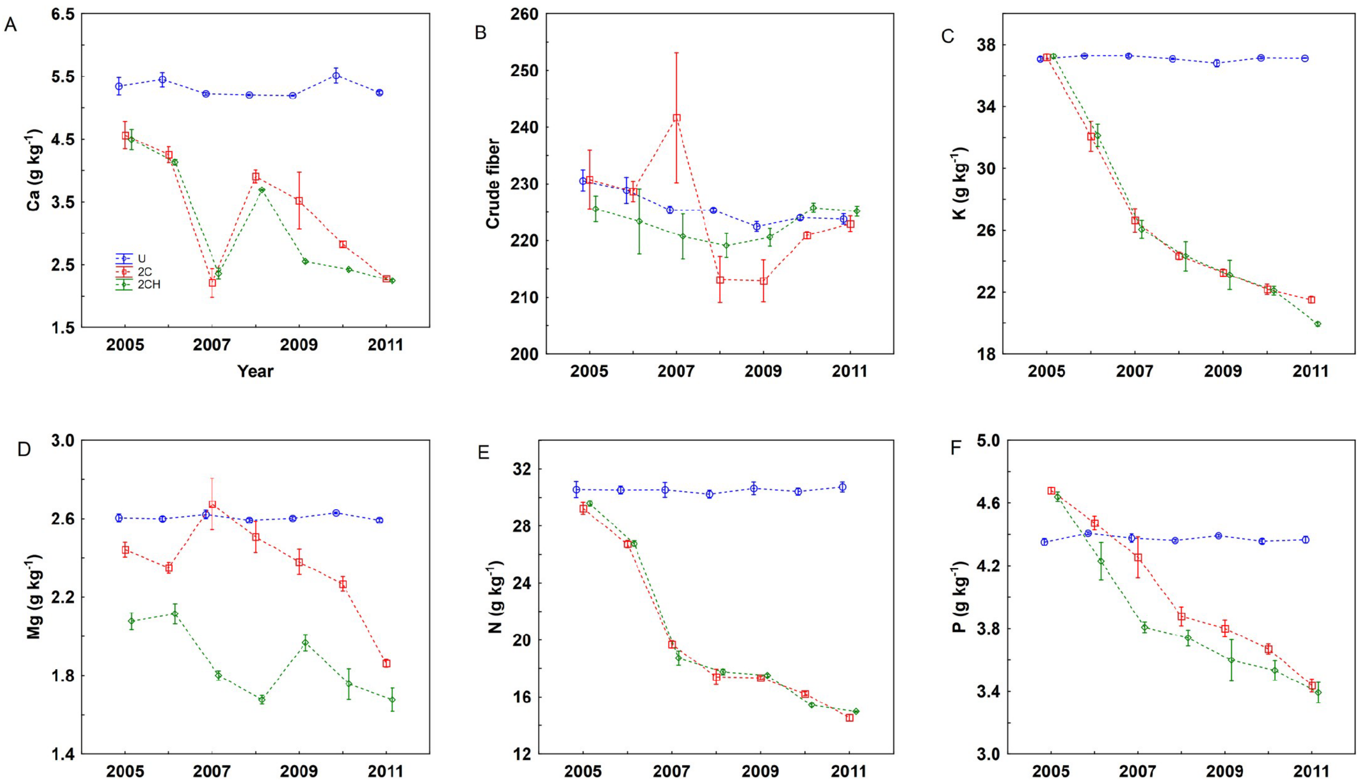

Fig 2. Concentration of Ca (A), Crude fiber (B), K (C), Mg (D), N (E) and P (F) in the herbage. Error bars represent standard error of the means (SE). For treatment abbreviation (U, 2C, 2CH) see Table 1.

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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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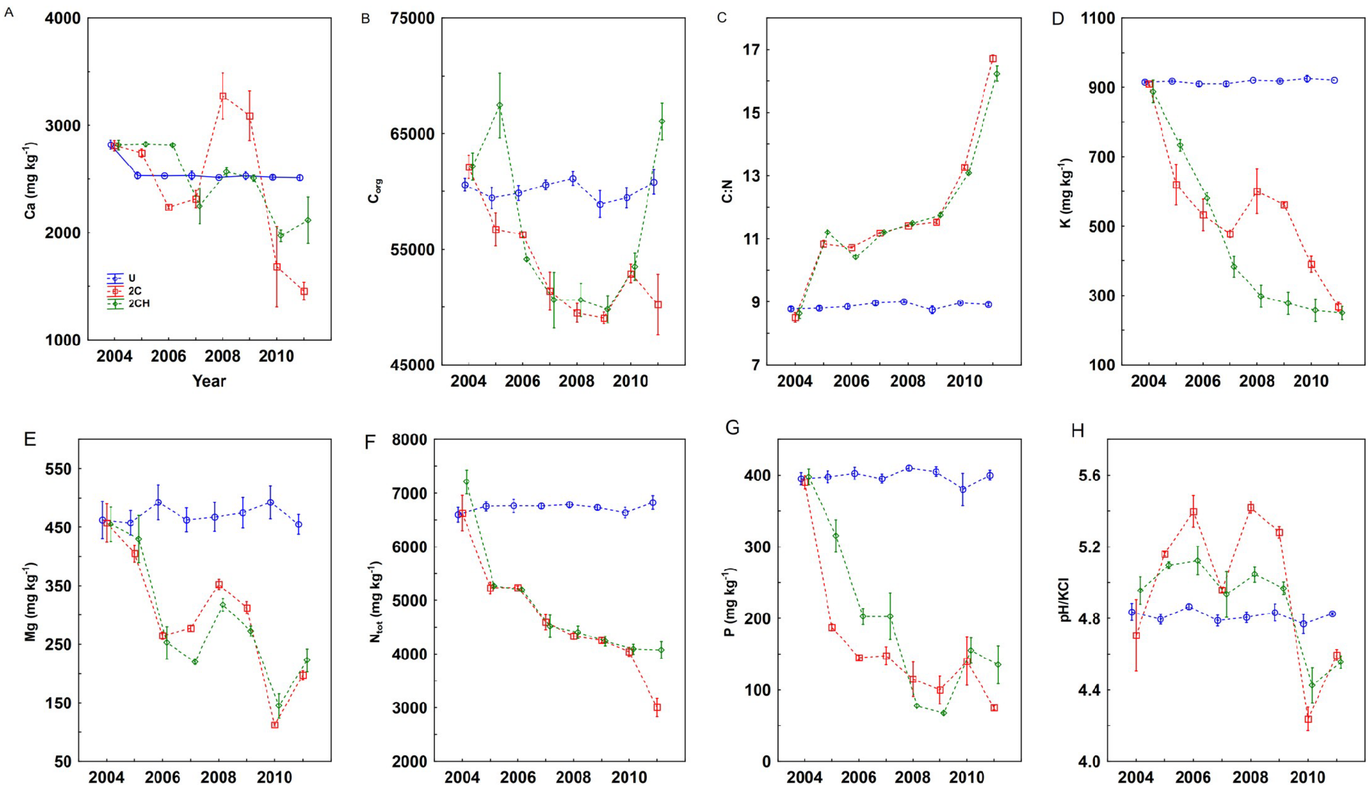

Fig 3. Concentrations of Ca (A), C_{org} (B), C: N (C), K (D), Mg (E), Total N (F), P (G) and pH/KCl (H), in the soil (0–10 cm). Error bars represent standard error of the means (SE). For treatment abbreviation (U, 2C, 2CH) see Table 1.

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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*

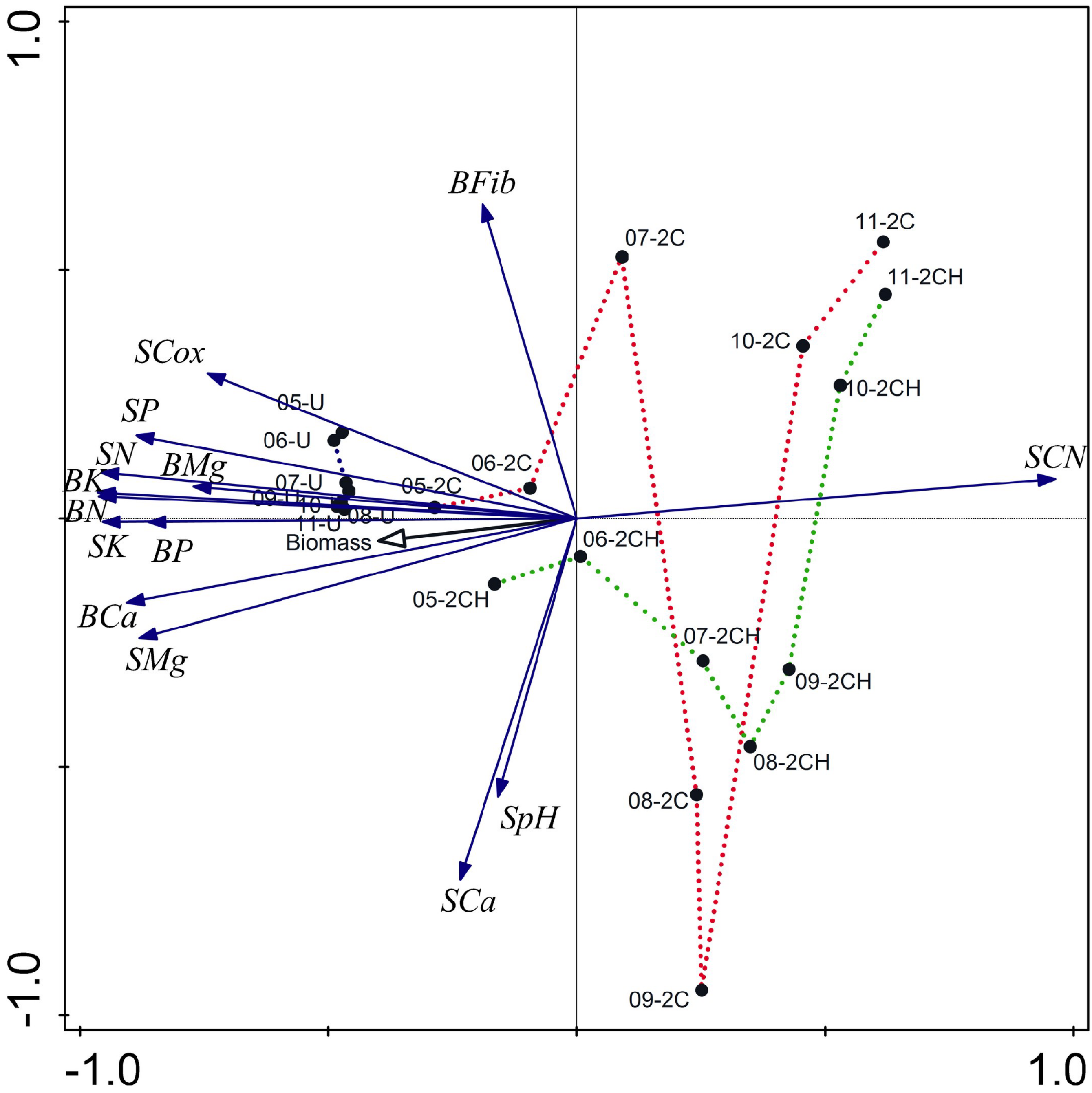

Fig 4. Principal component analysis (pPCA) of the nutrient concentrations in the herbage and in the soil indicating the influence of treatment and its development over the years from 2005 to 2011. The first and the second axis explain 64% and 10%, respectively. Labels include nutrient names and abbreviations: 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).

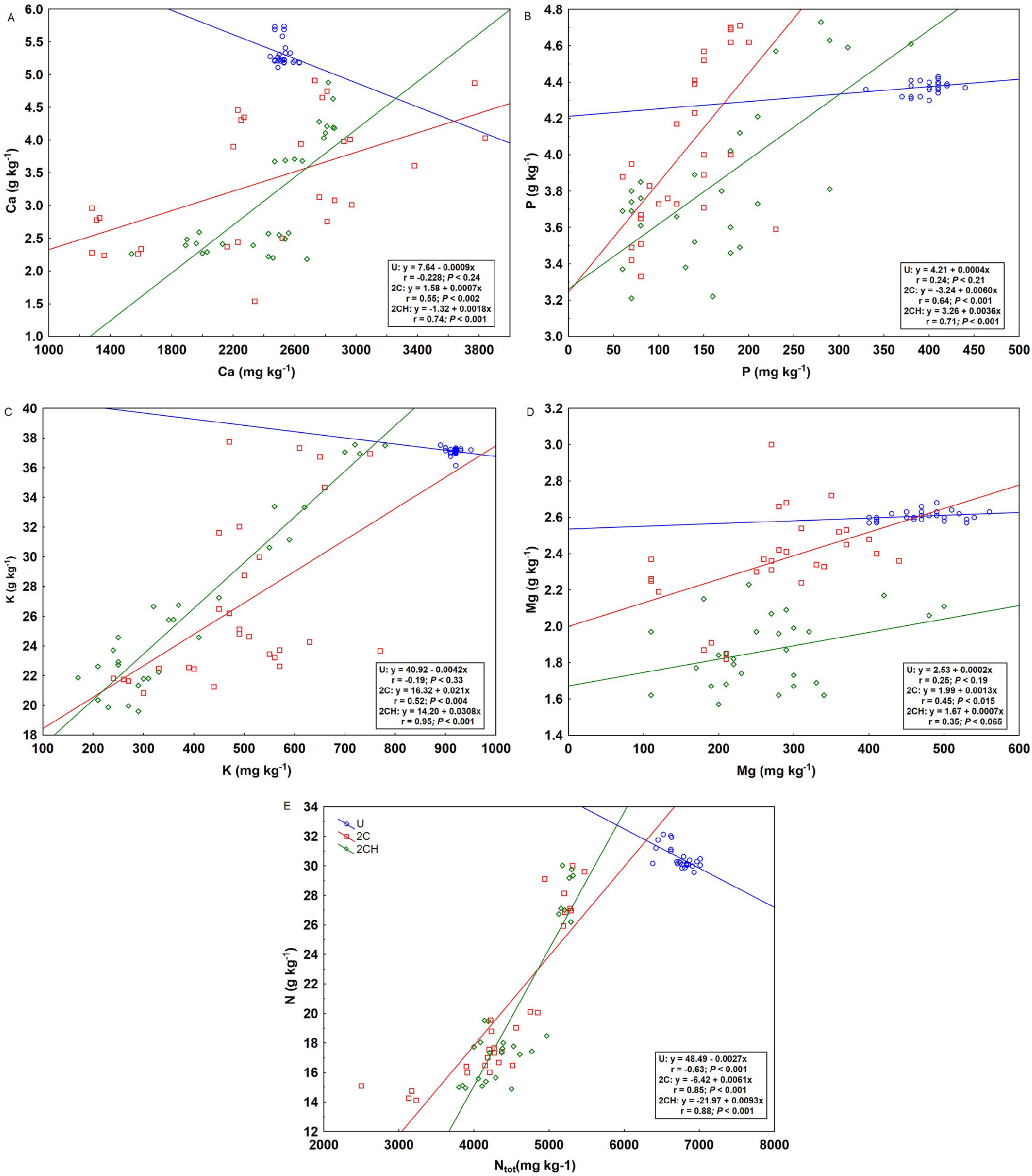

Fig 5. Relationship between concentrations of calcium (A), phosphorus (B), potassium (C), magnesium (D) and nitrogen (E) in the herbage and in the soil. For treatment abbreviation (U, 2C, 2CH) see Table 1.

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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 15^{th} 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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Formal analysis: Teowdroes Kassahun, Petr Blažek.

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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 6

The effect of 19 years of restoration managements on forage quality and herbage soil relationships within improved upland grassland

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Manuscript

The effect of 19 years of restoration managements on forage quality and herbage soil relationships within improved upland grassland

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ABSTRACT

Restoration managements based on extensification strategies are commonly used to improve biodiversity on formerly improved grasslands. This paper reports novel data relating to the long-term impacts of extensification strategies when applied to reseeded marginal grasslands common to less favoured areas.

The long-term effects of six different extensive management regimes on forage quality, soil/herbage/sward characteristics and their relationships were studied and compared with a conventional, intensively managed, control. The treatments were: sheep grazing, with and without lime application; hay cutting only, with and without lime application; and hay cutting followed by aftermath grazing, with and without lime application; with the control treatment continuing the previous site management (liming, NPK application and continuous sheep grazing).

Higher proportions of forbs were linked to higher numbers of plant species in restoration managements incorporating cutting (hay or hay/grazed defoliation) resulted in higher quality of the forage than forage from only grazed treatments. The dry matter standing biomass and concentrations of crude protein, neutral detergent fiber and K in the herbage were negatively correlated with total number plant species and cover of forbs, whereas *in vitro* organic matter digestibility and concentrations of Ca, Mg and Na were positively correlated with number of plant species and forb cover. However, the quality of the forage indicated it was suitable only for sheep or beef cattle feeding. A positive relationship between P and K concentration in the soil and in the herbage was recorded. No effect of previous liming on forage quality was found.

Overall, introducing long-term restoration managements to support biodiversity by postponing the timing of the first defoliation (hay cutting) to mid growing season (end July) increased forage quality in comparison with continual grazing in species rich upland grassland.

Keywords: Restoration, Grazing, Cutting, Nutrients, Herbage, Soil

1. Introduction

Improvement of grazed grasslands through the application of N, P and K fertilizers and of lime increases plant and livestock productivity, but by changing nutrient availability simultaneously reduces plant species richness (Bakker et al., 2002; Hejcman et al., 2010; Pavlů et al., 2011; Humbert et al., 2016). The majority of European grasslands underwent such improvement in the second half of the 20th century, leading to the predominance of productive, but species-poor, grasslands with high residual nutrient availability in the soil (Pegtel et al., 1996; Isselstein et al., 2005; Pavlů et al., 2011). Restoration management strategies effective at removing soil nutrients (Hansson and Fogelfors, 2000; Van Diggelen and Marrs, 2003) and increase plant species diversity are long-term hay-making with biomass removal or grazing without fertilization (Pavlů et al., 2021). Through such long-term extensification management practices we can affect soil chemical properties, botanical composition and forage quality (Pavlů et al., 2006; Pavlů et al., 2011). However, while there have been many studies exploring changes in vegetation structure and soil nutrient status in response to different types of extensification management, very little is known about related impacts on forage quality (Hofman and Isselstein, 2005; French, 2017). To introduce such extensive species-rich grasslands as a source of ruminants feeding could support not only sustainable farming but also simultaneously improve livestock welfare and health (French, 2017). It is hypothesised that increases in protein supply associated with a greater legume and forb proportion within the diet together with the more rapid particle breakdown of these within the rumen (Jamot and Grenet, 1991; Waghorn et al., 1989) would offset the greater maturity and related fibre concentrations of the grass fraction.

The study was conducted using the Brignant long-term plots at he Pwllpeiran Upland Research Centre (UK). In a previous study (Pavlů et al., 2021) it was found that defoliation type was the key driver influencing plant species diversity (hay cutting followed by aftermath grazing >hay cutting>grazing). Further, higher concentrations of Ca and Mg in the soil in treatments with former liming had no effect on species richness and plant species composition. In this paper we described forage quality after 19 years of continual exposure to various alternative restoration regimes.

In this paper we compare forage quality and its relationship with species richness and soil chemical properties when managed according to one of seven regimes that represent the common and best practices in less favoured areas dominated by temperate European upland grassland. We aimed to answer the following questions: (i) what are the effects of long-term restoration managements and previous liming on herbage quality characteristics? (ii) what relationships exist between minerals in the soil and in the herbage and between herbage quality and sward characteristics?

2 Materials and Methods

2.1. Experimental design

The experimental plots used (the Brignant plots) were set up in 1994 at the Pwllpeiran Upland Research Centre on permanent pasture that had been ploughed and reseeded in 1973, and which had received regular inputs of fertilizer and lime. The plots are located 310 m a.s.l. (O.S. Ref: SN752757) on free-draining typical brown podzolic soils. The area receives a mean annual rainfall of approximately 1850 mm, and has minimum and maximum air temperatures of 5.2 °C and 11.9 °C respectively. The plots are arranged in a randomized block design with three blocks and a total of seven grassland management regimes imposed. The treatments are: sheep grazing, with (GL+) and without (GL-) lime application; hay cutting only, with (HL+) and without (HL-) lime application; and hay cutting followed by aftermath sheep grazing, with (HGL+) and without (HGL-) lime application. Control (CO) plots continuing the previous site management (i.e. limed, fertilized and continually grazed by sheep) are also included within each block. These receive an annual application of 60 kg ha^{-1} N and 30 kg P ha^{-1} with K also applied as required to maintain an index of 2+ (ADAS, 1983). All the lime treatments received a single application of lime in 1998 with the intention of maintaining a soil pH of 6.0. Treatments are imposed on plots 0.08 ha (hay cut only) or 0.15 ha (grazed) in size. The schematic block design of the experiments and an aerial photo are provided in Appendix A, Figs. S1 and S2.

The plots are stocked with sheep (usually Welsh Hill Speckled Faced yearlings) with numbers adjusted to maintain a sward surface height of approximately 4 to 6 cm. Turnout occurs late April/early May, when there is sufficient biomass to sustain stock. The HL+, HL-, HGL+ and HGL- plots have a single hay harvest taken annually after the 15th of July, as and when weather conditions allow. Stock are subsequently restocked on the HGL+ and HGL- plots after a short period of sward re-growth. All stock are removed end of September/early October, depending on seasonal climatic conditions and related biomass growth.

2.2. Measurements

Sward biomass was sampled in July 2012 by cutting the herbage within a 14 cm × 100 cm quadrat to ground level using electric shears at three random sites across each plot. The fresh herbage biomass samples were weighed, oven dried (48 h at 60°C) and then re-weighed to determine dry matter (DM) standing biomass (DMSB), which was expressed on a *per* ha basis. The samples were then milled to pass through a 1 mm sieve prior to chemical analysis.

Total N was determined using a Leco FP 428 nitrogen analyser (Leco Corporation, St. Joseph, MI, USA) and then multiplied by 6.25 to obtain crude protein (CP) concentrations. Concentrations of Ca, K, Mg, Na and P were determined by inductively coupled plasma optical emission spectrometry. *In vitro* organic matter digestibility (IVOMD) was determined according to the two-stage method of Tilley and Terry (1963), adapted for the ANKOM

DAISY[II] 220 incubator system (ANKOM Technology Corporation, Fairport, NY, USA). Water soluble carbohydrate (WSC) concentrations were determined using an automated anthrone method (Thomas, 1977) for extracting sugars using a sulphuric acid reagent. Neutral detergent fibre (NDF) was calculated using the Gerhart fibrecap system (Kitcherside, Glen and Webster, 2000). Reagents were as described by Van Soest et al. (1991), with the following exceptions; sodium sulphite was omitted and Termamyl (NCBE Enzymes, Reading, UK) replaced α-amylase. Ash was defined as the remainder after ignition at 550 °C, so that all C is removed. Ash was assumed to contain all the inorganic residue of the DM. All analyses were conducted in an accredited laboratory at Aberystwyth University.

2.3. Data analysis

As explanatory variables we used: i) all study treatments: CO, GL+, GL-, HL+, HL-, HGL+, HGL-; ii) type of defoliation management: Grazed (GL+, GL-, CO), Hay (HL+, HL-), Hay and Grazed (HGL+, HGL-); iii) liming: Limed (GL+, CO, HL+, HGL+), No limed (GL-, HL-, GL-).

A linear mixed-effects model (LMM) with fixed effects of explanatory variables (treatment, defoliation management and liming) and random effect of experimental block was used to analyse the effects of explanatory variables on herbage quality characteristics. If necessary, data was log-transformed to meet LMM assumptions. In order to control for false-discovery rate (FDR) we applied Benjamini-Hochberg's correction. To identify significant differences between individual treatments a post-hoc comparison using Tukey’s HSD test was applied. Relationships between minerals in the soil and in the herbage and between herbage quality and sward characteristics were analysed by linear regression analysis. Soil chemical properties and vegetation (total number of plant species, cover of forbs and graminoids) data were as reported previously (Pavlů et al., 2021). All LMM and regression analyses were performed in Statistica 13.1 (Dell Inc., Texas, 2016).

Redundancy analysis (RDA) in the CANOCO 5 program (ter Braak and Šmilauer, 2012) was used to evaluate multivariate herbage chemical properties data. All data in RDA were logarithmically transformed [y = log (y + 1)]. For all analyses 999 permutations were performed, with blocks used as covariables to restrict permutations into blocks. To visualize the results of the RDA analysis a standard biplot ordination diagram was used.

3. Results

3.1. Herbage quality

The RDA analysis showed strict discrimination on the first ordination axis according to management regime (Fig. 1, Table 1, Analysis 1). In particular, the CP, NDF, P and K concentrations and N:P, K:P ratios in the herbage were positively correlated with grazed only treatments (GL+, GL-, CO), whereas IVOMD, WSC, Na, Mg and Ca concentrations were positively correlated with all treatments where cutting was introduced (HL+, HL–, HGL+, HGL–). The defoliation management was the second best explanatory variable, whereas liming had no effect on herbage chemical properties (Table 2, Analyses 2 – 3).

Based on LMM results, there were significant effects of treatment (Table 2) and the type of defoliation (Table 3) on the majority of nutrient characteristics of the herbage, however liming did not have any effect (Table 4).

Organic components DMSB, NDF and IVOMD were significantly influenced by treatment and by the type of defoliation. The DMSB ranged from 217.9 ± 9.6 (HL+) to 404.6 ± 70.9 g m^{-2} (GL-), NDF ranged from 58.8 ± 1.3(HGL-) to 65.2 ± 0.2 g kg^{-1} (GL-) and IVOMD ranged from 48.0 ± 0.9 (GL-) to 53.2 ± 1.1 % (HGL-) (Table 2). Both DMSB and NDF were significantly supported by grazing-only defoliation (CO, GL+, GL-), whereas IVOMD was supported by defoliation that incorporated cutting (hay and hay/grazed defoliation) (Table 3). Concentrations of CP were not influenced by treatment or type of defoliation (Tables 2, 3) and ranged from 96.9 ± 8.2 (HGL^+) to 144.0 ± 17.3 g kg^{-1} (CO). WSC was significantly influenced only by treatment, and ranged from 5.6 ± 0.3 (CO) to 9.3 ± 0.7 g kg^{-1} (HL+) (Table 2).

Minerals Ca, Mg and Na were significantly lower under grazed only defoliation than under treatments that incorporated cutting (Tables 2, 3), whereas liming had no effect on mineral concentrations (Table 4). Concentrations of Ca ranged from 2.6 ± 0.6 (CO) to 5.6 ± 0.6 g kg^{-1} (HGL-), concentrations of Mg from 1.1 ± 0.3 (CO) to 2.0 ± 0.2 g kg^{-1} (HL+), and concentrations of Na from 0.5 ± 0.1 (CO) to 3.3 ± 0.7 g kg^{-1} (HL-) (Table 2).

The concentrations of ash, P and K in the herbage were not influenced by the treatment or the type of defoliation (Tables 2, 3). Ash concentration ranged from 5.2 ± 0.4 (GL-) to 6.8 ± 0.1 g kg^{-1} (HGL-), P concentration from 1.6 ± 0.2 (HGL+) to 2.3 ± 0.3 g kg^{-1} (HL-), and K concentration from 7.3 ± 0.7 (HL-) to 11.8 ± 0.2 g kg^{-1} (GL+) (Table 2). However, significant differences in the K:P ratio were recorded between treatments (Table 2) and between types of defoliation (Table 3). The lowest K:P ratio was 3.4 ± 0.1 (HL-) and the highest ratio 5.9 ± 0.5 (GL+) (Table 2). In contrast, there was no effect of the treatment or the type of defoliation on the N:P and N:K ratios in the herbage (Tables 2, 3), which ranged from 7.8 ± 0.9 (HL-) to 13.3 ± 5.6 (CO) and from 1.6 ± 0.1(GL+) to 2.3 ± 1.2 (CO), respectively (Table 2).

3.4. Relationships between minerals in the soil and in the herbage and between herbage quality and sward characteristics

Positive relationships between P and K concentrations in the soil and in the herbage were revealed ($P = 0.003$, $r = 0.62$, $P = 0.031$, $r = 0.47$), respectively. In contrast, there were no relationships between bivalent cation (Ca and Mg) concentrations in the herbage and in the soil.

IVOMD and concentrations of WSC, Ca, Mg and Na in the herbage were significantly and positively correlated with total number of plant species, whereas DMBS, CP and NDF that were correlated negatively. No correlation with total number of plant species was found for any of the other herbage characteristics (P, K and ash) (Table 5). DMBS and concentrations of CP, NDF and K in the herbage were significantly and positively correlated with total cover of graminoids, whereas IVOMD and concentrations of Ca, Mg and Na were correlated negatively with grass cover. Conversely, relationships between herbage quality properties and total cover of forbs were opposite to those revealed for the total cover of graminoids.

4. Discussion

Compared to cattle, sheep are more selective grazers (Dumont et al., 2011), and choose the best quality components (especially forbs) during grazing (Garcia et al., 2003), regardless of grazing intensity. As species richness in temperate grasslands is usually associated with higher proportions of forbs in the sward (e.g. Hansson and Fogelfors, 2000; Heinsoo et al., 2020), species-poor pastures are formed as a result of this selectivity (Marriott et al., 2009; Pavlů et al., 2021).

The sheep grazing not only reduced the cover of forbs in our experiment (Pavlů et al., 2021) and but the extensivity of grazing led to large proportion of ungrazed matured grasses with dead biomass remaining on the pasture (unpublished observation). The standard methodology used to measure sward surface heights (Barthram, 1986) only records hits of green, vegetative growth and so does not take into account this type of material. The rejected vegetation resulted in the estimated forage quality based on DOMD and NDF being lower for vegetation within grazed only treatments compared to treatments which included cutting (hay and hay/grazed treatments), where almost all biomass was removed annually, rejuvenating the sward. The NDF and CP concentrations recorded out in our experiment were typical for UK species rich grasslands (French, 2017; Hayes et al., 2016), and due to the low IVOMD values none of forages met the requirements of high productive lactating animals; only those of sheep or beef cattle (NRC, 1985; NRC, 2000).

The highest concentrations of Ca, Mg and Na were found under treatments which comprised cutting (hay and hay/grazed defoliation) regardless of liming. It was probably because of the higher proportion of forbs in these treatments (Pavlů et al., 2021), as generally forbs contain higher mineral concentrations than grasses (Whitehead, 2000; Pirhofer-Walzl et al., 2011; Liebisch et al., 2013). Although previous liming positively affected Ca and Mg concentrations in the soil (Pavlů et al., 2021), it had no effect on these elements in the forage. It seems that forage quality was considerably affected by botanical composition which reflected applied management. In our experiment mineral concentrations in the herbage was in the range observed for species rich grasslands in UK (French, 2017) and more suitable for sheep and beef feeding (Whitehead, 2000; NRC, 1985). Some mineral imbalances in the forage can be easily solved by supplying livestock with free-choice mineral supplements (Suttle, 2010).

In our experiment the forage quality was positively affected by the higher total number of plant species, which was almost exclusively increased via the forb species. Species rich grasslands with high proportions of forbs can have higher concentrations of protein and minerals (French, 2017), and higher Ca and Mg concentrations in particular have been linked to higher digestibility (Mládková et al., 2018). Therefore, this type of grassland can have a higher forage quality than unimproved ones with dominance of grasses. While the positive relationships between P and K concentration in the soil and in the herbage recorded have also been reported in earlier studies (Schaffers et al., 1998; Pavlů et al., 2013, 2016), only a few authors have found a similar relationship in the case of P (Pavlů et al., 2016).

5. Conclusion

Restoration managements for supporting biodiversity that postponed the timing of the first defoliation (hay cutting) to mid growing season (mid July) improved forage quality in comparison to grazed only treatments. No effect of previous liming on forage quality was found. Lower forage quality in grazed only treatments was due to a combination of low IVOMD, high NDF, low divalent cations (Ca, Mg) and low Na, and was exclusively connected with the reduction of forbs cover, presumably as a result of long-term selective grazing. The forage quality in all treatments was suitable only for sheep or beef cattle feeding.

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Table 1 Results of the redundancy analyses for three different H0 analyses (A1-A3); % expl. = explained variation by axis 1 (adjusted explained variation by all ordination axes), a measure of the explanatory power of the explanatory variables; *F*-ratio = *F*-statistics for the test of a particular analysis; *P*-value = corresponding probability value obtained by the Monte Carlo permutation test. Treatment abbreviations are given in Fig. 1.

Analysis	Expl. var.	Covariables	% expl.	*F*-ratio	*P*-value
A1 Different grassland managed regimes have no effect on herbage quality characteristics	CO, HGL+, HGL-, HL+, HL-, GL+, GL-	blocks	49.3 (61.6)	2.1 (3.5)	0.001 (0.001)
A2 Different defoliation management regimes have no effect on herbage quality characteristics	Grazing, Hay, Grazing+Hay	blocks	44.8 (48.6)	6.9 (8.1)	0.001 (0.001)
A3 Liming have no effect on herbage quality characteristics	Limed+, Limed-	blocks	8.8	1.6	0.167

Table 2 Herbage quality characteristics per plot under the different treatments in 2012. Treatment abbreviations (CO, GL+, GL, HL+, HL-, HGL+, HGL-) are explained in Table 1. Numbers represent average of three replicates ± standard error of the mean (SE); *F*—ratio = *F*—statistics for the test of a particular analysis; P—value = corresponding probability value. In cases of significant differences obtained by LMM after table-wise Benjamini-Hochberg's FDR correction (highlighted in bold), the post hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments. Differences are indicated by different small letters. Herbage quality characteristics and treatment abbreviations are given in Fig. 1.

			Treatment						
Herbage quality characteristics	*F*—ratio	*P*—value	CO	GL+	GL-	HL+	HL-	HGL+	HGL-
DMSB g m^{-2}	5.62	**0.006**	304.42±31.31ab	319.72±46.32ab	404.62±70.92a	217.86±9.59b	253.10±10.08b	262.77±23.06ab	223.41±24.37b
IVOMD %	5.87	**0.005**	50.32±0.42ab	49.24±0.23ab	47.95±0.92b	53.11±0.61a	52.85±0.21a	50.99±1.74ab	53.21±1.14a
CP g kg^{-1}	2.70	0.070	143.96±17.32	118.13±4.88	111.46±4.21	108.33±8.87	106.88±3.48	96.88±8.16	100.63±5.00
NDF g kg^{-1}	10.31	**<0.001**	63.67±0.77ab	63.09±1.25abc	65.26±0.24a	59.54±0.49cd	60.52±1.05bcd	60.64±0.45bcd	58.76±1.34d
WSC g kg^{-1}	5.00	**0.009**	5.64±0.27b	8.88±1.07a	7.51±0.56ab	9.29±0.73a	8.89±0.19a	8.50±0.44a	8.36±0.55ab
Ash g kg^{-1}	2.24	0.110	6.07±0.24	6.20±0.50	5.22±0.43	5.89±0.16	5.67±0.50	6.59±0.43	6.84±0.10
P g kg^{-1}	0.84	0.562	2.20 ± 0.56	2.03 ± 0.17	1.93 ± 0.12	1.83 ± 0.18	2.27 ± 0.30	1.63 ± 0.17	1.93 ± 0.09
K g kg^{-1}	1.44	0.278	11.70 ± 3.18	11.80 ± 0.21	9.13 ± 0.58	8.33 ± 0.44	7.27 ± 0.69	8.27 ± 0.50	9.53 ± 0.85
Ca g kg^{-1}	7.72	**0.001**	2.63 ± 0.62 c	3.20 ± 0.10 bc	3.20 ± 0.10 bc	4.97 ± 0.18ab	4.07 ± 0.35abc	5.07 ± 0.47 ab	5.57 ± 0.55a
Mg g kg^{-1}	6.77	**0.003**	1.10 ± 0.31c	1.37 ± 0.09abc	1.27 ± 0.15bc	2.03 ± 0.15a	1.97 ± 0.12 ab	1.83 ± 0.15 ab	1.90 ± 0.15 ab
Na g kg^{-1}	13.34	**<0.001**	0.47 ± 0.09c	0.73 ± 0.12c	1.17 ± 0.23bc	2.50 ± 0.30ab	3.30 ± 0.69a	2.70 ± 0.35ab	2.80 ± 0.42a
N:P	0.47	0.819	13.29 ± 5.58	9.43 ± 0.94	9.34 ± 0.97	9.49 ± 0.33	7.78 ± 0.93	9.67 ± 1.14	8.36 ± 0.53
N:K	0.54	0.771	2.59 ± 1.18	1.60 ± 0.09	1.98 ± 0.19	2.07 ± 0.08	2.39 ± 0.20	1.90± 0.25	1.72 ± 0.17
K:P	4.31	**0.015**	5.26 ± 0.37ab	5.88 ± 0.45a	4.73 ± 0.19ab	4.59 ± 0.21ab	3.24 ± 0.14b	5.25 ± 0.92ab	4.99 ± 0.65ab

Table 3 Herbage quality characteristics per plot under the different type of defoliation in 2012. Numbers represent average of three replicates ± standard error of the mean (SE); *F*—ratio = *F*—statistics for the test of a particular analysis; *P*—value = corresponding probability value. In cases of significant differences obtained by LMM after table-wise Benjamini-Hochberg's FDR correction (highlighted in bold), the post hoc comparison using the Tukey's HSD test was applied to identify significant differences between the types of defoliation. Differences are indicated by different small letters. Herbage characteristics abbreviations are given in Fig. 1.

			Type of defoliation		
Herbage quality characteristics	*F*-ratio	*P*-value	Grazed	Hay	Hay and Grazed
DMBS g m^{-2}	9.88	**0.002**	342.92±30.37a	235.48±10.04b	243.09±17.39b
IVOMD %	11.56	**0.001**	49.17±0.45b	52.98±0.30a	52.10±1.06a
CP g kg^{-1}	4.24	0.033	124.51±7.28	107.60±4.27	98.75±4.36
NDF g kg^{-1}	20.70	**<0.001**	64.00±0.54a	60.03±0.56b	59.70±0.76b
WSC g kg^{-1}	3.84	0.043	7.34±0.59	9.09±0.35	8.43±0.31
Ash g kg^{-1}	4.23	0.034	5.83±0.26	5.78±0.24	6.72±0.21
P g kg^{-1}	0.99	0.392	2.06±0.18	2.05±0.18	1.78±0.11
K g kg^{-1}	3.79	0.045	10.88±1.03	7.80±0.44	8.90±0.52
Ca g kg^{-1}	20.23	**<0.001**	3.01±0.21b	4.52±0.27a	5.32±0.34a
Mg g kg^{-1}	22.30	**<0.001**	1.24±0.11b	2.00±0.09a	1.87±0.10a
Na g kg^{-1}	32.98	**<0.001**	0.79±0.13b	2.90±0.38a	2.75± 0.24a
N:P	0.57	0.578	10.69±1.78	8.64±0.58	9.01±0.63
N:K	0.89	0.430	2.06±0.37	2.23±0.12	1.81±0.14
K:P	5.50	**0.015**	5.29±0.24a	3.91±0.32b	5.12±0.51ab

Table 4 Herbage quality characteristics per plot under the limed or no limed conditions in 2012. Numbers represent average of three replicates ± standard error of the mean (SE); *F*-ratio = *F*-statistics for the test of a particular analysis; *P*-value = corresponding probability value obtained by LMM. Herbage characteristics abbreviations are given in Fig, 1.

Herbage quality characteristics	*F*-ratio	*P*-value	Limed	No limed
DMBS g m^{-2}	0.10	0.762	276.19±17.69	293.71±35.56
IVOMD %	0.16	0.698	50.91±0.59	51.34±0.95
CP g kg^{-1}	1.40	0.252	116.82±6.98	106.32±2.65
NDF g kg^{-1}	0.03	0.854	61.73±0.62	61.51±1.09
WSC g kg^{-1}	0.07	0.795	8.08±0.52	8.25±0.31
Ash g kg^{-1}	0.68	0.421	6.19±0.17	5.91±0.31
P g kg^{-1}	0.45	0.510	1.93±0.15	2.04±0.11
K g kg^{-1}	1.61	0.222	10.03±0.87	8.64±0.50
Ca g kg^{-1}	0.30	0.591	3.97±0.37	4.28±0.39
Mg g kg^{-1}	0.43	0.520	1.58±0.14	1.71±0.13
Na g kg^{-1}	2.56	0.128	1.60±0.32	2.42±0.40
N:P	1.91	0.185	10.47±1.33	8.49±0.48
N:K	0.10	0.755	2.04±0.28	2.03±0.14
K:P	4.56	0.047	5.24±0.27	4.32±0.34

Table 5 Correlation (r) between herbage quality characteristics and sward characteristics. Data of sward characteristics are from previous study (Pavlů et al. 2021). Herbage characteristics abbreviations are given in Fig. 1. Asterisks indicate significant differences (*P < 0.05; **P < 0.01; ***P < 0.001). P – value = corresponding probability value.

Sward characteristics	Herbage quality characteristics										
	DMBS	IVOMD	CP	NDF	WSC	Ash	P	K	Ca	Mg	Na
Total number of plant species	-0.52*	0.65**	-0.58**	-0.74***	0.55**	0.43	-0.09	-0.36	0.84***	0.79***	0.79***
Total cover of graminoids	0.61**	-0.74***	0.56**	0.80***	-0.47*	-0.34	0.11	0.47*	-0.86***	-0.81***	-0.83***
Total cover forbs	-0.63**	0.75***	-0.54*	-0.78***	0.49*	0.30	-0.13	-0.49*	0.81***	0.81***	0.85***

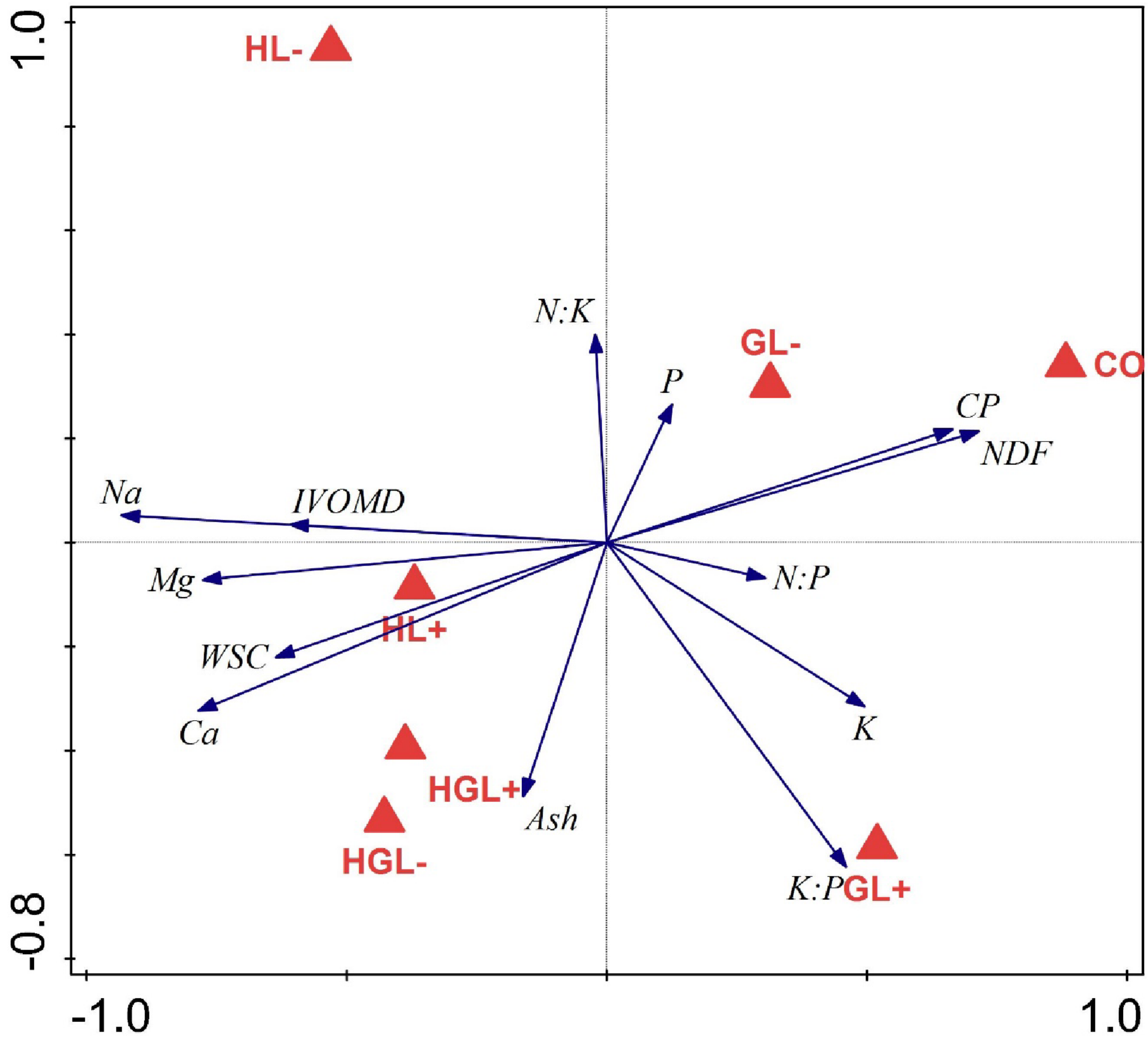

Figure 1. Ordination diagram of the results of RDA analysis showing changes in nutrient concentration in the herbage, with treatments used as predictors (see Table 1, Analysis 1 for details). Treatment abbreviations are: sheep grazing, with (GL+) and without (GL-) lime application; hay cutting only, with (HL+) and without (HL-) lime application; and hay cutting followed by aftermath sheep grazing, with (HGL+) and without (HGL-) lime application; control (CO) continuing the previous site management (limed, fertilized and continually grazed by sheep. Abbreviations: DMSB—dry matter standing biomass, IVOMD—in vitro organic matter digestibility, CP—crude protein, NDF—neutral detergent fiber, WSC— water soluble carbohydrates detergent; P, K, Ca, Mg-nutrient concentration in the herbage; N:P, N:K, K:P-ratios in the herbage.

Appendix A

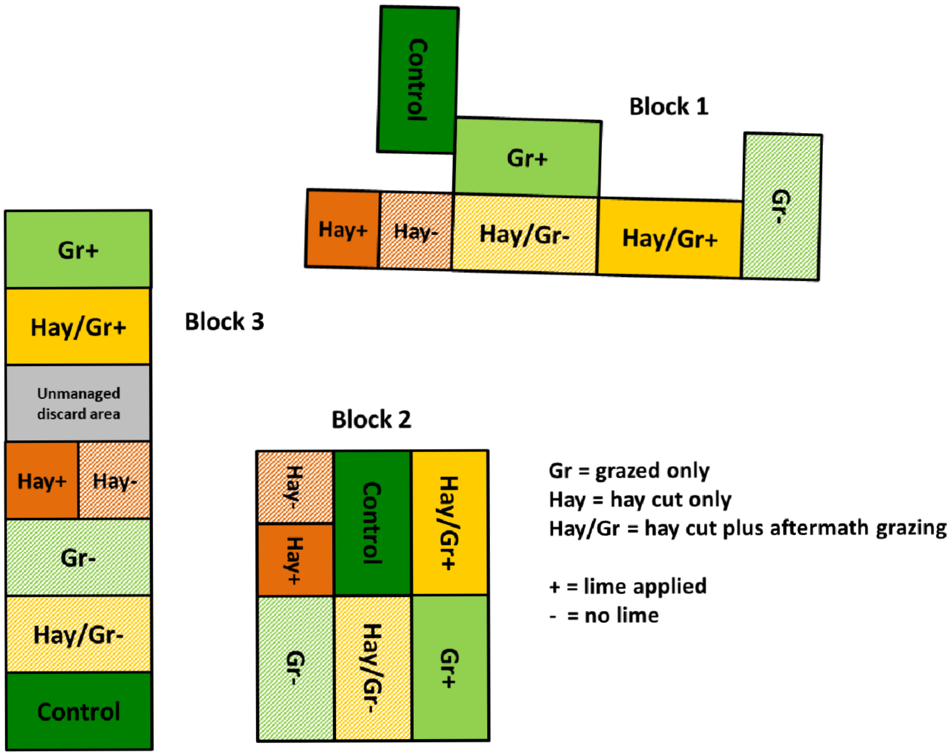

Figure S1: Schematic block design of the Brignant Long-term Plots.

Figure S2: Aerial view of the Brignant Long-term Plots (© Google 2015).

CHAPTER 7

The main results and overview discussion

7.1 The main results

7.1.1 The effects of first defoliation and previous management intensity on forage quality of a semi-natural species-rich grassland (Chapter 3)

In the **early part** of the grazing **season DMSB** had **similar development till** the beginning of **June in both treatments**. The **highest** mean value of **DMSB** in the **extensive treatment** was **recorded in July.**

During the **early part** of the grazing **season** a **sharp decline** in **IVOMD** was recorded in **both treatments**. The mean values of **IVOMD** were significantly **higher** in the **intensive treatment** than in the extensive treatment.

Concentrations of **CP** and **both fibres** (ADF, NDF) showed **opposite development trends** over the **whole period** of the grazing **season**.

In the **early part** of the grazing **season CP** concentration was significantly **higher** in the **intensive treatment** than in the extensive treatment.

The **both fibre** concentrations (ADF, NDF) were **higher** in the **extensive treatment** in comparison with the intensive treatment during the **early part** of grazing **season**.

The **sharp decrease of P** concentration in the **herbage** was **recorded from May to June** for **both treatments.**

In the **early part** of the grazing **season** the **K** concentration **reached** its **highest peak** in **May** under **both treatments**, then there was a declining trend for the rest of the grazing season in both treatments.

The concentrations of **Ca** and **Mg** in the **herbage** were significantly **higher** in the **intensive treatment** than in the extensive treatment in the **early part** of the grazing **season**.

From June onwards **Ca** concentration **in the herbage** tended to be **higher** in the **extensive treatment** than in the intensive treatment, whereas **Mg** concentration was **similar** in **both treatments** for the **remainder** of the **season**.

In the **early part** of grazing **season Na** concentration in the **herbage** was significantly **higher** in the **intensive** treatment than in the extensive treatment; this concentration of **Na** was **decreasing during the whole** of the grazing **season** in **both treatments**.

In the **early part** of the grazing **season the K/(Ca+Mg)** ratio showed a **slow decline** in **both treatments** and this ratio was significantly **higher** in the **extensive treatment**. **From June throughout** the **rest** of the grazing **season** the mean values for this **ratio** were predominantly **higher** in the **intensive treatment** than in the extensive treatment**.**

7.1.2 Effect of grazing intensity and dung on herbage and soil nutrients (Chapter 4)

The **highest** values for **SH**, **DM** content and **DMSB** were found under **tall patches** in the **extensive treatment without** and **with** presence of **dung.**

The **highest** values for **dead biomass** under **tall patches** in the **extensive treatment without** presence of **dung** and under **grazed** patches in the **extensive treatment** were revealed.

The **highest N**, **P**, **K** concentrations in the **herbage** were revealed under **tall patches** in the **intensive treatment with** presence of **dung**, whereas the **highest Ca and Mg** concentration was found in **grazed** patches in the **intensive treatment.** The **highest Mg** concentration was found **under tall patches** in the **intensive treatment with** presence **of dung.**

The **presence** of **dung** under **tall sward-height patches** in the **extensive grazing** had **no effect** either on **SH**, **DM** content and **DMSB** or on **N**, **P**, **K** concentrations in the **herbage**. The **lowest SH, DM** content**, DMSB** were under grazed patches in the **intensive grazing** which is linked with age of sward.

Type of **patch** did **not show** any significant **effect** on the concentrations of **N_{tot}, C_{org}**, and **plan**t **available** nutrients **P, K, Ca**, and **Mg** in the **soil**.

7.1.3 Restoration management of cattle resting place in mountain grassland (Chapter 5)

During the course of the **experiment**, significant **amount 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.
The concentrations of **N, P, K, Ca and Mg** in the herbage were **higher** under **unmanaged treatment** in comparison with both cut treatments.

The mean **plant available** concentrations of **P, K, Mg** in the soil and **N_{tot}, pH/KCl** were **higher** under **unmanaged treatment** compared to both cut treatments whereas **plant available** concentration of **Ca** were the **highest** under **unmanaged treatment** and **cut treatment** with **herbicide** application.

In the **both cutting 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**.

Under **unmanaged treatment**, the concentrations of **Ca, K** and **N** in the **herbage** was **negatively related** to the concentrations of **plant available Ca, K** and **N**. In contrast, the concentrations of **P** and **Mg** in the **unmanaged treatment** were **related positively**.

7.1.4 The effect of 19 years of restoration managements on forage quality and herbage soil relationships within improved upland grassland (Chapter 6)

The **DMSB** and **NDF** were significantly **supported** by **only grazed type** of defoliation, whereas **IVOMD** was **supported** by the type of **defoliation** that comprised **cutting** (hay and hay/grazed defoliation) **regardless** of **liming**.

The concentrations of **Ca, Mg** and **Na** in the **herbage** were significantly **lower** under

only grazed defoliation type than under the type of defoliation that incorporated cutting regardless of liming.

Although concentration of **WSC** was significantly **influenced** by the **treatment**, the type of defoliation did not have any effect on it. The **lowest** concentration of **WSC** was **recorded** under **control treatment**, conversely the **highest** concentration was under **hay cutting treatment with lime** application.

The concentrations of **CP, ash, P** and **K** in the **herbage** were **not influenced** by the **treatment** or the **type of defoliation**.

The **lowest K:P ratio** was under **hay cutting treatment without lime** application and the **highest** was under **sheep grazing treatment with lime** application. In contrast, there was **no effect** of the **treatment** or the **type of defoliation** on the **N:P** and **N:K** ratios in the **herbage.**

The **IVOMD** and concentrations of **WSC, Ca, Mg** and **Na** in the **herbage** were significantly **positively correlated** with number of **all vascular plant species**, in contrast to **CP, NDF** and **N** that were **correlated negatively**. On the contrary for concentrations of **P, K** and **ash no correlation** was found.

7.2 Overview discussion

Experiment abbreviations: BRE - Brignant Restoration Experiment (UK); **OFQE** - Oldřichov Forage Quality Experiment (Czechia); **OPE** - Oldřichov Patch Experiment (Czechia); **RPE** - Resting Place Experiment (Slovakia)

7.2.1 Herbage production

The highest yield of DM biomass for temperate grassland is usually in August-September, whereas the optimum concentration of minerals for cattle nutrition is in May-June (McDowell and Valle, 2000; Whitehead, 2000). Generally, DM biomass yield ranges from 2 to 4 t ha^{-1} year $^{-1}$ for Central European conditions (Smit et al., 2008), but it can have a wider range from 1 to 12 t ha $^{-1}$ year $^{-1}$. This range depends on the method of management and ecological conditions of the habitat (Frame, 1992; Kollárová et al., 2007).

The mean thirteen-year total DM biomass production was from 2.4 to 5.0 t ha^{-1} under intensive and from 2.3 to 4.7 t ha^{-1} under extensive grazing (OFQE, chapter 3). These values roughly corresponded with average yields of dry matter forage biomass in pasture and reflected typical biomass growth at the study site (Kassahun, 2016). When measuring biomass production through grazing season, usually a higher DM biomass yield was found under continuous intensive grazing, than extensive one, with two peaks in spring and summer (Pavlů et al., 2006b). However, when we measured DMSB (biomass growth without any disturbation in the vegetation season - cutting or grazing) we got the different result. In the OFQE (chapter 3) the highest mean value of DMSB was found under previous extensive grazing in September (5.9 t ha^{-1}) and under previous intensive grazing in October (5.3 t ha^{-1}). These differences were affected by the different phenology under various grazing pressure. Although biomass production was affected by the presence of faeces on pasture, it was not confirmed for biomass production of tall sward-height patches under extensive grazing in September (OPE, chapter 4).

In a very nutrient rich conditions in former cattle resting place (RPE, chapter 5) DM biomass production ranged from 6 to 7.4 t ha^{-1} per year. The variation in the dry matter biomass production observed during the early period of this experiment could be attributed to climatic conditions and altitude such as temperature and precipitation distribution during the vegetation season, as well as the species composition, and management applied (Mpokos et al., 2014; Smith et al., 2008). Such variability in biomass production is expected and similar results have been reported in other long-term studies in Central Europe (Hrevušová et al., 2009; Smits et al., 2008; Pavlů et al., 2006b). Also, other authors (Hejcman et al., 2010; Klimeš and Klimešová, 2002) declare the influence of the type of management to the height of vegetation and biomass yield. However, one of major outcomes from the RPE study was that biomass production did not change either in response to the cutting or the combined herbicide-reseeding-cutting management. It stabilized throughout the experiment period after the first year under both these cutting treatments.

Liming is used as an important tool in the restoration of species-rich grassland habitats (De Graaf et al., 1998), which can also increase availability of nutrients and consequently biomass production. Nevertheless, we did not find any effect of liming on DM biomass production in the restoration experiment (BRE, chapter 6) and although soil pH was higher in the treatments with liming than without it (Pavlů et al., 2021), it did not have any effect on biomass production.

7.2.2 Minerals

The concentrations of minerals in the herbage are affected by the plant available nutrient concentration (Schaffers, 2002), further also by phenophases, representation of individual agro-botanical groups in the grassland (Mládek et al., 2011), shading intensity, soil moisture and acidity and other ecological factors during the vegetation season (Schaffers, 2002). Herbage at the early part of growing season has very high concentration of minerals (Duru and Ducrocq 1996; Pavlů and Velich 1998). In the OFQE (chapter 3), dealing with the forage maturation during the grazing season, a significant decline of P, K and Na concentrations was found in the herbage during the grazing season. It is consequence of the 'dilution effect', it means, that during the maturation the herbage biomass increases, whereas mineral contents decrease (Duru and and Ducrocq, 1997; Mládek et al., 2011). Similarly, Míka (1997) stated that dry matter biomass increases more than total amount of minerals in the herbage.

Nevertheless, the highest N, P and K concentrations in the herbage were not found in younger forage under intensive grazed patches, but in tall patches with dung under intensive grazing (OPE, chapter 4). The main factor, which increased P and K concentrations in the herbage of these tall patches, was presence of dung (Scheile et al., 2018).

There were also found the high concentrations of Ca and K in the herbage under all treatments in the RPE (chapter 5), especially during the early periods of the experiment, as a result of long-term defecation of the animals.

On the other hand, the highest concentrations of Mg, Ca and Na (BRE, chapter 6) were recorded in the treatments where cutting, that supported the cover of forbs, was included (only hay or hay-grazed defoliation) regardless of liming (Pavlů et al., 2021).

Higher concentrations of Mg and Ca (OFQE, chapter 3) under previous intensive grazing treatment in the beginning of the growing season were probably caused by higher proportion of legumes in the sward (Novák, 2008; Whitehead, 2000) and also by higher proportion of *Taraxacum* species, which are known for their high concentration of Ca (Ata et al., 2011; Grzegorczyk et al., 2013; Harrington et al., 2006; Suttle, 2010; Wilman and Derrick, 1994; Whitehead, 2000). In the early part of grazing season (OFQE) the concentration of Ca was sufficiently high in both intensive and extensive treatments to meet nutritional requirements for dairy cows (4–6.0 g kg^{-1}; Whitehead, 2000). However, concentration of Mg in the herbage fulfilled the requirements for dairy cows (at least 2.0 g kg^{-1}) in the early part of the grazing season only in the intensive treatment.

In the later period of grazing season (OFQE, chapter 3) under the previous intensive treatment there were decline in concentrations of Ca and Mg in the forage. It was due to the reduction of proportions of legumes and *Taraxacum* species with increased growth of grasses (*Agrostis capillaris*, *Festuca rubra* agg., *Poa trivialis*), which generally have lower mineral concentrations than forbs (Královec, 2001; Liebisch et al., 2013; Pirhofer-Walzl, 2011; Whitehead, 2000). On the contrary, under the previous extensive treatment the proportion of several tall forbs (*Aegopodium podagraria, Galium mollugo agg., Hypericum maculatum*) in the sward increased at the expense of the grasses (unpublished result), so higher concentration of Ca in the herbage in the previous extensive than intensive treatment in the later period of grazing season could be caused by seasonal development of different plant species composition, as was also described by Mládek et al. (2011). Thus, in this period under the previous intensive grazing treatment, Ca concentration met requirements only for low productive milking cows (threshold 3.0 g kg^{-1}) and beef cattle (threshold 2.9 g kg^{-1}), whereas Mg concentration was mostly suitable only for beef cattle (1.6 g kg^{-1}) in both treatments (Whitehead, 2000).

The physiological requirements of K for animals are significantly less than it is usually present in the herbage, therefore animal intake of K is higher, than their optimal need (Suttle, 2010; Whitehead, 2000). Thus, K was the only element in the herbage that exceeded the recommended range for cattle nutrition (5–9 g kg^{-1}; Whitehead, 2000) during the whole grazing season in both treatments (OFQE, chapter 3). The concentration of K in the biomass was particularly high in the spring, but during the vegetation season this concentration gradually decreased. Similar decline was also described by Pelletier et al. (2006). The highest concentrations of K occur in forbs, then in grasses and the lowest in legumes (Královec, 2001).

The requirements of Na for dairy cows (2.0 g kg^{-1}) as well as beef cattle (1.0 g kg^{-1}) usually exceed Na concentration present in the herbage (Whitehead, 2000). Also, in the OFQE (chapter 3) concentration of Na in the forage did not meet the requirements of either dairy cows or beef cattle in both treatments during the whole grazing season too. Similarly, according to results of Pavlů et al. (2006a) concentration of K in the forage based on grasses tends to be higher than animal requirements, whereas concentrations of Mg and Na (alternatively Ca) tend to be in deficit.

The concentration of P in the herbage (OFQE, chapter 3) met the requirements of productive animals (2.4–4.0 g kg^{-1}; Whitehead, 2000) in both the extensive and intensive treatments only until early part of June. In the RPE (chapter 5) a very high concentration of P in the herbage was found in all treatments. Although the decline in the concentration of P in the herbage was observed from the beginning to the end of experiment under both cutting treatments, P concentration in the herbage was still high and thus biomass growth was not limited by P (Hejcman et al., 2012). This high concentration of P in the early part of the RPE could be caused by high representation of weedy *U. dioica* and *R. obtusifolius*, for which high concentrations of N and P are typical (Taylor, 2009; Thompson et al., 1997). Nevertheless, high herbage concentration of P in this study site was predominantly based on the previous management (cattle resting place) which considerably enriched the soil with nutrients.

There were generally lower concentrations of P, K and K/Ca+Mg ratio but higher concentrations of Na in the herbage in the OFQE (chapter 3) in comparison with another experiment in Jizerské hory Mountains based on rotation and continuous grazing regime (Pavlů and Velich, 1998). These differences were caused by lower concentration of plant-available nutrients in the OFQE caused by the different management.

Recommended K/(Ca+Mg) ratio should not exceed the value 2.20 (Novák, 2008), but according to various authors this ratio can be up to 2,50–3 (Grunes et al., 1970; Pavlů and Velich, 1998; Voisin, 1963). This exceeding ratio can cause grazing tetany (Novák, 2008). In the OFQE (chapter 3), the grass tetany ratio K/(Ca+Mg) in meq. of 2.5 (Grunes et al., 1970; Voisin, 1963) has never exceeded, due to high Ca and Mg concentrations in the herbage. In general, mineral imbalances is possible to solve by supplying with free-choice mineral supplements to livestock (McDowell and Valle, 2000; Suttle, 2010).

7.2.3 Organic components

Young herbage in vegetative state has higher digestibility values and higher concentrations of CP than the mature forage (Koidou et al., 2019; Rychnovská, 1985; Whitehead, 2000). A gradual decrease of digestibility is associated with an increasing accumulation of structural carbohydrates and lignification during the maturity (Frame, 1992; Rymer, 2000). The optimal values of forage digestibility are higher than 67% for dairy cows (Frame, 1992), at least 60% for beef cattle (NRC, 2000) and around 50% as maintenance value (ARC, 1980). The digestibility of forage is affected not only by the intensity of grazing during the recording period, as some previous studies show (Bruinenberg et al., 2002; Motazedian and Sharrow, 1990; Pelve et al., 2012; Stejskalová et al., 2013; Tallowin and Jefferson, 1999), but it can be also affected by previous grazing intensity.

The optimum level of IVOMD in forage in the OFQE (chapter 3), which meets the requirements for dairy cows was until early part of June in the previously intensive grazing treatment but only during the first two weeks in May in the previously extensive grazing treatment. In these both treatments (OFQE) the optimum values of IVOMD for feeding of beef cattle were suitable during the whole early part of the grazing season, because beef cattle do not have high digestibility requirements as dairy cows (NRC, 2000). The sufficient maintenance values of digestibility for feeding cattle were until July under the both treatments. Similar development of digestibility is typical for upland European grasslands (e.g. Andueza et al., 2014; Koidou et al., 2019).

Values of IVOMD and CP concentrations showed similar patterns in the OFQE (chapter 3) over the course of the grazing season. In both treatments a decline was recorded from the early part to the end of the grazing season. The highest concentrations of CP were reached in the early part of May, after this period there was a sharp decline until mid of June, when values were about 100 g kg^{-1} regardless of treatment, this level was still optimal for the requirements for beef cattle (80 g kg^{-1}) (Thumm et al., 2009). After mid of June CP concentrations remained unchanged until the end of the growing season. The higher proportion of legumes or *Taraxacum* species in the sward could cause the higher CP concentration in the herbage in the early part of the grazing season under the previous intensive grazing treatment. These plant species usually have higher CP concentrations than grasses (e.g. Biel et al., 2017; Elgersma and Søegaard, 2016; Jancovic and Holubek, 1999). The optimum concentrations of CP that can meet the requirements of dairy cows (>160 g kg^{-1}) (Thumm et al., 2009) were only in the first two weeks of May in both treatments.

The high concentration of CP in the herbage in the RPE (chapter 5) was also found during the early period in all treatments, after this period there was found a decline in both cutting treatments. It seems that regular biomass removal can reduce N in the soil, which is usually linked with CP concentration in the herbage.

The highest CP concentration in the herbage in the OPE (chapter 4) was not surprisingly found in intensive grazed patches, where biomass is not matured, but under tall patches with dung in intensive grazed plots, which increased concentration of CP in the herbage biomass.

Level of NDF and CP concentrations found out in the BRE (chapter 6) was typical for UK species rich grasslands (French, 2017; Hayes et al. 2016) and because of low IVOMD in all treatments the forage was not suitable for high productive lactating animals, but only for sheep or beef cattle (NRC, 1985; NRC, 2000).

The forage quality in terms of NDF concentration was not suitable for dairy cows in both treatments of the OFQE (chapter 3), as their recommended values are about 300–400 g kg^{-1} (Kudrna et al., 1998; NRC, 2001), higher concentrations are usable only for beef cattle (NRC, 2000). The high concentrations of ADF in forage were in both treatments in the OFQE, except for the first week in May under the previous intensive grazing treatment. These values were not acceptable for dairy nutrition, their recommended values are about 190–240 g kg^{-1} (Kudrna et al., 1998; NRC, 2001). After early part of June, the both ADF and NDF concentrations in the herbage increased and were suitable only for beef cattle (NRC, 2000).

The herbage biomass in the OFQE (chapter 3) harvested after July was of very low quality and was not suitable as the only food source for cattle nutrition, but this biomass can be used for combustion (Boob et al., 2019).

7.2.2 Soil chemical properties

Cutting management with biomass removal usually results in decreasing of plant available P and K in the soil as well as consequently in the herbage (Hejcman et al., 2010; Pavlů et al., 2011). The reduction of K concentration from the soil by cutting and removing herbage is generally quick (Pavlů et al., 2013). The RPE (chapter 5) showed a decline in N and the major plant available nutrients (P, K and Mg) in the soil over the duration of the study under both cutting treatments with biomass removal. The decline in all these nutrients was similar in both soil layer (0-10 and 10-20 cm). However, the reductions of plant available concentrations of Ca and Mg under both cutting treatments were relatively small. It can be explained by the limited duration of the experiment, because significant nutrient removal requires a long-term period (Hansson and Fogelfors, 2000; Hejcman et al., 2010; Perring et al., 2009; Schnitzler and Muller, 1998). Conversely nutrients in the soil remained relatively stable under the unmanaged treatment throughout the experiment period, which was caused by the absence of management.

Dung is a potential source of available nutrients for plants and has a significant effect on the chemical status of the soil (Aarons et al., 2004). Typical rates of deposition of elements in dung pats are equivalent to 150–170 kg K, 125–400 kg P and 650–850 kg N per ha per year (Pearson and Ison, 1997), a recovery of nitrogen by urine in to the soil is about 60–70% (Ball and Keeney, 1983). In the OPE (chapter 4) the type of patch did not show any significant effect on the plant available nutrients (P, K, Ca, Mg) and concentrations of N_{tot}, $C_{org,}$ in the soil. The regression analysis showed no relationship between the concentrations of nutrients in the herbage and in the soil. According to Dickinson and Craig (1990) and Scheile et al. (2018) nutrient losses from dung are not necessary connected with increases in nutrients in the soil, as these nutrients might have been used immediately by plants under the dung. Nevertheless, other studies (Aarons et al., 2009; MacDiarmid and Watkin, 1972; Yoshitake et al., 2014) have noted direct positive effect of dung-derived nutrients on the nutrient concentrations in the soil.

Although Pavlů et al. (2021) found a positive effect of previous liming on Ca and Mg concentration in the soil in the BRE (chapter 6) no effect of previous liming on mineral concentration in the forage was found in this study. So it seems that forage quality was predominantly affected by botanical composition which reflected applied management. In the BRE the positive relationship between the concentrations of P and K in the herbage and plant available concentrations of P and K in the soil was revealed. Similarly, several studies (Schaffers et al., 1998; Pavlů et al., 2013, 2016) has reported this positive linear relationship for K, but only a few authors (Pavlů et al., 2016) have found a similar relationship in the case of P.

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CHAPTER 8

Summary of results

Future recommendation

8.1 Summary of results

The previous extensive management had a carry-over effect which significantly reduced the organic parameters of quality (IVOMD, ADF, NDF, CP) and Ca, Mg and Na concentrations in herbage of *Agrostis capillaris-Festuca rubra* dominated grassland until mid-June. Herbage mineral concentrations declined over the course of the sward maturation. 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. The diversification of DMSB occurred after early part of June and it tended to be higher under previously extensively managed treatment. 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 to after mid-June, but also when extensive management is required.

The intensity of grazing management can influence utilization of nutrients released from dung. In tall sward-height patches in extensive grazing, the sward was almost ungrazed and thus did not need nutrients to regrow. In addition the presence of dung in extensive grazing did not have any influence on herbage properties and nutrients were leached without utilization. On the contrary intensive grazing supported frequency of grazing of tall patches and some nutrients from dung were utilized for regeneration of pasture sward. As the presence of dung did not have any influence on the soil nutrient concentrations in any type of patches we suppose that the non-utilized nutrients were leached and thus soil nutrient enrichment was very low.

The introduction of cutting management as well as a combination of cutting with herbicide application and reseeding had effect on herbage production and nutrient concentration in the herbage as well as in the soil. Finally, considering the result from this experiment and other similar studies, we can see treatment with herbicide application combined with cutting did not demonstrate significant difference in removing nutrients from the soil/herbage compared to the nature friendly only cut treatment. 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.

Due to higher proportion of forbs the forage from restoration managements including cutting defoliation had higher quality than only grazed treatments. There was not found any effect of previous liming on forage quality. The positive relationship between P, K concentrations in the soil and in the herbage was revealed and their high concentration in the soil was connected with lower species richness. Concentrations of Ca, Mg and Na in the herbage were positively correlated with plant species richness, whereas concentration of N had the opposite effect. The restoration managements with the postponing the timing of the first cutting defoliation (non-selective) to mid growing season practiced in an upland grassland may not deteriorate forage quality and can be used as a forage for not highly productive herbivores.

8.2 Future recommendation

As there is only little information about forage quality of semi-natural grasslands further research should be focused on the qualitative and quantitative herbage parameters during the vegetation season in relation to the different stage of phenophase and plant species composition. Research should be focused not only on whole grassland community but also on the particular dominant plant species. For these studies, it would be appropriate to evaluate these parameters in relation to the soil properties, because examining of herbage biomass properties without knowledge of the soil parameters is not meaningful. In general, future research of this topic should be more focused on the relationship between nutrients in the soil and the herbage.

The grazing experiments should study the influence of animal's excrements (urine and dung), because they have a significant effect on the nutrient cycle in the herbage and the soil. Their effect on the grassland has not been much examined yet, although their influence is crucial on the nutrient concentration in the herbage and the soil, botanical composition and behaviour of grazing animals (avoiding these places). This research should be also associated with the decomposition time of dung from which nutrients are released on the pasture. Understanding of the nutrient cycle is very important for ecosystem functions, but also for botanical composition, as well as for animal nutrition in the grassland.

CHAPTER 9

Curriculum vitae

List of publications

9.1 Curriculum vitae

Personal information

Name: Klára Pavlů

Date of birth: 15.8. 1990

Nationality: Czech

Gender: Female

Address: Oldřichov v Hájích, 122, 463 31

Contact: kpavlu@fzp.czu.cz; (+420) 721 258 761

Work experience

2015 – Present: Researcher at the Department of Ecology, Czech University of Life Sciences (CULS), Prague; branch in Liberec – joint workplace of CULS and Crop Research Institute "Ecology and management of grasslands"

Content of the work: work on projects, biomass sampling, data processing, articles writing, active participation in conferences, assistance with leading excursions for students

2015 – 2019: Researcher at the Department of Nutrition and Feeding of Farm animals, Institute of Animal Science, Prague

Content of the work: working in laboratory, data processing, articles writing, active participation in conferences

Education

2015 – Present: Czech University of Life Sciences, Prague

Faculty: Faculty of Environmental Sciences

Field: Ecology, PhD.

2013 – 2015: Czech University of Life Sciences, Prague

Faculty: Faculty of Agrobiology, Food and Natural Resources

Field: Masters of Animal Nutrition and Dietology, Ing.

2010 – 2013: Czech University of Life Sciences, Prague

Faculty: Faculty of Agrobiology, Food and Natural Resources

Field: Bachelor of Animal-assisted Therapies and Activities, Bc.

Personal skills

Mother tongue(s): Czech

Foreign language(s): English: B2

Job-related skills

Basic knowledge of Microsoft Office (Word, Excel, PowerPoint), Statistica

Projects

EU, Interreg SN/CZ r.č. 100264999 Sustainable grassland management for biodiversity support, 2017-2019, research worker

EU, Interreg CZ/PL r.č. CZ.114.120/0.0/0.0/16_026/0001092 Grassland biomass as a renewable source of energy Biodiversity-Biomass-Biogas, 2017-2021, research worker

Internship

15/04/2019 – 15/09/2019: Georg-August-Universität Göttingen, Institute of Grassland Science (supervisor- Prof. Dr. Johannes Isselstein)

Work on two experiments:

- Determination of plant functional traits in relation to the grazing intensity
- Measuring root production under varying grazing intensities in different sward patches

9.2 List of publications

Scientific articles

i) Scientific articles with IF

Pavlů K., Kassahun T., Pavlů V., 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. *PlosOne*, 16(3): e0248804.

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Gaisler J., Pavlů L., Nwaogu Ch., **Pavlů K.**, Hejcman M., Pavlů V. 2019. Long- term effects of mulching, traditional cutting and no management on plant species composition of improved upland grassland in the Czech Republic. *Grass Forage Science*, 74: 463-475.

Pavlů K., Kassahun T., Pavlů L., Pavlů V., Fraser M. D. The effect of 19 years of restoration managements on forage quality and herbage soil relationships within improved upland grassland. Manuscript

ii) Scientific articles without IF

Titěra J., Haase H., Kassahun T., Nwaogu Ch., **Pavlů K.**, Kändler M., Pavlů L., Gaisler J., Paška F., Heidenreich H., Liepelt G., Jonášová I., Pavlů V. 2018. Divergrass - A cross border project to promote sustainable management of grasslands. *ACC Journal*, 24: 61-78.

Conferences

Pavlů K., Kassahun T., Nwaogu Ch., Pavlů V., Pavlů L. Gaisler J., Homolka P. 2018. The effect of previous contrasting grazing intensity on the content of nutrients in pasture forage. *Grassland Science in Europe.* 23:313-315. International Scientific Conference EGF (European Grassland Federation), Cork, Ireland, 17–21 June, 2018.

Kassahun T., **Pavlů K.**, Pavlů V., Pavlů L., Gaisler J. 2018. Biomass production and forage quality under intensive and extensive grazing. *Grassland Science in Europe.* 23:262-264. International scientific conference EGF (European Grassland Federation), Cork, Ireland, 17–21 June, 2018.

Pavlů K., Kassahun T., Nwaogu Ch., Pavlů V., Pavlů L., Gaisler J., Homolka P. 2017.The nutrient concentration in the soil and above-ground biomass of patches under intensive and extensive grazing intensities of *Agrostis capillaris* grassland. In: Harabiš F., Solský M. (eds). Kostelec inspire 2017. ISBN 978-80-213-2790-0, Kostelec inspire 2017. Conference of Czech University of Life Sciences in Prague, Faculty of Environmental Sciences, Kostelec, 23–24 November, 2017.

Pavlů K., Kassahun T., Nwaogu Ch., Pavlů V., Pavlů L., Gaisler J., Homolka P. 2017. Soil and herbage nutrients under pasture patches. In: Mertens J., Tropek R. (eds.). 6. Conference of Czech Society for Ecology. ECOLOGY 2017. 25–27 September, 2017.

Pavlů K., Homolka P., Hejcman M., Pavlů V. 2016. The effect of previous contrasting grazing intensity on the content of nutrients in pasture forage. *Grassland Science in Europe.* 21: 266-268. International Scientific Conference EGF (European Grassland Federation), Trondheim, Norway, 4– 8 September, 2016.

Pavlů K. 2016. The mineral content of herbaceous biomass influenced by previous varying intensity grazing. In: Šimůnková K. a M. Solský (eds.), Biodiversity 2016, Book of Abstracts, Czech University of Life Sciences in Prague, pp. 16. ISBN 978-80-213-2625-5, Biodiversity 2016. Conference of Czech University of Life Sciences in Prague, Faculty of Environmental Sciences, Chloumek, 23– 24 January, 2016.