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Long-term effects of different grassland management types on plant species composition in protected landscape areas

DISSERTATION

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AUTHOR’S STATEMENT/DECLARATION

I declare that this Ph.D. thesis was written in accordance with all the requirements necessary for the gaining of the Ph.D. degree in the Department of Ecology, Faculty of Environmental Sciences at the Czech University of Life Sciences Prague. I declare that I was writing this thesis independently with the help of my supervisor, consultant and coauthors of my scientific papers. I declare that all the relevant literature sources used in the thesis are properly cited and any plagiarism in all thesis had not occurred.

Ing. Jan Titěra

Prague, Czech Republic, 2023

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General introduction

Preface

This dissertation is focused on the detection of long-term effects of different management types, fertilization, expansive species such as *Typha latifolia* and weed species such as *Rumex obtusifolius* on plant species composition and species diversity in protected landscape areas and in the Sudetes Mountains in Poland and Czechia. All the research was carried out on the long-term grasslands experiments with different amounts of nutrients in the soil and under different humid and temperature conditions and in the Sudetes Mountains on a landscape scale. Diverse plant species composition under different long-term experiments and at various landscape types represents natural variability between the different types of grasslands. The effects which are generally understood as destroying the natural communities and have a negative impact on plant species composition and species diversity seems to be not as harmful as they were considered before the beginning of experiments and landscape studies. On the other hand, some hidden effects can appear in the long-term and in some areas with unique soil and climatic conditions on a landscape scale and they can largely affect plant communities. Therefore, long-term experiments are necessary for the collection of large data sets, which represent the development of plant communities throughout many years and examine various effects of different management types as well as landscape studies including many types of grasslands in variable conditions. Individual plant species, plant traits, functional groups, number of species, species diversity, plant species composition, the dynamic of plant communities, productivity, sward height, soil nutrients, and many other response variables are possible to investigate in the long-term grassland experiments and on a landscape scale. Changes in plant communities over the time and the development of grasslands in long-term as well as landscape studies including many types of grasslands can provide answers to many research questions. Parts of my dissertation are results from four long-term grassland experiments located in Austria, Germany, Slovakia, and Czechia and one landscape study in the Sudetes Mountains (Central Europe). These experiments and various landscape types include different grassland communities, developed throughout specific management history. All the results here are used to expand knowledge about the positive and negative effects in grassland communities, which are part of the cultural landscape.

Grasslands and their management

The majority of temperate grasslands are related to human activities and farming practices. These practices formed their nutrient status, plant species composition, and species richness. The first of them had been created during the Neolithic Age (Ellenberg, 1996). Semi-natural and improved (intensive) grasslands were created by burning off the original forests or by changes of natural grasslands, predetermined by environmental conditions and wild herbivores. The development of human society and the changes in the political situation largely affected the dynamics of the grassland areas in Central Europe throughout history (Hejcman et al., 2013). The oldest management type on these grasslands was grazing. Grasslands were part of the first semi-intensive agriculture. Different types of animals were used for grassland management, especially domesticated herbivores for the production of milk, meat, and wool (Prins, 1998). Maintenance of non-natural grasslands depends on regular disturbances. Extensive grassland management (mowing, grazing) needs to be carried out at least once every few years to reduce shrubs, woody species, and tree seedlings overgrowing the original vegetation. If it is not the case, typical grassland flora will decline and long-term abandonment will result in a closed forest (Hansson & Fogelfors, 2000).

Large areas of the grassland were affected by excessive fertilization, liming, reseeding of productive grass/clover mixtures and by drainage systems after the intensification in the second half of 20th century in the Czech Republic. These changes led to a decrease in biodiversity in the majority of grasslands. Grasslands with high nature value have been preserved only at a low percentage from the total grassland areas. The grasslands on the steep slopes and difficult reachable places such as Mountains and uplands areas in central Europe were abandoned, because of low demand for hay (Mašková et al., 2009). Conservation of the remaining species-rich grasslands is the primary goal for nature protection. This could be secured by the utilization of traditional management types such as extensive grazing, scythe mowing, or ash fertilization (Isselstein et al., 2005). Nowadays, financial sources from the agro-environmental climate measures and financial sources for setting up of management plans for farmers could serve as instruments for sustainable grassland management. The aim of these measures is to find a balance between the economical possibilities of the farmers, human food security, and environmental sustainability. Part of the agro-environmental climate schemes are measures such as organic farming, integrated production, reduced inputs of fertilizers and/or pesticides, crop rotation, increasing areas of habitats for wildlife, introducing buffer strips and optimal livestock grazing pressure to provide desirable plant species composition. These measures prevent the risk of soil erosion and preserve the original genetic resources of the native plant species populations. Overall these measures help to decrease pressures of population growth and associated intensification in agriculture and the grasslands management to nature (SFEP, 2017).

Semi-natural grasslands represent important sources of biodiversity (Pfestorf et al., 2013). They are threatened by many changes in the management in central Europe, such as intensive fertilization, afforestation, or abandonment (Kahmen & Poschlod, 2008). One of the appropriate management types for biodiversity conservation could be extensive grazing, which limits shrubs encroachment (Kesting et al., 2009). Regular mowing could be another suitable management type (Lennartsson et al., 2012). It must be taken into account, that different management types affect differently not only plant species composition, but also available nutrients in the soil and soil microbial communities (Šimek et al., 2001).

Long-term grassland experiments in Europe

Long-term grasslands experiments in Europe can answer numerous research questions. They are source of plant species with different adaptation strategies and unique genetics developed under different management conditions (fertilization, cutting). They also provide a diversity of functional traits in relation to prediction, variability, and influence of ecosystem services of permanent grasslands. They can also elucidate the relationship between soil nutrient status and plant species in equilibrium. Plant species traits as well as their changes in time can be also observed. Long-term grassland experiments can also show effects of climate changes on plant species composition, plant traits, soil edaphon and productivity through long-time period. Simulation models can be used to study differences between biocoenoses developed under different long-term management in the same meadow. Most of them are in balance with biotic and abiotic factors and could be compared with the biocoenoses from other long-term grassland experiments developed under the same management types. It is also expected, that new research questions will come in the future and only the data from long-term grassland experiments will be able to answer them in detail (Pötsch et al., 2015).

The oldest grassland experiment in the world is The Park Grass Experiment, which was already established in 1856. The aim of this experiment was to answer some agricultural questions at the beginning. Then this experiment became a demonstration of the evolution of adaptation at a very local scale and reveal genetic detail about the long-term dynamics of plant populations (Silwertown et al., 2006).

Other long-term grassland experiment is Palace Leas Hay Meadow Experiment, which is located 30 km north of Newcastle. This experiment was established in 1896 and nowadays is over 120 years old. The experiment consisted of treatments with farmyard manure and mineral fertilizers application. Long-term impacts of some types of fertilizers on plant species composition, and responses of functional groups and individual plants, showed pathways and mechanisms that drive ecosystem responses in relation to their application. An example could be ammonium sulphate, which caused acidification after long-term application. Based on these findings, maximal utilization of farmyard manure was recommended as an optimal fertilization method (Kidd et al., 2017).

The Steinach Grassland Experiment is one of the oldest still-running grassland experiments in the Continental Europe. This experiment was established in 1933 and provides detailed information about the long-term effects of different fertilizers on plant species composition, biomass productivity, and soil nutrient content. Decades-long individual applications of N, P and Ca or their combination were observed on alluvial meadows with high nutrient status and dominance of *Alopecurus pratensis*. Fertilizer application altered the plant species composition, but the diversification of plant communities is not as high as in other fertilized grassland experiments with lower nutrient status at the beginning. Survival of plants adapted to high nutrient availability is therefore secured even in unfertilized control. Species richness is negatively affected directly by soil acidification or indirectly by increased competition for light, especially after long-term N fertilizer application with a combination of other nutrients such as P, K, and Ca. This experiment shows that alluvial grasslands with dominance of *A. pratensis* are substantially less affected by fertilizer application than other low-productive grasslands (Hejcman et al., 2014).

The Rengen Grassland experiment was established in 1941 in the Eifel Mountains in Germany on low productive grassland. This experiment belongs to the oldest grasslands experiments with different fertilizer applications in Europe. There are fertilizer treatments with Ca, N, P, and K nutrient applications. Long-term fertilization caused significant diversification of plant communities under different treatments. The largest differences in plant species composition and biomass production are between the treatments fertilized with P with other nutrients such as N and Ca and treatments fertilized without P. This is because P is a limiting nutrient in this experimental site (Hejcman et al., 2007).

The Admont grassland experiment in Austria was established in 1946 in an alluvial wet meadow. The experiment consists of treatments with different N, P, K nutrient applications and their combinations and one control treatment without any fertilization. Fertilization as well as the form of application determines plant species composition. P and K fertilization support legumes and forbs, whilst N fertilization supports short plant species. The highest number of plant species occurs in the P treatment.

The Ossekampen Grassland Experiment was established in 1958 on mesophilic grassland classified as the *Arrhenaterion* alliance. This experiment is near Wageningen in the Netherlands. This is more than 60-year-old field experiment with different fertilizer applications such as superphosphate, potassium sulphate, ammonium nitrate, and lime marl. It was found, that species richness in all treatments declined, but after 25 years began to increase again. The strongest decline was observed in NPK fertilized treatments associated with a strong divergence of plant trait composition from the control treatment. The trait composition remained stable after the subsequent period of increase in species richness in the NPK treatments. This could be because the species responsible for the recovery are not necessarily the same as the ones that disappeared. These are mainly the species adapted to nutrient-poor conditions, which have become increasingly rare in the last decades and require special protection (Pierik et al., 2011; Korevaar & Geerts, 2015).

A long-term Permanent Grassland Experiment at Vel’ká Lúka in Central Slovakia was established in 1961 in a flat wet meadow. The experiment shows the effects of mineral fertilizers, such as N, P, K and their combinations in comparison with no fertilization on plant functional groups and productivity. Long-term mineral fertilization led to a decrease of legumes and forbs and an increase in grasses. NPK treatment that received the highest doses of fertilizers has the highest biomass yield. The experiment demonstrates that medium fertilization seems to be an acceptable to meet either productivity requirements or environmental aspects (Vargová et al., 2020).

Grasslands management

Grazing

Grazing is a natural type of feeding herbivores and it is the most common grassland management in the world. Grazing animals have a direct impact on the structure and composition of plant communities by trampling and excretion of feaces and urine. It affects the distribution of nutrients in the pasture and creates mosaic vegetation structures with different patche types. Moderate grazing regimes usually increase level of diversity and follow the patterns of traditional grassland managements. Therefore grazing is often used as a tool for sustainable grassland management in nature protection. Grazing can preserve target species in the plant communities and simultaneously conserve different landscape types (WallisDeVries et al., 1998).

Grazing is sustainable grassland management in long-term, because the nutrients return back into the vegetation through feaces and urine of grazing animals in contrast with cutting, which contributes to lower nutrients content in the soil throughout the regular biomass removal.

Cutting

Cutting of herbage with biomass removal is a type of management, which is practiced since medieval times, when the scythe was invented. Biomass is usually removed immediately from the area after cutting (Gaisler et al, 2013). The frequency of cutting is one of the most important factor affecting grassland community, especially plant species composition and the amount of nutrients in the aboveground biomass and in the soil. Mowing can also affect belowground conditions such as the activity of soil decomposers (Ilmarinen & Mikola, 2009). Plant species composition and presence of individual plant species as well as their covers (%) in the vegetation are strongly affected not only by frequency, but also a time of cutting during the vegetation season. Long-term regular cutting with biomass removal can cause gradual mineral depletion and can shift plant species composition toward species-poor oligotrophic community, especially in mountain meadows (Mašková et al., 2009).

Abandonment

An increasing percentage of grasslands has been abandoned since the early nineties of 20th century in central and Eastern Europe. Grassland management was either changed to less intensive livestock production or less favoured areas of grasslands have no longer been utilized at all (Isselstein et al., 2005). Nowadays, large areas of low-productive grasslands especially in the mountains are abandoned, because of their lower use for forage production (Mašková et al., 2009). The tall and highly competitive species begin to predominate and original vegetation is overgrown by shrubs and tree seedlings (Isselstein et al., 2005). The small portion of dominants with high light requirements, but not necessarily with high requirements for N, usually prevail in the vegetation (Bartha et al., 2014). Abandonment usually leads to a decrease in the number of plant species and species richness (Gaisler et al., 2013; Mašková et al., 2009) and in long-term leads to a change of the secondary grasslands into the forests.

Nutrients applications

Different types of fertilizers were used by humans for centuries to improve the herbage biomass yield of the grasslands. The organic fertilizers (manure, slurry, compost) were the most important way, how to increase production till the middle of 19th century, when the production of mineral fertilizers began. Fertilizer application is related to the changes in plant species composition (Isselstein et al, 2005). The changes in plant species composition after fertilization were observed in many long-term grasslands experiments (Kidd et al., 2017; Hejcman et al., 2014; Silvertown et al., 2006). Application of nutrients has also large impact on community biodiversity and productivity and the stability of ecosystem services. Before the application of fertilizers, the knowledge gained from long-term grassland fertilization experiments should be considered to avoid their potentially negative effects, which might take several generations (Melts et al., 2018). Long-term application of different fertilizers has also large impact on bacterial communities in grassland soils (Pan et al., 2014).

N application

Despite the general meaning, that N fertilization is detrimental to vascular plant diversity, the presence of three orchid species, *B. media* and short sedges and, the absence of tall grasses in the N treatment in Rengen Grassland Experiment showed that this must not necessarily be the case. Then a large change in the plant species composition occurs only, when the N is applied together with another limiting nutrient, such as P (Hejcman et al., 2007). The application of N fertilizer only is detrimental especially for legumes, when it is applied in higher doses. Therefore it is necessary to choose N-rates appropriately to the local nutrient conditions to allow legume establishment or their maintenance in the sward. Yearly N rates should be at least below 85 kg N ha^{-1} on the permanent grasslands with three cut regimes in central Europe. The type of N fertilizer application, mineral or slurry was shown to be less important (Weggler et al., 2019). But fertilization by farmyard manure could be preferred over mineral fertilizers due to its ability to release nutrients more slowly and also because of its micronutrients content (Čop et al., 2009). The presence of N-fixing legumes in the sward can increase N availability for other species (Mulder et al., 2002) and partly replace N, which would have to be applied by mineral fertilization.

A large meta-analysis of temperate mountain grasslands (including montane, subalpine, and alpine zones) showed the effects of N fertilization on species richness. N fertilization usually leads to an increase of phytomass and a reduction in plant species richness, similarly as in the case of lowland grasslands. Negative effects on species richness are stronger by increased dose (ha^{-1} $year^{-1}$) and longer duration of N application (years) in an additive manner. Sustained application of low to moderate levels of N over time has similar effects as a short-term application of high N doses in temperate mountain grasslands. Negative effects of N fertilization on species richness and diversity are more pronounced in mountain grasslands experiencing warm rather than cool summers. Therefore climate changes can strengthen the effects of nutrients on plant species diversity, richness, functioning, and resilience of mountain grasslands. The greater the phytomass response to N addition is, the greater is the decline in species richness. Plant community changes are not only affected by N fertilization alone, but also by the interaction of this fertilization with other abiotic factors and local management (Humbert et al., 2015). N application usually supports grasses, but if it is combined with P, a more balanced plant species composition is secured (Papanastasis & Koukoulakis, 2006). N fertilization also affects arbuscular mycorrhizal fungi (AMF) communities. These fungi are affected by soil N availability, N:P supply ratio, and by host-plant photosynthetic strategy (C3 vs. C4). In the soils with limited P (high N:P ratio) N fertilization usually increases AMF productivity, species richness, and diversity, contrary in the P-rich soils (low N:P ratio) decreases, due to the loss of rare AMF species (Egerton-Warburton et al., 2007). It seems that the abundance and activity of soil micro-organisms are regulated more by plant species traits than by direct effect of N fertilization (100 kg ha^{-1}) in upland grasslands. The differences between traits can be in the variations of root exudation patterns and/or in the efficiency of nutrient acquisition (Bardgett et al., 2002).

P application

Grasslands soils are usually poorly supplied with P, therefore P application usually increases their biomass yield (Papanastasis & Koukoulakis, 2006). Short-term P application has usually limited influence on changes in microbial community structure, if the P application rate not exceeds 40 kg P ha^{-1} in the sward dominated by timothy. But the process of microorganisms involved in soil P cycling is affected by P fertilization (Shi et al., 2020). The acquisition of P by plants is largely affected by arbuscular mycorrhizal fungi (AMF). P use efficiency increases after the colonization of the roots by AMF. High soil P availability can negatively affect the abundance of AMF. Long-term moderate levels of P fertilization usually help to keep stable and not decrease AMF abundance over time. AMF can help to save financial costs for additional P nutrient applications in intensively-managed grasslands (Fornara et al., 2020).

K application

Application of K can increase biomass yield a lot, especially on soils poor on K^+ such as alfisols (Papanastasis & Koukoulakis, 2006). A merely high content of K in the soil is usually related with high plant species diversity, in contrast to high levels of P in the soil, which have usually the opposite effect. The losses of K are usually greater than of P, because it is more leachable nutrient than phosphorus and the plants take higher quantities of K per year (cca 200 kg ha^{-1} $year^{-1}$) than of P (Janssens et al., 1998).

Combined applications of nutrients

Phosphorus and nitrogen are the most limiting nutrients in many types of grassland. Application of both nutrients usually distinctly increases above-ground biomass yield, but decreases species richness (Willems et al., 1993). Responses to the application of fertilizer also depend on the nutrient status of the soil and weather conditions, usually only a moderate rate of NPK fertilizer application increases herbage yield considerably (Čop et al., 2009). Combined application P and K increases dry matter biomass production in comparison with unfertilized or limed plots. An extra increase in dry matter biomass production and sward height occurs especially when the N is applied together with P and K. This is followed by a decrease in species numbers (Honsová et al., 2007; Korevaar & Geerts, 2015). NPK fertilization also decreases cover (%) of N-fixing legumes and increases cover of tall grasses (Hrevušová et al., 2014). The application of PK nutrients usually supports functional group herbs, if the N is not applied together. On the contrary functional group grasses usually decrease cover under PK fertilization without N application (Honsová et al., 2007). Long-term PK fertilization often supports N-fixing legumes (Hrevušová et al., 2014). A medium amount of fertilizers is necessary to apply to meet both productivity of biomass and maintain quality and quantity of soil organic matter at the same time. Fertilization with N, P, and K nutrients application can also affect the C:N, C:P, N:P and N:K ratios in aboveground plant biomass (Vargová et al., 2020).

Nutrients applications and pH

Changes in the pH can alter soil processes and plant growth. The application of different fertilizers results in a wide range of soil pH from acid to neutral. Especially the form of fertilizer application is very important for the soil pH. The availability of many nutrients for the plants and accumulation of soil organic matter can be affected by soil acidification. Neutral soils with Ca application are better for microbial activity and lead to a lower C:N ratio due to the lowest content of soil organic matter (Hejcman et al., 2014). The availability of P and N for the plants can be reduced under acid soils (Gough et al., 2001; Rejšek & Vácha, 2018). Fertilization by mineral N can increase the acidification of the soil if it is added in the form of ammonium sulphate $(NH_4)_2SO_4$. The pH can reach equilibrium, if it is applied in long-term and then no further acidification does occur. The evidence for this was observed on long-term Park Grass and Palace Leas grasslands experiments. The pH equilibrium has been already reached in Park Grass experiment and it is expected to be reached in Palace Leas experiment as well and therefore a subsequent dramatic decline in soil pH is unlikely to be anticipated. Application of farmyard manure seems to be better than mineral fertilization to resist acidification, due to its presence of Ca^{2+} and Mg^{+} ions (Kidd et al., 2017). Application of P can increase soil pH, if it is added as Thomas slag, which also contains Ca and at the same time Ca is removed by harvested biomass and leaching is lower (Hejcman et al., 2014). Application of Ca usually led in increase of plant species diversity due to the increase of pH (Korevaar & Geerts, 2015). This is because a higher number of plant species is adapted in calcareous base-rich soils rather than in acid. As one of the explanations can be the fact, that during the Pleistocene time acid soils were much rarer than base-rich soils. As a result of ecological drift determined by historical bottleneck more acidophilous than calciphilous species died out (Ewald, 2003). Permanent removal of base-rich nutrients with the harvested biomass can also cause soil acidification under treatments with no fertilization (Vargová et al., 2020). On one side low pH of the soil can reduce activities of soil microorganisms, on other side higher pH can contribute to faster mineralization and cycle of soil organic matter (Duffková et al., 2005).

Long-term effects of nutrients applications

Fertilization has also often long-term after-effects on plant species composition and species richness. It seems that the removal of N from the soil is faster than that of P. One example can be the experiment on calcareous grasslands in the Netherlands. N was removed in less than 10 years, contrary P was still present 25 years after the fertilizer application was finished under one-year cutting regime in August. The effects of P surplus were still observed in species composition, biomass yield, and in soil chemistry (Smits et al., 2008).

Plant species communities dynamics

Mechanism stabilizing community temporal fluctuations usually declines after the loss of biodiversity. The greater variability in individual plant species populations and the synchronicity of their fluctuations is, the lower is community stability. This can appear when the decrease in temporal stability of total biomass is related to the decrease in species richness. The weakening of stabilizing effect „portfolio effect" related with the decline of species richness is a crucial driver of community destabilization. Stabilizing effect can be strengthened by compensatory dynamics, when the decrease of one individual plant species cover (%) is compensated by the increased cover (%) of other species (Lepš et al., 2018). The number and relative abundances of plant species in a community at equilibrium are determined by competition for essential resources. Among the most important resources for plants belong nutrients and light. Each individual plant species has some essential source that limits its growth. If two species are competing for the same essential resource, the winner of the competition will be the one able to tolerate its lowest concentration (Silvertown et al., 2006). Theoretical models can be used to explore the stabilities and uncertainties of grassland ecosystems and estimate equilibrium states of soil moisture, living biomass, and wilted biomass. These models can be used to find key issues of grassland equilibrium state instability (Sun & Mu, 2012). The equilibrium of guild composition is however dynamic, continually perturbed by year-to-year changes in the weather (Silvertown et al., 2006) or microbial changes in the soil in the long-term under different management types.

Types of grasslands in a research sites

Violion caninae meadows

Violion caninae submontane short grassland occurs mainly on nutrient-poor soils (Chytrý et al., 2009). *Violion caninae* alliance is often species-rich and can contain rare species such as *Arnica montana*, *Hieracium lactucella* or *Pedicularis sylvatica* (Matějková et al., 2003) and other submontane (*Violion caninae*) species (Krahulec, 1990). The species occurring in *Violion caninae* alliance can be *Nardus stricta*, *Potentilla erecta*, *Viola canina*, *Danthonia decumbens* (Havlová et al., 2004), *Polygala vulgaris*, *Pilossella officinarum*, *Carex pallescens*, *Dianthus deltoides*, *Silene nutans* and *Fragaria vesca* (Škodová & Janišová, 2008; Škodová et al., 2015). The main abiotic factors temperature and moisture indicate extension of *Violion caninae* vegetation, which occurs mostly at colline sites in contrast with *Nardion strictae* vegetation, which is widespread in high altitudes at montane sites (Maurice et al., 2012).

***Trisetion* meadows**

Polygono–Trisetion semi-natural grasslands occur mainly as small islands over the calcareous bedrocks across Europe (Hegedüšová et al., 2011). Altitude seems to be the most important factor, which forms plant species composition in a central European mountain range. This reflects the natural transition of *Arrhenatheretalia* meadows into the *Polygono–Trisetion* along the altitudinal gradient, so that *Polygono–Trisetion* occupies higher localities (Pruchniewicz & Zolniers, 2014). Species-rich mountain hay meadows (*Polygono-Trisetion*) with very low available P content in the soil is possible to leave unmanaged for several years due to only small changes in plant species composition throughout the time. Thus lack of management is not detrimental to the preservation of the target vegetation despite the changes in covers of some dominant species. Therefore cutting management combined with several years of no management seems to be a suitable strategy for the maintenance of *Polygono-Trisetion* grasslands. This can save financial costs for the cutting management with regard to low demand for forage from *Polygono-Trisetion* grasslands and thus no financial gain from the mowed biomass (Pavlů et al., 2011). But some of the *Polygono bistortae-Trisetion flavescentis* meadows are not cut in long-term and they are seriously endangered by succession, afforestation, or by conversion to downhill courses. All these factors represent great risk for species-rich *Polygono–Trisetion* semi-natural grasslands (Hegedüšová et al., 2011).

***Arrhenatherion* meadows**

Species-rich *Arrhenatherion* communities occur especially on semi-natural grasslands older than 100 years. They had been used by traditional historical managements such as sheep grazing. Conservation efforts should therefore focus on implementing these traditional land use practices to maintain these communities. Medium-rich *Arrhenatherion* grasslands are usually not older than 30 years. They were predominantly established on formerly arable fields with lower productivity. Therefore they are not the primary goal of nature conservation as species-rich *Arrhenatherion* communities (Krause & Culmsee, 2013).

Increasing pressure for the cattle slurry application on *Arrhenatherion* grasslands in central Europe may cause a decrease in their natural value. Requirements of farmers for high soil fertility and adequate herbage production, environmental requirements for reduction of soil and water pollution and nature conservation requirements for the species-rich grasslands need to be taken into account. An acceptable compromise in maximal annual application of cattle slurry between all these partial interests of all stakeholders seems to be a suitable rate of 120 kg N ha $^{-1}$ on *Arrhenatherion* grasslands with three cut regime (Duffková et al., 2015). Mineral fertilizer application with calcium ammonium nitrate under 2-3 cut management in May, August and in some years in October should be applied with lower rates than organic fertilizers. It seems that already an application of 56 kg N ha^{-1} can increase the cover of nitrophilous species, tall graminoids and decrease cover of short graminoids in *Arrhenatherion* meadows (Čámská & Skálová, 2012). Intensive NPK fertilization usually leads in increased yield and changes in the proportion of plant functional groups under three cut regimes on *Arrhenatherion elatioris*-type meadows. Declines of forbs and legumes under NPK applications are then also followed by changes in the forage quality of *Arrhenatherion* grasslands (Dindová et al., 2019). *Arrhenatherion* grasslands can be managed more intensively, than nutrients poorer *Trisetion* and *Nardion* grasslands.

Wet *Cirsium* meadows

Species-rich wet meadows are among the most endangered grassland types in Central Europe, threatened by land use change and intensification. The majority of floodplain meadows were fragmented and replaced by other land use types, usually by species-poor, intensively managed grasslands or arable fields. Then a lot of local effects such as fertilization and drainage pronouncedly affected these meadows. Conservation of large-scale floodplain meadows, in areas where remnants of historically old grasslands persist, is a primary goal of nature protection (Krause et al., 2011). Total species richness declined over time and the most affected plant species were habitat specialists, as they disappeared from the most of wet grasslands (Diekmann et al., 2019). A variance of plant species composition on the wet grasslands is best explained by altitude, soil pH, geographical gradient, frequency of flooding, mean annual temperature, date of mowing, humidity, annual amount of precipitation, and other factors such as plant-available phosphorous and amount of Ca^{2+} ions in the soil.

Changes in the water level, excessive phosphorous addition, and extreme acidification of the soil are harmful factors affecting the remaining species-rich wet grasslands in Central Europe (Zelnik & Čarni, 2013). Higher levels of soil extractable P are likely to be detrimental to wet grasslands (Critchey et al., 2002). N and P co-limitation usually led to a decrease in biomass production of wet meadow vegetation. Nutrients surplus caused by flooding with heavily eutrophicated surface water could lead to long-term changes in plant species composition (Hoek et al., 2004). Conservation of wet meadows and fens depends on moderate grazing or cutting, which prevents successional changes, which can cause local extinction of certain plant species. Development of a litter layer and lower competitiveness of short-lived species, such as *Viola palustris* about the light in the abandoned vegetation could lead to a decrease in species richness and could cause the local extinction of typical fen and wet meadow species (Jensen & Meyer, 2001).

Typha spp.

Typha spp. belongs between expansive wetland macrophytes reducing the diversity of wetland species. *Typha* spp. expansion can be suppressed by physical removal such as hand clipping, flooding or by control techniques using a fire (Apfelbaum, 1985). Growth of *T. latifolia* in deep water can be suppressed by cutting aerial shoots below the water surface. This eliminates the supply of oxygen into the rhizomes (Murkin & Ward, 1980; Sale & Wetzel, 1983). This is related especially to the older stands of *Typha* spp. As Lishava et al. (2017) suggest, that cut above the water is sufficient to control young stands of this species. Mowing *Typha* spp. seems to be suitable in shallow water, but in deep water burning, if it is possible to carry out, seems to be better option, because of the lower management costs (Ball, 1990). A positive effect of *Typha* spp. is their ability to reduce nitrates in the water and harvesting of these species can reduce their function in wetlands (Martin et al., 2003). *T. latifolia* is well adapted to humid conditions, therefore drought periods can negatively affect its performance (Li et al., 2004). Then water depth can also reduce vitality of *T. latifolia* by decreasing incidence of flowering and shoot density (Grace, 1989).

Weed species on the grasslands (*Rumex obtusifolius* and *Urtica dioica*)

Rumex obtusifolius (broad-leaved dock) is a widely distributed weed in managed grasslands (Hann et al., 2012) and belongs to one of the most troublesome weeds for forage production (Gilgen et al., 2010). The presence of this species in the pastures usually led to the decrease of yield and fodder quality and its non-chemical measures are often limited (Zaller, 2006; Stilmant et al., 2010). High Ca and Mg concentrations in the topsoil usually reduce the density of this species. *R. obtusifolius* infestation on soils with a high amounts of these nutrients is possible to reduce with management extensification (Hann et al., 2012). Despite that seedling emergence and early development stages of *R. obtusifolius* are usually negatively affected by N availability, fully developed rosettes of this species can tolerate very high N supply. Generally for the growth of stems and flowering and seed ripening usually high N as well as P nutrients supplies are necessary (Křišťálová et al., 2011). *R. obtusifolius* is also species with high water use efficiency and seems to be well adapted to drought periods. With respect of stronger effects of clime changes in the future, weed pressure by *R. obtusifolius* will demand additional management measures to limit its success (Gilgen et al., 2010). A promising grassland management to control *R. obtusifolius* seems to be sheep grazing. Reduced regrowth potential of this species as a direct effect as well as creation of a dense grassland sward to reduce the abundance of this weed as an indirect effect could be supported by optimal grazing intensity (Zaller, 2006). Unmanaged grasslands with higher nutrient availability can decrease mortality of *R. obtusifolius* plants. But no grassland management for several years under conditions of low nutrient availability in the soil could be an effective strategy to successfully control *R. obtusifolius* infestation (Hejcman et al., 2012). Digging out taproots of *R. obtusifolius* from the depth of 15 cm seems to be suitable technique in organically managed grasslands to reduce this species. Although this procedure can eliminated majority of *R. obtusifolius* plants, it is time and laborers demanding (Hujerová et al., 2016). Stilmant et al. (2010) suggest, that *R. obtusifolius* plants in permanent grasslands is possible to reduce only either with high grazing or cutting intensity (Van Evert et al., 2020).

Urtica dioica is a highly competitive shade-tolerant ruderal species, which is widely naturalized in a range of habitats (Taylor, 2009). *U. dioica* is related to disturbed soils, which are often enriched by excreta of animals or by wood-ash (Pigott, 1971). N is considered as the most important element for the *U. dioica* nutrition (Rutto et al., 2012). Cutting twice per year seems to be not sufficient to suppress the growth of *U. dioica* on the soils with high N, P, and K availability. Therefore, this species also survive in frequently cut-intensive grasslands with high nutrients supply (Müllerová et al., 2014). It seems that *U. dioica* is well adapted on heavy grassing pressure by increasing the number of stinging trichomes, their density and stinging capacity. Then induced defense in *U. dioica* dissuade most vertebrate herbivores they interact with (Pullin & Gilbert, 1989).

Plant traits

Plant functional traits-based approach is increasingly used in nature science. Morphological, physiological, and phenological plant properties can be used to reveal ecosystem functioning. Trait-based approach can be also used in grassland research. Main trade-offs among plant traits and their variability can be observed in different plant species as a response to a nutrient gradient. The plant trait-based approach is ideal tool how to relate grassland science and functional ecology (Schellberg & Pontes, 2012). It is possible to observe large scale of plant functional traits such as lamina proportions, organic matter digestibility of grasslands and foliar traits (specific leaf area, leaf dry matter content). Plant traits can also reveal relationship between a set of vegetation and digestibility characteristics (Andueza et al., 2010). By using traits it is also possible to distinguish plant life forms such as grasses, rosettes, upright forbs, and legumes. Defoliation regime and nutrient availability usually lead to development of certain plant life forms with special plant traits in the studied grasslands. Simple method such as monitoring targeted plant traits can be used for assessing the effects of land-use change on grassland ecosystem behavior (Ansquer et al., 2009). Traits analysis can help to understand the mechanisms of plant responses and predict changes in plant species composition under different grazing regimes throughout the season. Plant traits differ due to changes in the mechanism of species responses to grazing. Heavier summer grazing usually increase gap colonization ability, spring and winter grazing increase plant regrowth ability (Bullock et al., 2001). Not only aboveground plant traits, but also below ground plant traits are important, because they can sometimes better explain responses of individual plant species to studied environmental gradient (Schrama et al., 2013). Responses of traits can be related with differences in a wide range of abiotic conditions. To predict effects of soil moisture, root length, canopy height and average leaf area can be used. These plant functional traits that respond to water availability can be mediated by grasslands land use changes (Gross et al., 2008). Traits shifts among the different species can be coherent, but the magnitude of changes depends on the individual plant species or functional groups (monocots vs. dicots). Root morphological traits (specific root length, specific root surface area, root diameter, root tissue mass density and root N concentration) may be used as valuable indicators of environmental changes, changes in fertilization or in management (Leuschner et al., 2013). Leaf dry matter content weighted by species abundance at plant community level can be used as a plant trait that responds to stress and disturbance related to the weather conditions and cutting or grazing grassland managements. Based on the single trait approach it is possible to distinguish two basic plant species groups. First, species with the exploitative strategy adapted to low stress (high temperature and nutrients), second, species with conservative strategy adapted to high stress (low temperature and nutrients). Not only these two, but also variety of other plant community functional compositions can be related with the combinations of management practices and environmental factors (Duru et al., 2012). Then plant traits and microbial functional traits can be used as well for the determination of C sequestration and soil N retention. Exploitative species with higher specific leaf area, leaf N concentrations and lower leaf dry matter content, along with soil microbial communities dominated by bacteria have poor C and N retention. Contrary conservative species with opposite traits, along with soil microbial communities dominated with fungi and bacteria with slow activities have greater soil C storage and N retention (Grigulis et al., 2013). Differences in aboveground community biomass in semi-natural grasslands may be best explained by abiotic factors, trait values of the dominant species and functional trait diversity in combination, as their isolated consideration may be misleading (Schumacher & Roscher, 2009). Plant species have a different functional adaptations represented by their traits combinations. Plants with the same development changes due to mowing or plants under selective pressure induced by grazing can be related with these special traits combinations (Catorci et al., 2011). Some of the traits can be associated with the treatment frequency and nutrient conditions such as plant height or specific leaf area, others can be associated with the timing of the treatments such as start and duration of flowering. Convergent response of functional traits to different management treatments can help to understand the mechanisms behind the changes in vegetation and predict future changes despite floristically divergent plant communities (Kahmen & Poschlod, 2008). Then it is also possible to observe similar trait responses to succession. The more the development of plant community toward succession is, the more proportion of species with plant height > 0.6 m, leaves distributed regularly along the stem, vegetative spread >100 mm, start of flowering later than May and duration of flowering 1-2 months, will increase (Kahmen & Poschlod, 2004). Traits indicators can be used for the detection of changes in community structure and for the identification of causes and possible consequences of the altered plant community composition in relation with environmental conditions and management regimes (Pfestorf et al., 2013). Variations in above-ground net primary productivity related with the changes in cutting management and N supply are not controlled only one single trait, but rather by group of plant functional traits. These traits can be average flowering date, tiller density per unit land area, leaf dry matter content or dry matter fresh mass (Pontes et al., 2010). Several key functional traits can reflect processes in the grassland ecosystems such as strong decline in community growth during the summer drought followed by increased growth two months later. As an example can be the resilience of mountain grasslands to summer drought, which seems to be high with not a lot of changes in plant species and trait compositions (Deléglise et al., 2015). Plant traits can be used as a tool to link changes in species composition with grasslands ecosystem processes at the community level (Quested et al., 2007).

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Research questions

Central European semi-natural hay grasslands

(i) In this study, we specifically asked: How uneven is the distribution of biomass among co-occurring plant species in grasslands?

(ii) What is the role of widely distributed and rare (in terms of frequency) species in biomass production in grasslands?

(iii) How do different functional groups (grasses, sedges and rushes, legumes and other non-legume short and tall forbs) contribute to these frequency distribution classes?

(iv) Does functional diversity explain biomass inequality among different species?

(v) What are the key factors affecting s-AGB?

Rengen Grassland Experiment

(i) what was the effect of long-term different fertilizer applications on species richness and plant species composition during ten years of observation?

(ii) was there any detectable trend in species richness and plant species composition during the ten years of observation?

Admont Grassland Experiment

What is the effect of long-term different treatments of fertilizer application on:

(i) vegetation (plant species diversity, plant species composition, dry matter biomass yield and sward height)

(ii) the soil properties of alluvial meadow?

Nízké Tatry Mts. grassland experiment

(i) is there any effect of cutting twice per year on *R. obtusifolius* or *U. dioica* suppression?

(ii) can cutting twice per year carried out throughout all study period decrease *R. obtusifolius* or *U. dioica* infestation after single-use herbicide application and reseeding by grasses/forbs mixture in the first year?

(iii) have abandonment of regular cutting and leaving mountain grasslands without any other management effect on *R. obtusifolius* or *U. dioica* inhibition?

Malá Strana grassland experiment

(i) What is the effect of long-term cutting at different frequencies (once or twice per year, relative to no cutting management) on *T. latifolia* characteristics (cover, number and height of tillers, dry matter biomass (DMB) yield and litter) and on sward characteristics of wet meadow (species diversity, sward height, DMB yield, plant species composition and functional groups)?

(ii) Is there any effect of biomass removal after cutting on *T. latifolia* characteristics and on sward characteristics of wet meadow?

Chapter II

Drivers of species-specific contributions to the total live aboveground plant biomass in Central European semi-natural hay grasslands

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Abstract

Semi-natural grasslands are known to provide numerous ecosystem services, of which one of the most important is production of biomass. However, the contribution of individual plant species to the total biomass is much less well understood. This study addressed questions concerning community structure and responses of species-specific biomass (s-AGB) to gradients in soil acidity and fertility, topographical and climatic features, and disturbance regimes in mown and abandoned grasslands in the Sudetes Mountains (Central Europe). It identified pH as the most significant environmental gradient affecting turnover in s-AGB, and mowing cessation, temperature, and precipitation also had significant effects. Further, it showed high inequality in biomass among co-occurring plant species. It also showed that biomass inequality (measured by the Gini coefficient) among interacting species decreases with increasing functional diversity (Rao's index). This study highlights that common plant species (in terms of frequency) play a major role in contributing to the total aboveground biomass (t-AGB). However, less frequent species are also significant contributors to the t-AGB. Thus, the combined contribution of infrequent species to the t-AGB should not be neglected. Our findings support the mass ratio hypothesis stating that ecosystem functions such as biomass production depend on dominant species. On the other hand, high niche differentiation ensures the coexistence of less competitive species with the dominants by the variety and complementarity of functional traits. Infrequent and non-dominant species were the core of the diversity seen in the studied grasslands. The maintenance of species diversity in grasslands should be prioritized in nature conservation policies to ensure the sustainability of ecosystem services.

Keywords: Abandonment; Climate; Functional diversity; Biomass inequality; Niche differentiation; Soil

1. Introduction

Grasslands are one of the most widespread ecosystems in the world, covering up to approximately 40% of the land surface (Gibson, 2009). These ecosystems are mostly dominated by graminoids (grass and grass-like species). However, they host a high diversity of other life-forms (Biurrun et al., 2021; Dengler et al., 2014; Gibson, 2009; Raduła et al., 2020; Veen et al., 2009). They are a habitat for wildlife (Bonari et al., 2017; Zulka et al., 2014), and provide numerous ecosystem services, such as soil and water protection (Habel et al., 2013; Zhao et al., 2020). There are many different types of grasslands, but their common feature is the production of biomass, and they therefore play a crucial role in the world's carbon cycle, in addition to being essential for the animal production industry and for global food security (O'Mara, 2012).

Grassland productivity has been the subject of numerous studies in both basic and applied ecology (Scurlock et al., 2002). Aboveground biomass production is the most frequently studied component of plant productivity, and measurements of peak aboveground biomass are often used as a surrogate for net aboveground primary productivity (Bhandari and Zhang, 2019; Kahmen et al., 2005; Xia et al., 2018), with it most often measured as the total clipped standing biomass (Hejcman et al., 2010; Kahmen et al., 2005; Pavlů et al., 2013). Overall biomass sample figures are also sometimes divided into living and dead matter components (Bedia and Busqué, 2013; Gross et al., 2009; Lanta et al., 2009), or partitioned into functional groups (Kassahun et al., 2021; Pokorny et al., 2004; Semmartin et al., 2007; Zeeman et al., 2019). A number of local experiments have focused on the variation of aboveground biomass production in temperate grasslands in different European regions (Bernhardt-Römermann et al., 2011; Dodd et al., 1994; Galvánek and Lepš, 2012; Hector et al., 1999; Hejcman et al., 2010; Lepš, 2004; Maire et al., 2012). However, no study has yet examined the fine-scale biomass distribution of coexisting species and the causes of variation in species-specific aboveground biomass (s-AGB) across a broad range of gradients in soil acidity and fertility, topographical and climatic features, and disturbance regimes. The assessment of aboveground biomass distribution among species in grassland ecosystems has been extremely rare in both observational (Andueza et al., 2016; Qi et al., 2021) and experimental studies (Lepš, 2004). This approach has also been rare even in experimental manipulations of species-poor artificial plant assemblages (Maire et al., 2012), or has been limited to dominants only (Hejcman et al., 2007).

In an ecological context, the amount of s-AGB serves as a measure of species' success in the plant community (Colgan & Asner, 2014). Species abundance is influenced by species interactions and is therefore subject to the effects of competition for available resources (Herben et al., 2007). European semi-natural grasslands are mostly dominated by grasses (Padullés Cubino et al., 2022; Večeřa et al., 2021). The ecological success of grasses can be linked to wind-pollination, effective long-distance dispersal, establishment and short germination rates, and resilience to continuous disturbance (Linder et al., 2018). However, semi-natural grasslands can have a rich diversity of species, including various sedges, rushes and forbs, including legumes. Many species of these groups are poor competitors and have scarce distribution in semi-natural grassland communities; despite this, they are strongly associated with grassland habitats (Lennon et al., 2011; Markham, 2015). It has long been recognized that plant communities typically consist of a few common species (often dominant) and many rare ones (Preston, 1948). The mass ratio hypothesis (Grime, 1998) states that ecosystem properties are primarily determined by the traits of the dominant species. However, several recent studies have shed light on the importance of rare species for ecosystem functioning (Lyons et al., 2005; Soliveres et al., 2016). Thus, even if a single, infrequently occurring species is meaningless relative to overall biomass production, the combined contribution of such infrequent species to total biomass production should not be neglected. This assumption was based on the high species diversity seen in semi-natural grasslands, resulting from the co-occurrence of species with contrasting frequencies (Pärtel et al., 2001). Semi-natural grasslands are an example of ecosystems with few broadly distributed species and many species with low frequency distribution (the core-satellite hypothesis; Hanski, 1982).

There is limited understanding of the factors that cause inequality of biomass (size inequality) distribution among coexisting species at the plant community level in grassland ecosystems. An inequality of species abundance is a universal phenomenon in both animal and plant communities (Ulrich et al., 2010), which can be measured by using an index intended to study the income inequality, known as the Gini coefficient (Weiner & Solbrig, 1984). Identifying the mechanisms driving species relative abundance is a primary concern in ecology (McGill, 2010). The recent study by Qi et al. (2021) showed that species-dimension biomass inequality in stressful alpine grassland ecosystems is dependent on environmental and productivity gradients. In this study we employed the trait-based approach, which is particularly useful in elucidating plant coexistence patterns in competitive communities (Maire et al., 2012; Padullés Cubino et al., 2022). Among various plant functional traits, the leaf-height-seed (LHS) strategy scheme represents fundamental plant functions and major dimensions of plant adaptation strategies (Westoby, 1998). An important leaf trait is specific leaf area (SLA), which is related to resource acquisition and relative growth rate; plant height at maturity reflects the competitive ability for light; and seed mass describes the reproduction strategy (Díaz et al., 2016; Westoby, 1998; Westoby et al., 2002).

It is noteworthy that recent studies have shown that species abundance and their distributions in grasslands depend on deterministic processes, and more specifically on habitat filtering, which promotes the dominance of phylogenetically related species (mostly grasses), whereas niche differentiation has a positive effect on the species diversity of less competitive plants (Maire et al., 2012; Padullés Cubino et al., 2022). Based on this hypothesis, we predict high biomass inequality among coexisting plant species, in particular between dominant grasses and infrequent forbs. We also predict that high variation in functional traits at the plant community level (high functional diversity) has a stabilizing effect on biomass inequality among species. Building on the hypothesis that most of the plant diversity in the community is maintained by niche differentiation, we predict species turnover and associated variation in s-AGB along environmental gradients which presumably influence the availability of particular plant species niches.

In this study, we specifically asked: How uneven is the distribution of biomass among co-occurring plant species in grasslands? What is the role of widely distributed and rare (in terms of frequency) species in biomass production in grasslands. How do different functional groups (grasses, sedges and rushes, legumes and other non-legume short and tall forbs) contribute to these frequency distribution classes? Does functional diversity explain biomass inequality among different species? What are the key factors affecting s-AGB? To answer these questions, mown and abandoned species-rich mesic grasslands in the Sudetes Mountains of Central Europe were studied.

2. Methods

2.1. Study area and field sampling

The study was carried out in the Sudetes Mountains in Poland and the Czech Republic (49°54'–51°02'N, 14°30'–17°37'E), an area encompassing approximately 5500 km^2, in an area with elevations ranging from 350 to 950 m above sea level (Fig. 1). The mean annual temperature varies from 6.3°C to 9.3°C (mean 8.3°C) and the annual precipitation ranges from 721 to 1600 mm (mean 982 mm) (Karger et al., 2020). The bedrock consists mostly of acidic and poorly weathered igneous and metamorphic rocks.

Two types of Natura 2000 habitats listed on the EU Habitats Directive Annex I (Council Directive 92/43/EEC) were sampled: lowland hay meadows (habitat code 6510) and mountain hay meadows (habitat code 6520) These corresponded in phytosociological terms to *Arrhenatherion* and *Triseto-Polygonion* alliances, respectively (Chytrý et al., 2007; Kącki et al., 2021; Rodríguez-Rojo et al., 2017). According to the pan-European system for habitat identification, these vegetation types are recognized as low altitude mountain hay meadows and medium altitude mountain hay meadows (Chytrý et al., 2020). The vegetation in question is the type of traditionally managed (mown once or twice per year) and species-rich semi-natural hay meadow typically found on mesic soils.

Sampling was conducted in the years 2018-2019 during the peak of the growing season before the first cut at the end of June and into July. This is the traditional time for the first cut in Central Europe (Mašková et al., 2009). Unlike in other studies, this study utilized a measured weight of individual vascular plant species rather than their cover percentage, as the latter is a subjective estimate of the actual abundance (Chiarucci et al., 1999).

We sampled 28 regularly mown grasslands and 30 abandoned (5-15 years) grasslands. All abandoned sites were in the initial stages of secondary succession, with low and infrequent occurrences of late succession (woody) species (Fig. S-1, Table S-1, Supplementary material). At each grassland site, a 10 × 10 m focal plot was established. Live aboveground biomass (AGB) was measured by clipping individual plant species' shoots at ground level from four quadrat frames measuring 0.4 × 0.4 m (0.64 m^2), which were placed evenly in the four corners of the focal plot (1 m from focal plot borders). Fresh biomass was hand sorted into individual plant species at the site. The four plots combined were considered a sample. Sorted plant material was pre-dried indoors at room temperature for approximately 10 days and then dried in a forced-air circulation drying oven at 60°C for 24 h to a constant weight that facilitated the determination of the dry matter content of individual species per 0.64 m^2. Dead standing biomass and ground litter were excluded from sampling and weight measurements due to the inability to differentiate individual plant species.

This study focused on s-AGB, but it also reported the t-AGB. The t-AGB was expressed as the sum of the s-AGB recorded in each sample (sum of the s-AGB measured from four 0.4 × 0.4 m plots). The s-AGB and t-AGB were reported on a g/m^{-2} basis (Table S-1, Supplementary material). Semi-natural grasslands in the Sudetes Mountains were found to have high variation in s-AGB and t-AGB data (Table S-1, Supplementary material). The t-AGB ranged from 208.4 to 700.0 g m^{-2} (mean 365.6 ± 108.2 SD).

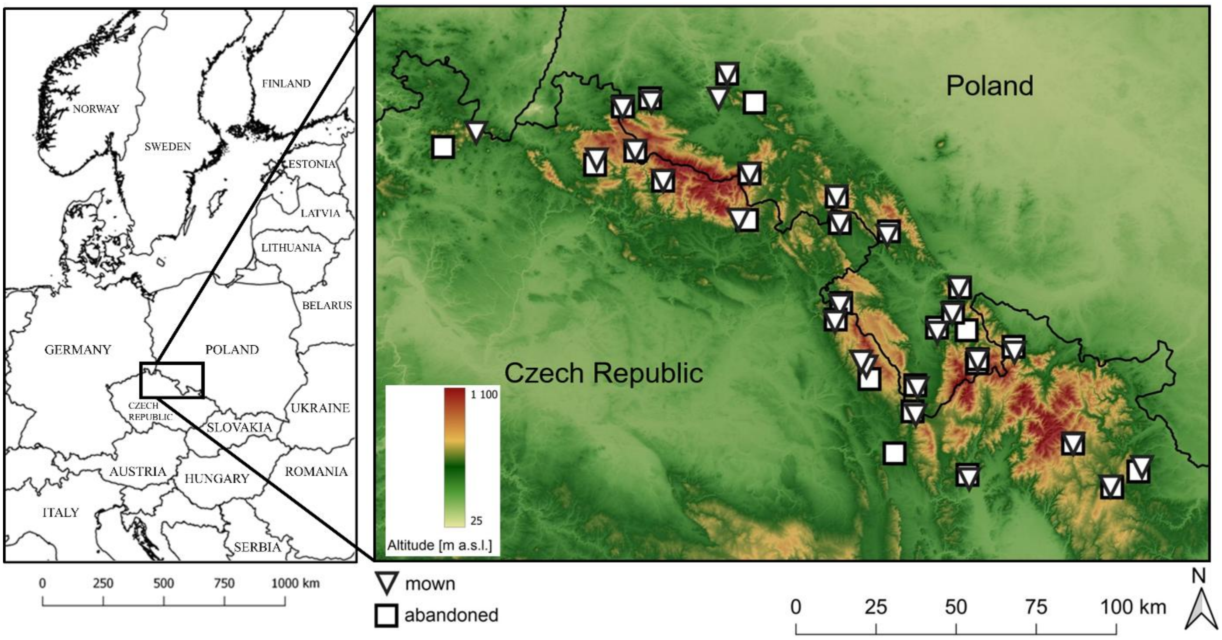

Fig. 1. Distribution of sampling sites in the study area.

2.2. Soil chemical analysis

To determine soil properties, five soil samples were collected from each focal plot using a 10-cm-deep soil core. These samples were then combined into a bulk sample. Soil samples were air-dried, biomass residues and roots were removed, and then the samples were ground in a mortar until the material could pass through a 2 mm sieve. All chemical analyses were performed in an accredited laboratory of the Crop Research Institute in Chomutov, Czech Republic. Plant-available calcium, potassium, magnesium, and phosphorus were extracted by the Mehlich III method (Mehlich, 1984), and concentrations were determined by inductively coupled plasma optical emission spectrometry (GBC Scientific Equipment Pty Ltd., Melbourne, Australia). Determination of pH ($CaCl_2$) was done using a pH meter (Sentron Wellinq, Leek, The Netherlands). Total nitrogen soil concentrations were determined using the Kjeldahl method, and organic carbon concentrations by the conventional oxidation procedure incorporating chromosulfuric acid and colorimetry (AOAC, 1984).

2.3. Statistical analyses

Two groups of species were distinguished based on their frequency distribution in the dataset. The group of frequent species included species with a relative frequency higher than 30%, whereas the group of rare species included species with a relative frequency lower than that threshold. Within these groups, the proportion of different growth forms was analyzed:

grasses (Poaceae), legumes (Fabaceae) and other non-legume short and tall forbs (herbaceous, dicotyledonous flowering plants), sedges and rushes (Cyperaceae and Juncaceae).

The proportional abundance of s-AGB was expressed as the proportional (0–1) ratio of s-AGB to the t-AGB in each sample. The relative proportion of s-AGB was used to determine the cumulative contribution of individual species relative to the t-AGB. The s-AGB data were sorted from highest to lowest values, which were then used to generate the cumulative proportion curve.

Plant biomass inequality was quantified for each sample using the Gini coefficient. The Gini coefficient was used as a measure of mean biomass difference among species at the plant community level. The values of Gini range from 0 to 1. The value zero corresponds to plant communities with perfect biomass equality, whereas a theoretical maximum of one corresponds to plant communities dominated by a single species. Linear regression was used to examine the relationship between the Gini coefficient and Rao’s functional diversity index (Lepš et al., 2006; Rao, 1982). Determination of Rao’s functional diversity index was based on traits including leaf specific area (SLA), plant height at maturity, and the seed mass. Functional trait data were retrieved from the LEDA database (a database on the life-history traits of the Northwest European flora) (Kleyer et al., 2008), and are a subset of a previously used data set by Lengyel et al. (2020). The Rao diversity index was used as an independent variable to explain variation in Gini using the linear regression model. Normality of data was checked with the Shapiro-Wilk test. The Breusch-Pagan test was used to test for heteroskedasticity. In addition, Spearman's rank correlation coefficient was used to express the coexistence patterns of species at the plant community level based on the raw s-AGB data.

Determinants of s-AGB were analyzed using redundancy analysis (RDA), with the ordination axes of RDA corresponding to the dominant gradients in species composition constrained by environmental factors. The raw s-AGB data were transformed using Hellinger transformation (Legendre and Gallagher, 2001). This type of transformation reduces the double-zero problem, which is common with vegetation-plot data, and gives low weights to species with low counts and many zeros. Hellinger-transformed s-AGB data were used as response variables in multivariate analyses. The ordination analyses utilized the set of explanatory variables acquired from direct field measurements and freely accessible databases. It then categorized them into five groups representing climate, topography, land management, soil properties, and vegetation parameters (Table 1). The annual sum of precipitation and the annual mean temperature representing the explanatory group climate were derived from CHELSEA (Climatologies at high resolution for the earth’s land surface areas) at a resolution of approximately 1 km × 1 km (Karger et al., 2020). The group topography included three topography-related indices calculated based on the Digital Elevation Model (EU-DEM) at 25 m resolution (www.eea.europa.eu; accessed on 15 January 2022). The analyses utilized the Topographic Wetness Index (TWI), Diurnal Anisotropic Heat Load (DAH), and Topographic Ruggedness Index (TRI), which all measure factors known to impact vegetation variability in mountainous areas (Moeslund et al., 2013). TWI quantifies terrain driven factors impacting soil water distribution. It was calculated with the MFD-md algorithm (Qin et al., 2011) according to the guidelines of Kopecký et al. (2021). DAH reflects the amount of solar energy potentially reaching the surface of the ground (Hengl and Reuter, 2009). TRI reflects local differentiation of altitude (Hengl and Reuter, 2009). The explanatory group soil included pH, total nitrogen (%), total organic carbon (%) and plant-available potassium, phosphorus, calcium and magnesium (mg kg^{-1}). The explanatory group vegetation parameters were comprised of vegetation cover (%) and ground litter cover (%), which were both estimated visually from the focal plot. For several sites information about time since abandonment was limited or uncertain, and therefore two strikingly different land management types (mown and abandoned) were used as explanatory variables. Only significant and nonlinearly related explanatory variables were selected by applying the forward selection procedure with false discovery rate (FDR) correction of the *p*-values. The forward selection procedure resulted in a parsimonious model that avoided collinearity among significant explanatory variables. The adjusted coefficient of determination (R^2 adj) was used as the measure of the ratio of the explained variation to the total variation in the global model. Permutation tests (with 999 permutations) were used to assess the significance of the relationships found in multivariate analyses.

In addition to the direct ordination analysis, we constructed the t-value biplots for s-AGB in order to test and visualize pairwise relationships between s-AGB and its controlling factor (Braak & Looman, 1994; Šmilauer & Lepš, 2014). The t-value biplot is a statistical technique which is used to approximate the t-value of the regression coefficients of a multiple regression model, where we used species as the response variable and environmental variable as the predictor. In the t-value biplot diagram, the plant species were individually shown by arrows. For each significant explanatory variable, a circle was drawn (known as Van Dobben circles) with its diameter equal to the length of that variable's arrow (here plotted as symbols). The t-value biplots show a positive or negative relationship at <.05 between s-AGB and the explanatory variable, if the t-values of respective regression coefficients were larger than 2 in the absolute value. Response variables with arrows that end in Van Dobben circles had significant relationships (red circle: positive; blue: negative) with particular explanatory variables. The shorter the arrow is for species within the circle in the t-value biplot diagram, the stronger the relationship with the explanatory variable.

Data were analyzed using R statistical software (http://www.r-project.org) with the "car", "DescTools", "dplyr", "ggpubr", and "vegan" packages. Ordination analyses were performed in CANOCO 5 (Šmilauer & Lepš, 2014). The nomenclature of taxa follows Euro+Med PlantBase (http://ww2.bgbm.org/EuroPlusMed/; accessed on 10 January 2022).

Table 1. Summary of explanatory variables employed in RDA.

Explanatory variables	min-max	Mean ± SD
Climate		
Temperature (°C)	6.3–9.3	8.3 ± 0.8
Precipitation (mm)	721–1600	982 ± 216
Topography		
DAH	-0.25–0.26	0.02 ± 0.12
TWI	9.0–15.0	11.3 ± 1.4
TRI	0.6–8.5	2.9 ± 1.6
Soil properties		
pH	3.4–6.9	4.7± 0.7
C (%)	3.0–17.4	5.3 ± 2.5
N (%)	0.3–1.4	0.5± 0.2
P mg kg^{-1}	8.6–238.9	60.8± 40.6
K mg kg^{-1}	113.9–705.9	233.8± 114.6
Ca mg kg^{-1}	311.3–7427.8	1729.3± 1370.5
Mg mg kg^{-1}	49.7–778.4	217.9 ± 131.7
Vegetation parameters		
Herb layer (%)	40.0–100.0	86.7± 13.1
Ground litter layer (%)	0.0–95.0	47.5 ± 37

Explanation: DAH – Diurnal Anisotropic Heat Load; TWI – Topographic Wetness Index; TRI – Topographic Ruggedness Index

3. Results

3.1. Contributions of species and species groups to the aboveground biomass

A total of 165 plant species were identified in biomass samples. This included 27 species of grasses, 85 species of short and 27 species of tall forbs, 16 species of legumes, and 10 species of sedges and rushes. A total of 28 species were frequent (species with a relative frequency >30%), and combined formed 79% of the t-AGB, on average (Table 2, Fig. 2). Almost a half of these species (46%) were grasses, 29% were short forbs, 14% were legumes, 7% were tall forbs, and 4% were sedges and rushes. A total of 137 species were rare (species with a relative frequency <30%), and combined formed 21% of the t-AGB, on average. More than half of these species were short forbs (56%), whereas grasses were only 10% of the species pool, 18% were tall forbs, 9% were legumes, and 7% were sedges and rushes.

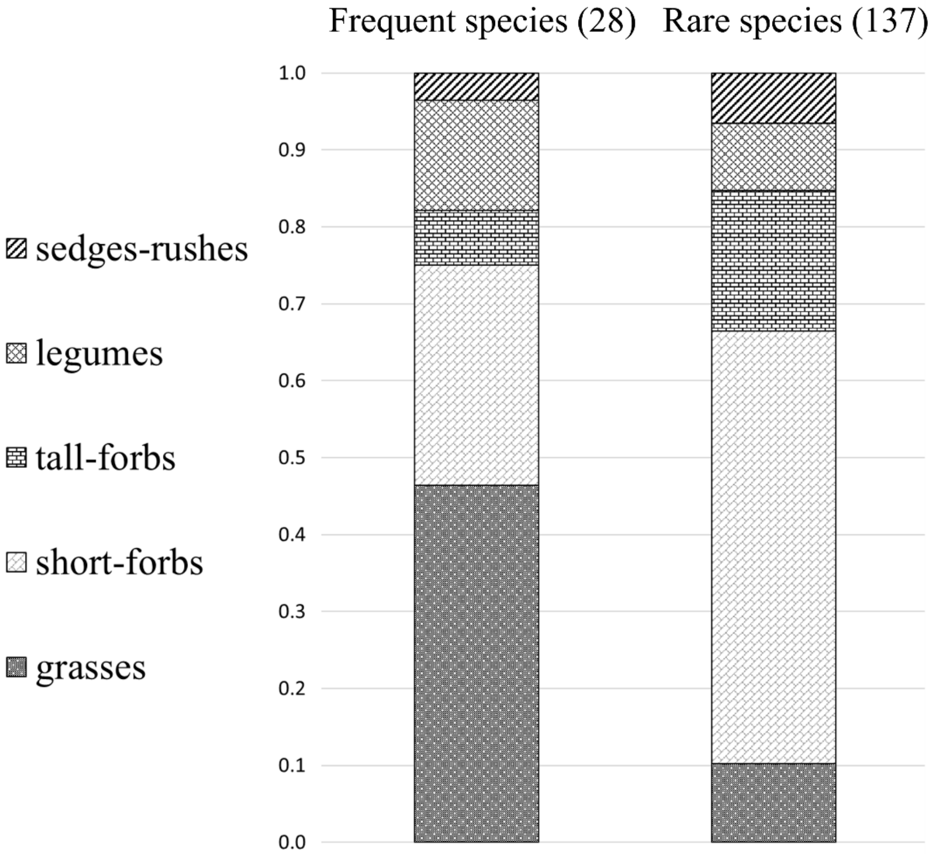

Fig. 2. Proportion of growth forms within the groups of frequent and rare species.

Table. 2. Summary of the most frequent species (>30%) and their correlations with other species. The five most productive species are underlined.

Species	Relative frequency (%)	Relative proportion ± SD	Strongest positive correlation (Spearman at p < .05)	Strongest negative correlation (Spearman at p < .05)
grass species				
<u>*Agrostis capillaris*</u>	84	10.8 ± 0.1	*Festuca rubra* (.55)	*Arrhenatherum elatius* (-.41)
<u>*Alopecurus pratensis*</u>	52	6.6 ± 0.2	*Trisetum flavescens* (.35)	*Campanula patula* (-32)
Anthoxanthum odoratum	45	0.4 ± 0.0	*Plantago lanceolata* (.47)	*Arrhenatherum elatius* (-.29)
<u>*Arrhenatherum elatius*</u>	72	8.7 ± 0.1	*Galium mollugo* (.45)	*Plantago lanceolata* (-.48)
<u>*Dactylis glomerata*</u>	76	6.0 ± 0.1	*Schedonorus pratensis* (.50)	*Potentilla erecta* (-.40)
Elytrigia repens	43	1.7 ± 0.0	*Phleum pratense* (.48)	*Geranium sylvaticum* (-.35)
<u>*Festuca rubra*</u>	95	17.1 ± 0.2	*Agrostis capillaris* (.55)	*Arrhenatherum elatius* (-.42)
Holcus lanatus	40	0.5 ± 0.0	*Achillea millefolium* (.50)	*Geranium sylvaticum* (-.33)
Holcus mollis	53	3.3 ± 0.1	*Hypericum maculatum* (.57)	*Schedonorus pratensis* (-.40)
Phleum pratense	47	1.4 ± 0.0	*Elytrigia repens* (.48)	-
Poa pratensis	88	1.8 ± 0.0	*Equisetum arvense* (.29)	*Leucanthemum vulgare* (-.44)
Schedonorus pratensis	47	1.9 ± 0.0	*Trisetum flavescens* (.62)	*Hypericum maculatum* (-.45)
Trisetum flavescens	62	1.9 ± 0.0	*Schedonorus pratensis* (.62)	*Holcus mollis* (-.40)
short-forbs				
Achillea millefolium	69	1.3 ± 0.0	*Holcus lanatus* (.50)	*Cirsium helenioides* (-.27)

Alchemilla monticola	47	0.6 ± 0.0	*Ranunculus acris* (.61)	*Arrhenatherum elatius* (-.30)
Galium mollugo	62	3.5 ± 0.1	*Arrhenatherum elatius* (.45)	*Scorzoneroides autumnalis* (-.36)
Plantago lanceolata	41	2.2 ± 0.1	*Trifolium repens* (.66)	*Arrhenatherum elatius* (-.48)
Ranunculus acris	41	0.5 ± 0.0	*Plantago lanceolata* (.63)	*Galium mollugo* (.33)
Rumex acetosa	74	0.2 ± 0.0	*Plantago lanceolata* (.50)	*Knautia arvensis* (-.42)
Stellaria graminea	45	0.3 ± 0.0	*Leucanthemum vulgare* (.40)	*Tragopogon pratensis* (-.29)
Veronica chamaedrys	88	1.8 ± 0.0	*Ranunculus acris* (.48)	-
tall-forbs				
Heracleum sphondylium	31	0.3 ± 0.0	*Angelica sylvestris* (.42)	*Leontodon hispidus* (-.28)
Hypericum maculatum	59	3.3 ± 0.1	*Holcus mollis* (.57)	*Schedonorus pratensis* (-.45)
legumes				
Lathyrus pratensis	50	1.4 ± 0.0	*Galium mollugo* (.40)	-
Trifolium repens	40	0.6 ± 0.0	*Trifolium pratense* (.72)	*Arrhenatherum elatius* (-.38)
Vicia cracca	53	0.4 ± 0.0	*Vicia sepium* (.38)	*Avenella flexuosa* (-.27)
Vicia sepium	36	0.2 ± 0.0	*Festuca rubra* (.45)	*Festuca rubra* (-.32)
sedges and rushes				
Luzula multiflora	31	0.3 ± 0.0	*Anthoxanthum odoratum* (.46)	*Dactylis glomerata* (-.31)

Species with low frequency including 14 species of grasses, 25 species of tall-forbs, 74 species of short-forbs, 12 species of legumes, 9 species of sedges and rushes, and 3 other species are not shown.

Species with the highest relative proportion to the t-AGB (≥5%) were *Festuca rubra* (17.1%), followed by *Agrostis capillaris* (10.8%), *Arrhenatherum elatius* (8.7%), *Alopecurus pratensis* (6.6%) and *Dactylis glomerata* (6.0%). These grass species dominated in biomass in both land management categories (Table S-1, Supplementary material). They formed approximately half (49.2 %) of the t-AGB. The remaining 50.8% of the t-AGB was dependent on 160 species. A total of 15 species with the relative proportion to the t-AGB <5% and ≥1% formed 29.6% of the t-AGB. The majority of species (145 out of 165) each accounted for less than 1% of the t-AGB, on average. The summed s-AGB of these species formed on average 21.2% of the t-AGB (Fig. 3). The distribution of s-AGB proportions was strikingly similar for both land management categories (Table S-2, Supplementary material).

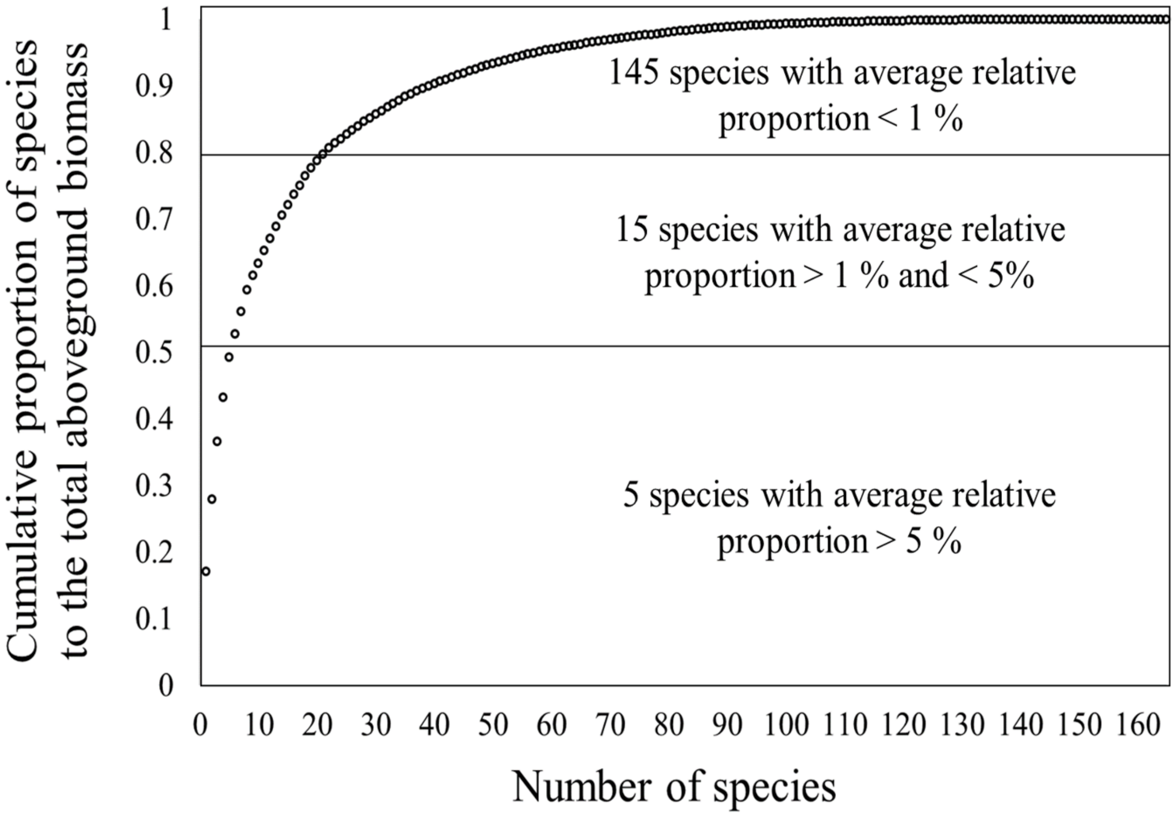

Fig. 3. The cumulative proportion of s-AGB to the t-AGB. See Table S-2 (Supplementary material) for details.

The Gini values ranged from moderately high (0.53) to very high (0.87), with the mean equal to 0.71 (± 0.08 SD). Linear regression showed a negative significant correlation between biomass inequality among coexisting species (Gini) and functional diversity (Rao) (Fig. 4). The inequality in biomass distribution among species decreased with increasing functional diversity of the community (R^2=0.2; p<.001). Also, a number of pairwise correlations were found between some s-AGB (Table 2, Table S-3, Supplementary material).

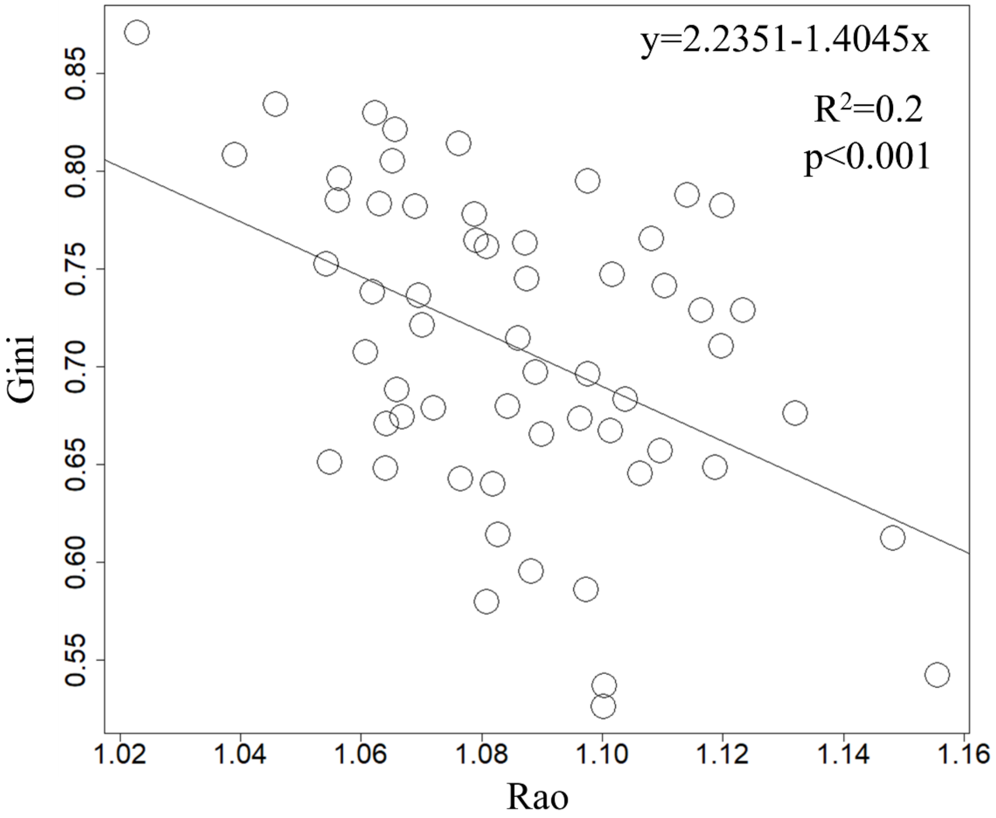

Fig. 4. Correlation between functional diversity (Rao) and biomass inequality among species (Gini).

3.2. Species responses to environmental and management factors

Explanatory variables explained 22.1% (R2 adj 16.3%) of the total variation in s-AGB data (Fig. 5A, B). The impact of four explanatory variables on s-AGB was found significant after the application of forward selection and the FDR correction of the *p*-values. The most influential variable on s-AGB data was pH (39.8% of the total variation explained; pseudo-F = 5.4; p(adj) = .003). Land management contributed 31.2% (pseudo-F = 4.5; p(adj) = .004), temperature 16.7% (pseudo-F=2.5; p(adj) = .009), and precipitation 12.2% (pseudo-F=2.5; p(adj) = .017) to the total variation explained. None of the explanatory variables from the explanatory group topography, nor vegetation parameters, were selected in the stepwise selection of variables.

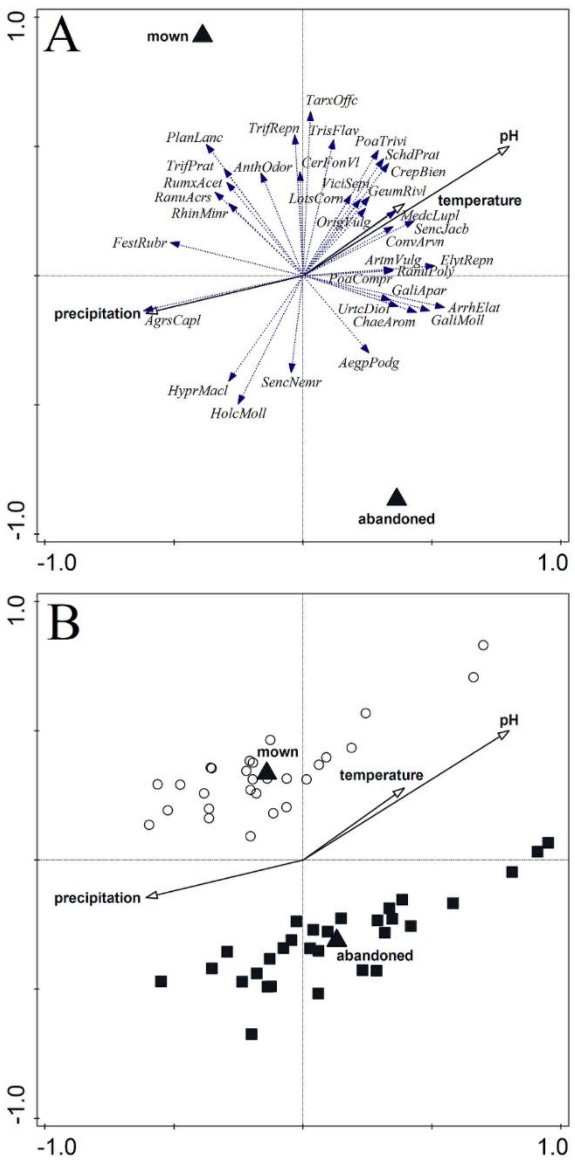

Fig. 5. The redundancy analysis (RDA) of s-AGB data. Significant and non-collinear explanatory variables (solid arrows and filled triangles) were plotted. The RDA diagram with 35 best-fitted species (dotted arrows) (A) and ordination of plots representing mown (open circles) and abandoned grasslands (filled squares) (B) are shown. Constrained RDA axis 1 (horizontal) explained 11.1% of the total variation in s-AGB data, and RDA axis 2 (vertical) explained 6.1% of the total variation in s-AGB data. Species abbrevations: AegpPodg – *Aegopodium podagraria*; *AgrsCapl* – *Agrostis capillaris*; *AnthOdor* – *Anthoxanthum odoratum*; *ArrhElat* – *Arrhenatherum elatius*; *ArtmVulg* – *Artemisia vulgaris*; *CerFonVl* – *Cerastium fontanum* subsp. *vulgare*; *ChaeArom* – *Chaerophyllum aromaticum*; *ConvArvn* – *Convolvulus arvensis*; *CrepBien* – *Crepis biennis*; *ElytRepn* – *Elytrigia repens*; *FestRubr* – *Festuca rubra*; *GaliApar* – *Galium aparine*; *GaliMoll* – *Galium mollugo*; *GeumRivl* – *Geum*

rivale; *HolcMoll – Holcus mollis*; *HyprMacl – Hypericum maculatum*; *LotsCorn – Lotus corniculatus*; *MedcLupl – Medicago lupulina*; *OrigVulg – Origanum vulgare*; *PlanLanc – Plantago lanceolata*; *PoaCompr – Poa compressa*; *PoaTrivi – Poa trivialis*; *RanuAcrs – Ranunculus acris*; *RanuPoly – Ranunculus polyanthemos*; *RhinMinr – Rhinanthus minor*; *RumxAcet – Rumex acetosa*; *SchdPrat – Schedonorus pratensis*; *SencJacb – Senecio jacobaea*; *SencNemr – Senecio nemorensis*; *TarxOffc – Taraxacum officinale*; *TrifPrat – Trifolium pratense*; *TrifRepn – Trifolium repens*; *TrisFlav – Trisetum flavescens*; *UrtcDioi – Urtica dioica*; *ViciSepi – Vicia sepium*.

The t-value biplots showed positive and negative correlations of s-AGB to particular explanatory variables (Fig. 6A–D). The highest number of correlations was found for soil pH and land management. Increasing pH was positively related to the grasses (*A. elatius, E. repens, P. compressa, P. trivialis, S. pratensis,* and *T. flavescens*), the short and tall forbs (*A. vulgaris, C. arvensis, C. aromaticum, C. biennis, G. mollugo, G. rivale, O. vulgare, R. polyanthemos, S. jacobea,* and *T. officinale*), and the legumes (*M. lupulina* and *V. sepium*). Several grass species (*A. capillaris*, *F. rubra*, and *H. mollis*) and a tall forb (*H. maculatum*) were negatively correlated with increasing soil pH. Twice as many species were found positively correlated with the mown grasslands than with abandoned ones. A positive correlation to mowing management was found for the grasses (*A. odoratum, F. rubra, P. trivialis, S. pratensis,* and *T. flavescens*), the short forbs (*C. fontanum* subsp. *vulgare, C. biennis, P. lanceolata, R. acris, R. minor, R. acetosa,* and *T. officinale*), and the legumes (*T. pratense* and *T. repens*). A negative relationship between s-AGB and mown grasslands (positive with abandonment) was found for the grasses (*A. elatius* and *H. mollis*) and the tall forbs (*A. podagraria, C. aromaticum, G. mollugo, S. nemorensis,* and *U. dioica*). Increasing precipitation was positively correlated with *A. capillaris*. There were no significant relationships found for s-AGB and precipitation.

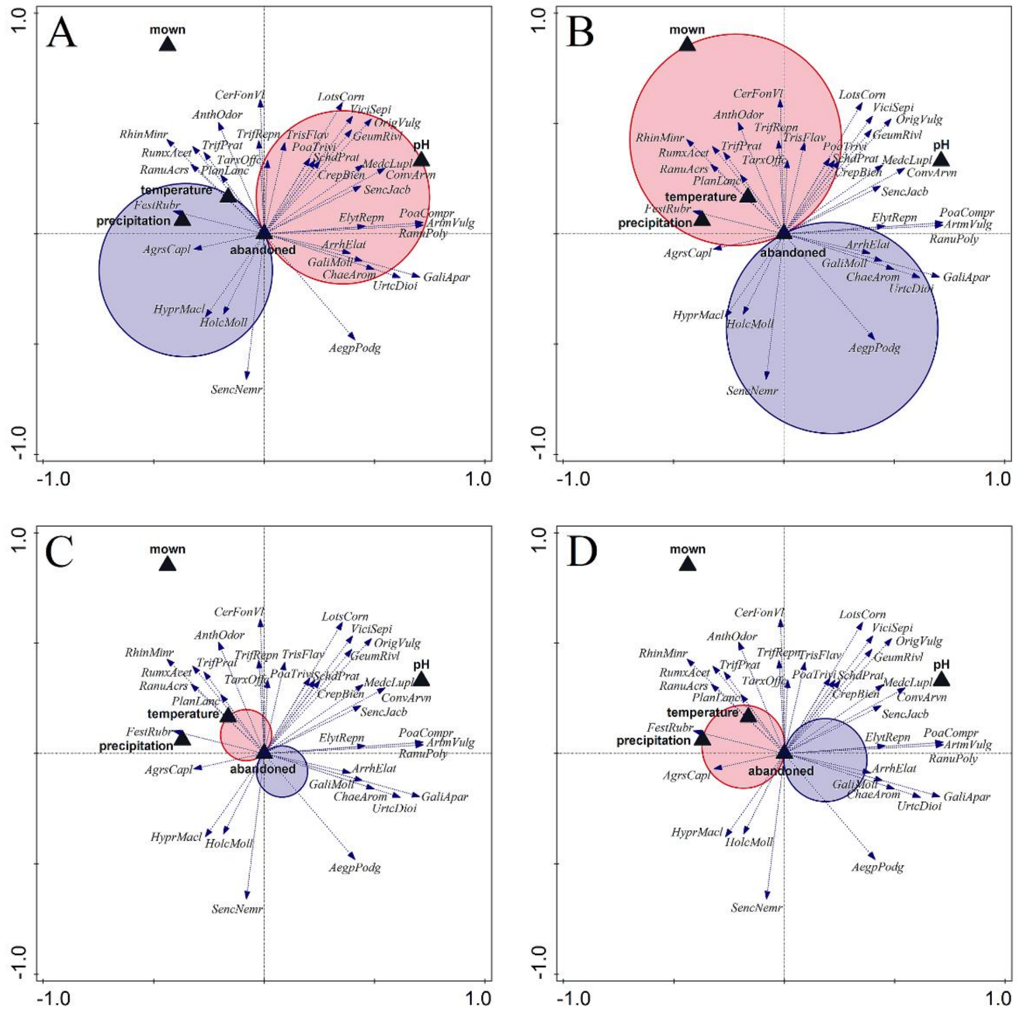

Fig. 6. T-value biplots of redundancy analysis (RDA) with Van Dobben circles drawn for pairwise relationships between s-AGB data and explanatory variables pH (A), land management (B), temperature (C), and precipitation (D). The s-AGB data (response variables) are displayed as dashed arrows. Explanatory variables are displayed as triangles. See Fig. 5 for species abbreviations.

4. Discussion

4.1. Contributions of species and species groups to the aboveground biomass

This study found a differing relative contribution of common and rare species to the t-AGB and high biomass inequality between coexisting species; as expected, it shows that widely distributed plant species are indeed most important in terms of biomass production in semi-natural grasslands, but the role of rare species should not be underestimated. Worth noting is that rare species constituted about three-fourths of all species recorded in this study. This study and others have shown that various forbs (also legumes) contribute significantly to grassland biomass and are indicative of high diversity in grassland communities (Li et al., 2016; Pokorny et al., 2004). It has been postulated that a variety of forbs is largely responsible for the taxonomic, phylogenetic, and functional diversity of grassland biome (Bråthen et al., 2021) and are crucial for the diversity of pollinators (Biesmeijer et al., 2006). Forbs contribute greatly to the richness of grass-dominated ecosystems worldwide across a wide range of climate regimes, including, for instance, prairies in North America; savannas in Africa; temperate European grasslands; Mongolian steppes; pampas, campos and cerrados in South America (see Table 1A, Supplementary material; Bråthen et al., 2021). Soliveres et al. (2016) underlined that rare species are the most important component of diversity because these species ensure ecosystem multifunctionality. Moreover, Schaub et al. (2020) underlined the importance of species-rich grasslands in forage production since high species diversity is positively correlated with increased biomass yields at rather constant forage quality. The balance between preserving high plant diversity and profitable yields in semi-natural grasslands should be prioritized in modern agriculture and nature conservation policies.

The relatively high values of the Gini coefficient found in the studied grasslands indicate an overall high biomass inequality among coexisting species. Biomass inequality can be ascribed to the five most widespread and most abundant grass species, which constituted on average about 49.2% of the t-AGB. All of them are widespread grassland species in Western and Central Europe; thus, their dominance in grassland biomass can be expected in the entire range of plant communities they form (Preislerová et al., 2022; Rodríguez-Rojo et al., 2017). The fact that grasses were found to have the highest relative proportion to the t-AGB is not surprising since this group of species is evolutionarily best adapted to open landscape habitats with continuous disturbance (Linder et al., 2018; Strömberg, 2011). According to the mass ratio hypothesis, ecosystem functioning is predominantly determined by the dominant species and their functional traits (Grime, 1998). However, it is equally important to highlight that nearly a half of the t-AGB was dependent on 160 species. Among them, there were 145 species with a very low relative proportion to the t-AGB (<1%). Non-dominant species can be of different sizes and have various functional traits and life strategies, and varying abilities to compete for resources. This study shows that the inequality in biomass distribution among species decreases with increasing functional diversity of the community. The distinctions in resource use between different plants (high niche differentiation) ensures the coexistence of less competitive species with the dominants by the variety and complementarity of species' functional traits (Hector et al., 1999; Jiang et al., 2020; Maire et al., 2012; Padullés Cubino et al., 2022; Tilman et al., 1997). The result of this study supports the belief that mass ratio hypothesis and niche complementarity in semi-natural grasslands are complementary to each other rather than mutually exclusive (Diaz et al., 2007; Maire et al., 2012; Mouillot et al., 2011). The complementarity of the mass ratio hypothesis and niche complementarity is also well supported by a number of pairwise positive correlations identified between dominant and non-dominant species (Table 2, Table S-3, Supplementary material). In contrast, many interactions between dominants and weak competitors could not be detected due to stochasticity in species distribution being affected by dispersal, environmental filtering, colonization, and successful competition.

4.2. Species responses to environmental and management factors

The highest portion of the variation in the s-AGB data was explained by the soil pH gradient. Soil pH has been indicated as one of the most important predictors of plant species composition in multiple studies based on species coverage data from wet grasslands (Hájek and Hájková, 2004; Zelnik and Čarni, 2008), dry mesic grasslands (Dvořáková et al., 2014), acidic grasslands (Stevens et al., 2011), and various different grassland types (Merunková and Chytrý, 2012). This study fills the gap of knowledge related to the effect of pH on s-AGB. In mountainous areas, changes in topographical features heavily impact pH, which is reflected in vegetation composition (Furley, 1974b, 1974a). The soil pH is typically lower on steep convex slopes exposed to weathering and higher in flat and concave areas (Merunková and Chytrý, 2012). In this study, the pH gradient was very wide, with a predominance of strongly acidic soils (47% of samples had pH of <4.5). The soil pH was negatively correlated with increasing precipitation, which positively correlated with increasing altitude, as there is a strong linear relationship between these factors in the Sudetes Mountains. The study found that there is a strong turnover in species composition along the pH gradient, and many more species correlated positively rather than negatively with increasing soil pH. Species with a negative correlation to pH (positive correlation to low pH), including *A. capillaris*, *F. rubra*, *H. mollis*, and *H. maculatum*, are diagnostic for nutrient-poor habitats on acidic bedrock (Chytrý et al., 2007; Kącki et al., 2021). These species are the most important in terms of productivity at higher elevations, possibly due to their physiological adaptations related to acidification. Alternatively, species with a positive correlation to pH are mostly lowland species that also thrive in low altitude grasslands in more nutrient-rich and less acidic habitats (Chytrý et al., 2007; Kącki et al., 2021). The limited abundance of these species in low pH habitats could be related to high phytotoxicity and nutrient deficiency (Tyler, 1996).

Land use management was found to be the second most important explanatory variable, and it accounted for about one-third of the variation explained in s-AGB. Short period of time since abandonment did not have as strong an effect as soil chemistry (pH in this case). With further successional changes it can probably be expected that abandonment will have an overriding role over the soil properties on the community structure and composition.

This and other studies documented a positive relationship between nitrogen-fixing legumes (here *T. pratense* and *T. repens*) and mowing management, while negative with abandonment (Ribeiro et al., 2014; Rudmann-Maurer et al., 2008). These results may indicate the low competitive ability of legumes in relation to the increasing dominance of strong competitors such as clonal grasses and tall forbs in the absence of mowing. This study also observed that mowing facilitates the development of less competitive plant species, especially low-productive short forbs, which are the core of the diversity in semi-natural grasslands (Pykälä et al., 2005). The results presented here show that the dominance of indigenous species may persist despite the cessation of mowing over a period of 5-15 years. Lepš (2004) highlighted that the high stability of dominant species is a property of semi-natural plant communities that have a naturally developed dominance structure. A similar role was found for less abundant species (Lyons & Schwartz, 2001). Pokorny et al. (2005) found that the dominance of indigenous species and their maximized niche complementarity is important in invasion resistance This could explain why alien species common in the Sudetes Mountains such as *Solidago gigantea*, *S. canadensis* and *Lupinus polyphyllus* (Czarniecka-Wiera et al., 2019) were poorly represented in abandoned grasslands (Table S-1, Supplementary material). Expansive species with high competitive abilities produce high amounts of biomass that can considerably exceed the productivity of non-invaded grassland ecosystems (McLeod et al., 2016; Teixeira et al., 2020). Abandonment of semi-natural grasslands is also often followed by the expansion of native tall forbs (Galvánek and Lepš, 2012; L. Pavlů et al., 2013), which was also found to be the case in this study (*A. podagraria*, *C. aromaticum*, *G. mollugo*, *S. nemorensis* and *U. dioica*). These species have their optimum in nutrient-rich and species-poor fringe communities. The progressive expansion of these species may lead to the exclusion of species with life-history strategies developed under mowing and grazing management.

Nutrient levels have been previously reported as one of the major determinants of species composition (Hejcman et al., 2007; Pavlů et al., 2022; Titěra et al., 2020) and productivity in grasslands with indications that phosphorus and nitrogen are limiting factors (Hejcman et al., 2010; Pavlů et al., 2013). This study found a wide range of nutrient levels, and therefore it is very difficult to generalize their effect on s-AGB. The lack of a unitary effect from nutrients could also be influenced by the predominant role of pH, which determines nutrient availability to plants (Heyburn et al., 2017; Tian et al., 2017).

The gradient in climatic variables is heavily dependent on altitude and mirrors that of pH. The significant response of s-AGB is the result of a wide gradient in mean annual temperature (6.3°C–9.6°C) and precipitation (721–1600 mm), but as individual variables both were weaker predictors of s-AGB than pH and land use management. This is reflected by a small number of correlations of individual species with these controlling variables. Two climatic variables together accounted for almost a third of the total variation explained in s-AGB data. The most pronounced effect of climatic factors on productivity was observed in macroecological studies carried out analyzing macro-climatic continentality and altitudinal gradients (Ni, 2004; Yang et al., 2009). Neither topography-related nor vegetation parameter variables were selectively significant in the model. The lack of any significant impact due to topography is most likely due to the wide and overlapping environmental requirements of mesic grassland species in relation to water availability (TWI), thermal conditions (DAH), and heterogeneity of the landscape (TRI).

5. Conclusions

From these findings it can be concluded that semi-natural grasslands consist of a number of species with a differing relative contribution to the total aboveground biomass. It was found that just a few grass species made up half of the total biomass, on average. However, less frequent and less abundant species (especially short forbs) combined to play an important role in the production of biomass. What is crucial is that they are the core of diversity in semi-natural grasslands. This study showed overall high biomass inequality among coexisting species, and that it is decreasing with increasing functional diversity of the community. These findings support the mass ratio hypothesis on one hand, but on the other hand they highlight the essential role of niche differentiation which ensures the coexistence of less competitive species with the dominants by the variety and complementarity of functional traits. This study identified pH as the most important predictor of s-AGB, followed by land management (mowing versus abandoned) and climatic explanatory variables. Soil and climatic conditions are important environmental filters that shape species composition in plant communities. However, mowing (or other disturbance regimes such as grazing) is essential for the conservation of high species and functional diversity in semi-natural grasslands. Regular biomass removal suppresses competitor species, thus preventing the competitive exclusion of multiple subordinate species which are an important component of grasslands productivity and diversity. The maintenance of species diversity in grasslands should be prioritized in nature conservation policies to ensure the sustainability of ecosystem functioning and services.

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Chapter III

Response of grassland vegetation composition to different fertilizer treatments recorded over ten years following 64 years of fertilizer applications in the Rengen Grassland Experiment

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Abstract

Questions: Fertilizer application is a key driver affecting the diversity and conservation value grassland vegetation. Using a long-term fertilization experiment in mountain grassland we addressed the following questions: i) what is the effect of long-term fertilizer applications on species richness and plant species composition, and ii) is there any detectable trend in plant species composition during ten years of continuous observation?

Location: the Rengen Grassland Experiment, Eifel Mountains, Germany (established in 1941).

Methods: Five treatments including different fertilizers applied annually and one unfertilized control were analysed: Ca (718 kg Ca ha^{-1}); CaN (752 Ca and 100 N kg ha^{-1}); CaNP (752 Ca, 100 N and 35 P kg ha^{-1}); CaNP-KCl (752 Ca, 100 N, 35 P and 133 K kg ha^{-1}); CaNP-K_2SO_4 (752 Ca, 100 N, 35 P and 133 K kg ha^{-1}). The experiment included five replicates per treatment in a fully randomised block design. All treatments were cut twice a year in late June or early July, and in mid-October. Percentage cover of individual plant species was estimated by visual observation in each plot in late June in the years 2005-2014.

Results and discussion: Despite inter-annual variability in the cover of the individual vascular plant species, the multivariate data analyses revealed relatively similar response of the plant community to the different fertilizer applications throughout the ten years. No differences in botanical composition among treatments with phosphorus application were found; however, they differed from other treatments without phosphorus application. In the unfertilized control there was a certain directed trend in plant species composition in response to ongoing nutrient impoverishment.

Conclusion: Species rich grasslands of high nature conservation value were only maintained under P-limitation in the control without fertilizer application (*Violion caninae*), in the liming treatment (*Polygono-Trisetion*), but also in the treatment with liming and pure N addition. It seems that some stage of equilibrium of grassland community was achieved in all treatments receiving any type of fertilizer application after 74 years, but less so in the unfertilized control treatment.

Keywords: sward, botanical composition, species richness, inter-annual variability, fertilizer applications, vegetation equilibrium, long-term experiment, grassland

Introduction

Fertilizer applications on grassland generally lead to increased biomass production, and to changes in botanical composition in favour of highly productive plants thereby resulting in a decrease of less-competitive species and overall species richness (e.g. Hejcman, Češková, Schellberg, & Pätzold, 2010; Humbert, Dwyeri, Andrey, & Arlettaz, 2016; Plantureux, Peeters, & McCracken, 2005; Silvertown et al., 2006). To determine the long-term effects of applications of fertilizers on vegetation requires long-term experiments (more than ten years), because only long-term data series can reveal succession of the community and fluctuations of individual species in the sward. Long-term data sets are also valuable for the testing of hypotheses and for prediction of future changes (Bakker, Poschlod, Strykstra, Bekker & Thompson, 1996).

Many authors have previously investigated the long-term effects of fertilization on grassland, but studies have often been based on experiments of limited duration, or with interrupted observations (e.g. Crawley et al., 2005; Hejcman et al., 2014; Kidd, Manning, Simkin, Paecock, & Stockdale, 2017) or with limitations on replication (e.g. Silvertown et al., 2006). Long-term studies require a commitment from research funders to maintain experimental sites and support the costs of data collection.

It is well documented that long-term fertilizer application in combination with a repeated defoliation management regime can lead to vegetation equilibrium, although this may take decades to be reached (Dodd, Silvertown, McConway, Potts, & Crawley, 1994; Silvertown, 1980). Vegetation equilibrium can be defined as species co-existence based on an ability of plant species to use different parts of the resource gradient by diverse phenology, ability to use light and rooting depth of plants (Lepš, 2013). The number and relative abundances of plant species in a community at equilibrium aredetermined by compensation dynamics, as competition for nutrients resources and irradiance penetrating the canopy (Tilman, 1982). Further, the equilibrium in guild composition is a dynamic one, continually perturbed by climate (Silvertown, 2006).

This study was performed on the Rengen Grassland Experiment (RGE) in the Eifel Mountains (Germany). It was established in 1941 and nowadays it is one of the oldest continuously managed long-term experiments on permanent grassland in mainland Europe. The first detailed botanical evaluation at RGE recorded in the year 1999 (Schellberg, Möseler, Kühbauch, & Rademacher, 1999) revealed significant effects of different fertilizer applications, not only on botanical composition (Hejcman, Klaudisová, Schellberg, &

Honsová, 2007) and on plant and soil chemical properties (Hejcman et al., 2010), but also on the type of phytocenological grassland community (Chytrý, Hejcman, Hennekens, & Schellberg, 2009). The multivariate data analysis (Redundancy Analysis) revealed a strong explanatory power (> 60 %) of fertilizer treatments (Hejcman et al., 2007) and of soil nutrient status (Hejcman et al., 2010) on plant species composition. Further, individual responses of plant species composition reflected the soil nutrient status, especially nutrient (N, P, K) limitations, and presumably an ongoing depletion of plant available P that prevented the realization of vegetation equilibrium in the treatments without P application (Hejcman et al., 2010). However, this result was based on one-year data observation only, as there were no data from longer periods to supporting this part of the investigation. In this context, this study aimed at appraising the effect of 74 years of long-term fertilizer applications on grassland community based on assessments over a 10-year period. The study attempted to address the following research questions: (i) what was the effect of long-term different fertilizer applications on species richness and plant species composition during ten years of observation and (ii) was there any detectable trend in species richness and plant species composition during the ten years of observation ?

Materials and methods

Study site

The fertilizer experiment was set up on the Rengen Grassland Research Station of the University of Bonn in the Eifel mountains (Germany, 50°13′ N, 6°51′ E; elevation 475 m above sea level) in 1941 (for details see (Schellberg et al., 1999)). Mean annual precipitation is 811 mm and mean annual temperature is 6.9 °C (Rengen meteorological station). The soil is classified as a Stagnic Cambisol. Mean chemical properties of soils for each treatment in 2015 are presented in Table 1.

Experimental design

The experiment is arranged in a complete randomised block design with five treatments and five replications with an individual plot size of 3 m × 5 m. The treatments are: A) zero fertilizer treatment as a control; B) calcium (Ca) application; C) Ca and nitrogen (N) application; D) Ca, N, and phosphorus (P) application; E) Ca, N, P and potassium chloride (KCl) application; and F) Ca, N, P and potassium sulphate (K_2SO_4) application. For a detailed description of type of applied fertilizer with the amounts of nutrients see Table 1. The control treatment (A) was introduced in 1998 in the vicinity of the experiment at a place that remained unfertilized but which had been mown together with all other plots since 1941. The experimental plots were mown and aboveground grassland biomass removed once a year in late summer from 1942 to 1944 and from 1950 to 1961. In the years 1945 – 1949, the experiment was temporarily abandoned without cutting or fertilization (Schellberg et al., 1999). All treatments in the experimental area have been mown twice per year (in late June/early July and in October) since 1962 (Schellberg et al., 1999). In response to different nutrient supplies due to the varying fertilizer applications, different grassland vegetation types (Chytrý et al., 2009) with different biomass productions developed during the period of the experiment (Table 2).

Data collection

The percentage cover (%) of all vascular plant species was visually estimated every year in June in the centre (1.8 m × 3.2 m) of each plot (Hejcman et al., 2007) by the same research group during the ten years of data collection (2005-2014). To avoid operator bias in the data observation, the first replicate of each fertilizer treatment was assessed by all of the four scientists in the research group, followed by the other plots each of which were monitored by a team of two. Nomenclature of vascular plant species follows the regional flora (Rothmahler, Jager, & Werner, 2000). Plant species diversity was evaluated by plant species richness, and Shannon and Simpsons species diversity indices (Begon, Townsend & Harper, 2005).

Data analysis

A linear mixed-effects model (GLM) with fixed effects of treatment, time (factor) and their interaction and random effect of replication was used to evaluate the cover of the most abundant vascular plant species and species richness. If necessary, data were log-transformed to meet ANOVA assumptions. For all univariate statistical analyses, software STATISTICA 13.2 was used (Dell Inc., Texas, 2016).

The programme CANOCO 5 was used for the evaluation of multivariate data. A redundancy analysis (RDA) was applied as the length of gradient was 3.2 SD (standard deviation) units. Species cover (%) data were logarithmically transformed [$y = \log(y + 1)$] for the purpose of RDA. Further, a Monte Carlo permutation test with 999 permutations in hierarchical design was used to reveal if the tested explanatory variables (environmental variables in this case) had a significant effect on plant species composition. Split-plot permutations in each plot during ten years were not used (independent across the whole plot). Whole-plot permutations were used as freely exchangeable. Year as a factor was used as covariate in all analyses. The effect of treatment was used as an explanatory variable. Results of the multivariate analysis were visualized in the form of a bi-plot ordination diagram.

As part of the RDA analysis, figures of species response curves for all individual treatments during the years of observation were created. Year was used as an explanatory variable (quantitative) and replications were used as covariates. Setting was the same as for RDA analyses.

Principal response curves (PRC) were used to analyse principal components of the treatment effects against time, expressing the treatment effects as deviations from the control treatment (reference coding). A year was used as a covariate and interaction treatment*year was used as explanatory variable. Set of hierarchical design was used by the same way as in the case of RDA. Set of species weights shown on the right side of the diagram was associated with each PRC.

The effect of treatment in a particular year (2005 – 2014) on plant species composition was evaluated by redundancy analysis (RDA) with 999 unrestricted permutations, where blocks were defined as a covariate.

PCA analysis as part of unconstrained multivariate statistical method was used to illustrate differences between treatment responses within individual years (2005-2014), where the effect of treatment was used as a supplementary variable.

To see if there is some type of temporal trend within individual treatments, variability partitioning as part of RDA was counted from sum of all eigenvalues to identify residual variability in vegetation data.

Results

Species richness and plant species composition

Throughout the study period (2005-2014), a total of 90 plant species was recorded across all treatments and replicates. The effect of treatment, year and treatment*year interaction was significant for the number of all species, the number of all species > 1%, Shannon index H, Simpson index D and for *Rhinanthus minor* and *Trifolium pratense* (Table 3).

The effect of treatment and year was significant for *Alopecurus pratensis, Arrhenatherum elatius, Galium mollugo, Plantago lanceolata* and *Trisetum flavescens.* Effect of treatment and treatment*year interaction was significant for *Briza media*, *Festuca rubra* agg., *Leucanthemum vulgare* and *Lotus corniculatus*. High year-to-year variability of the number of all species, the number of all species > 1%, Shannon index H, and Simpson index D with descendent trend during the study period was found (Fig. 1). The highest species richness was revealed in the control treatment (A) and in the treatment with Ca application only (treatment B).

The cover of tall grass species such as *A. elatius*, *A. pratensis* and *T. flavescens* was supported by P application, on average, in all study years (D, E, F) (Fig. 2). On the other hand, plant species such as *B. media* and *F. rubra* agg. exhibited higher cover in the treatments without P application (A, B, C). Between these two species, a strong negative correlation was revealed in B (*R* = - 0.58; *p* <0.001) and C (*R* = - 0.66; *p* <0.001) treatments. *Nardus stricta* was present predominantly in A and C treatments.

The tall forb *G. mollugo* had higher cover in the treatments with P application (D, E, F) than in the other treatments (A, B, C) (Fig. 3) throughout the ten years of observation. On the other hand, forbs such as *L. vulgare*, *P. lanceolata* and *R. minor* had higher cover in the treatments without P application (A, B, C). The cover of legumes such as *T. pratense* and *L. corniculatus* was supported predominantly by Ca application (treatment B).

Species response curves

The species response curves (based on RDA analysis) displayed the percentage cover development of plant species in the individual treatments (Appendix S2, S3, S4). A decrease in cover of species such as *B. media*, *F. rubra* agg., *R. minor*, *Lathyrus linifolius*, *Ranunculus nemorosus* and *L. catharticum* and an increase in cover of species such as *S. pratensis* and *Hypochaeris radicata* in A treatment was recorded over the duration of the experiment (Appendix S2a).

After the initial increase in the cover of *L. corniculatus*, *L. vulgare* and *F. rubra* agg., their successional decrease in B treatment (Appendix S2b) was revealed. The forbs *R. minor*, *Trifolium dubium* and *Ranunculus acris* showed decreasing cover during period of the experiment.

In treatment C, a mutual complementarity was recorded between *F. rubra* agg. and *B. media* (Appendix S3a). An increase in the cover of *F. rubra* agg. was accompanied by a decrease in the cover of *B. media* and vice versa. The cover of species such as *P. lanceolata* and *P. erecta* remained relatively stable during the study period.

Species such as *Taraxacum* sp., *F. pratensis, R. acris, R. nemorosus, T. pratense* and *T. dubium* decreased their cover in the D treatment during the course of the study (Appendix S3b). *Rumex acetosa*, *P. trivialis* and *Holcus lanathus* showed high temporal cover variability; their cover started to increase at the beginning of the study period but then decreased. The only species to show increased cover successively over the ten-year study period was *H. pubescens*

Similar responses of plant species in the E and F treatments have been found during ten-year of the study period (Appendix S4ab). For example, *A. elatius* started to increase its cover at the beginning of the study period and decreased thereafter. The opposite response was found for species such as *T. pratense, Crepis biennis* and *Trifolium hybridum.*

RDA and CCA analyses of community composition

Results of RDA analysis based on all ten years vegetation data showed that the effect of the treatments on plant species composition explained 54.25 % variability (*F*-value 67.6, $p = 0.001$) and 64.87 % variability (*F*-value 105.0, $p = 0.001$) on the first and all axes, respectively (Fig. 4). The treatments that had similar plant species composition according to the first ordination axis were sorted into two main groups: A, B, C treatments, and all treatments with Ca, N and P application (D, E, F). *Lathyrus linifolius* was strictly bounded to treatment A, whereas *Carex panicea* was related to treatment C. Species such as *Potentilla erecta, Succisa pratensis, Anthoxanthum odoratum, Linum catharticum* and *Carex pilulifera* were correlated with treatment B. All treatments with Ca, N and P application (D, E, F) had a similar effect on plant species composition and were related with the following tall grasses, *Poa pratensis, T. flavescens, Poa trivialis, A. pratensis* and *A. elatius.*

The effect of all treatments on individual plant species in the particular years based on RDA analysis (Appendix S5) showed that the percentage of explained variability on the first axis ranged from 53.21 to 62.46 % and the percentage of explained variability of all axis ranged from 66.73 to 75.77 %. It showed strong explanatory power of fertilizer application which was relatively stable over the years.

The PCA analyses displayed position of community composition for individual treatment in particular year (Appendix S6), which were relatively stable through ten years and reflected other multivariate data analyses, RDA (Fig. 4) and PRC (Appendix S7, S8, S9).

Principal response curves

The first PRC axis explained 76.20 % (*F*-value 5.7, $p = 0.001$), the second explained 8.10 % (*F*-value 0.7, $p = 0.001$) and third explained 4.70 % variability of plant species composition (*F*-value 0.5, $p = 0.001$). The PRC diagrams showed differences between the control treatment and the other treatments. The first vertical axis of the PRC diagram showed the primary effect of P application. There was a similar response of plant species composition in the treatments where P application was used (D, E, F) and in the treatments (A, B, C) without P application over the study period (Appendix S7). The second axis of PRC showed the effect of N application and so separated the C treatment from the others (Appendix S8). The third axis showed the effect of Ca application without N and P applications and separated the B treatment from the other treatments (Appendix S9).

Variability eigenvalues decomposition

Based on RDA, the temporal variability eigenvalues decomposition of individual treatments within the ten experimental years showed that the control treatment (A) had lower temporal residual variability and higher temporal explained variability compared with the other treatments (B, C, D, E, F). The lowest temporal explained variability was found in treatments fertilized with N (treatments C, D, E, F) (Appendix S10).

Discussion

Species richness and plant species composition

Long-term NPK and NP fertilization led to the shift in the grassland community becoming adapted to nutrient-rich conditions (Pierik, Ruijven, Bezemer, Geerts, & Berendse, 2011). This had a negative impact on species richness, which has been reported in many studies (e.g. Čop, & Eler, 2019; Korevaar, & Geerts, 2015; Hejcman, Strnad, Hejcmanová, & Pavlů, 2012; Pierik et al, 2011).

The decrease in Shannon and Simpson index values in all treatments over the study period is connected with the increasing number of plant species < 1%. According to Isbell et al. (2015) and Gaisler et al. (2019), species-rich grassland communities with higher numbers of species with cover of > 1% are more resistant to species fluctuation. Therefore, the treatments fertilized by NP and NPK (D, E, F) which have lower numbers of species with cover > 1% can show greater vulnerability of their grassland community to perturbations (Pressey &Taffs, 2001).

The higher cover of tall grasses such as *A. pratensis*, *A. elatius* and *T. flavescens* in the treatments fertilized by N in combination with Ca, P and K (treatments D, E, F) was in accordance with previous results of one year of data from the same experiment (Hejcman et al., 2010) as well as with results from other experiments conducted in Central European temperate grasslands (e.g. Hejcman et al., 2014; Kidd et al., 2017; Pavlů V., Gaisler, Pavlů, L., Hejcman, & Ludvíková, 2012). The variability of *B. media* and *F. rubra* agg. in B and C treatments can illustrate an example of compensatory dynamic (Lepš, Májeková, Vítová, Doležal, & De Bello, 2018), where the decrease in cover of one species is compensated by an increase of another species. This effect was probably because *B. media* is less tolerant to drought (Dixon, 2002), whereas *F. rubra* agg., which has many ecotypes (Grime, Hodgson, & Hunt, 1988), is well adapted to different abiotic conditions including drought. It seems, that *B. media* needs vegetation seasons with average or excessive precipitation to recover its dominant presence in the sward. The stability in the cover of *N. stricta* in the unfertilized control underlined its preference for, or adaptation to oligotrophic acid soils (Grime, Hodgson, & Hunt, 1988; Kurtogullari, Rieder, Arlettaz, & Humbert, 2020).

In our experiment *P. lanceolata* was one of the forb species present in all treatments and the addition of P reduced its cover significantly. Although this species is associated with moderately fertile soils (Grime, Hodgson, & Hunt, 1988), it can tolerate nutrient enrichment and was present even in the fully fertilized (NPK) treatments (E and F in this study). However, *P. lanceolata* in nutrient-rich soil cannot fully compete for light with grasses due to its leaf morphology (Stewart, 1996). Therefore, a few large plants of *P. lanceolata* were recorded (unpublished data) in the fully fertilized treatments only.

Both of the treatments without N application (A and B) promoted the annual facultative root hemi-parasite plant *R. minor* and the forb *L. vulgare*. *Rhinanthus minor* is suppressed in grasslands that have nutrient-rich soil (Jiang, Jeschke, Hartung, & Cameron, 2010; Kidd et al., 2017) and is unable to compete in fertilized grassland where annual herbage production exceeds about 5 t ha^{-1} DM (Hejcman et al., 2011). *Leucanthemum vulgare* is characterised as a species widely distributed on base-rich soil with pH>5, in grassland of intermediate level of productivity (Grime, Hodgson, & Hunt, 1988). It seems that increased biomass productivity (more than 3 t ha^{-1} DM) was detrimental for the competitive ability of both these species, as observed throughout the study period.

Leguminous species such as *T. pratense* and *L. corniculatus* are generally known for their positive response to PK and negative response to the high inputs of N, NP or NPK (e.g. Rabotnov, 1977; Hejcman, Strnad, Hejcmanová, & Pavlů, 2012; Čop, & Eler, 2019). This negative response of both species was also confirmed in all treatments with N application in this experiment for the majority of years. The highest cover of legumes in the B treatment was supported by lime application and higher soil pH (Storkey et al., 2015) without N fertilization.

This unique study of continuous vegetation recording over ten years in the RGE also showed year-to-year variability in the cover of most of the vascular plant species in all treatments. Such variability has been widely reported and is commonly attributed to variations in weather conditions (e.g. Deléglise et al., 2015; Grant, Kreyling, Dienstbach, Beierkuhnlein, & Jentsch, 2014; Herben, Krahulec, Hadincová, & Pecháčková, 2005; Louault et al., 2017).

Community composition

Despite year-to-year variability in the cover of individual plant species, the percentage of explained variability of RDA analyses for the first (53.2-62.4%) and all axes (66.7-75.8%) showed a similar response of grassland community to the treatments within particular years. This was underlined by the results of the PCA analysis revealing similar responses during the ten years period. The relative stability of the plant community was also underlined by the principal response curves results based on RDA. Similarly, Dodd, Silvertown, McConway, Potts & Crawley (1995) found at the Park Grass Experiment an equilibrium at the guild level, whereas individual vascular plant species changed their abundances and distribution because of weather conditions.

However, in the unfertilized control (*Violion caninae* alliance), a temporal trend and low residual eigenvalues variability were shown for the vegetation development (Appendix S9). This was probably caused by an ongoing oligotrophication through nutrient removal, even after 64 years, as there was more plant-available P and K in the soil than in the treatments with Ca and CaN applications (Hejcman et. al., 2010). Moreover, P is a slowly cycling nutrient and in the long-term perspective the low extractable P pool can resupply the bioavailable P pool (Roberts, & Johnston, 2015). Therefore its depletion in treatments without P application is not straightforward. It seems that a stage of community equilibrium was induced by the presence of high P levels in the soil in all treatments fertilized by P (D, E, F), in contrast to B and C treatments. The treatments with Ca (treatment B) and CaN application (C) had greater amounts of biomass DM yield, which led to higher P and K depletion from the soil in comparison with the control treatment. Plant-available P (<0.6 mg 100 g^{-1} of soil) and K (<2.5 mg 100 g^{-1} of soil) in treatments with Ca (B) and CaN (C) applications were very low (Hejcman et. al., 2010), and therefore it was probably very difficult to reduce them further. Therefore, grassland communities (*Polygono-Trisetion* alliance) in these treatments were adapted to very low plant available P and K. On the other hand, all treatments fertilized by P (treatments D, E, F) developed relatively stable grassland communities (*Arrhenatherion* alliance) with highly productive plant species already adapted to high levels of nutrients in the soil. Silvertown et al. (2006) concluded that equilibrium in plant species composition was already achieved after 40 years of fertilizer application in the Park Grass Experiment. Especially, in unfertilized grasslands, the time necessary to reach equilibrium may strongly depend on the initial nutrient status of the soil and the quantity of nutrients added per year.

Conclusion

The main finding of this ten-year study is that although year-to-year variability in the cover of individual vascular plant species was observed, the grassland community as a whole reacted rather similar to different fertilizer application treatments, indicating that as equilibrium has been reached. Surprisingly, in the unfertilized control we found the relatively strongest directional trend in plant species composition which may be interpreted as a response to ongoing nutrient impoverishment. Biomass production increased with increasing levels of fertilizer application (zero, Ca, CaN, CaNP and CaNPK) which led to a decline in species richness and evenness, especially in the numbers of species with cover > 1% with increasing productivity. In the long-term species rich swards were only maintained in the unfertilized (*Violion caninae* alliance) and limed (*Polygono-Trisetion* alliance) treatments, but also in the treatment with pure N-application and liming (*Polygono-Trisetion* alliance). Contrary, the combined application of growth-limiting macro-nutrients always resulted in a strong diversity decline. Our long-term results confirm the crucial role of low P-levels for the maintenance and restoration of species-rich mountain meadows.

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Author contributions

Pavlů, V., Pavlů, L., Hejcman, M. and Gaisler, J. collected the data. Titěra, J., Pavlů, L. and Pavlů, V. performed statistical analysis and wrote the paper. The research was conceived by Schellberg J. All authors discussed the results and commented on the manuscript.

Data Accessibility Statement

Data are accessible on request.

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List of Appendices

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Table 1. Soil chemical properties. Results of basic soil chemical analysis of the 0-10 cm layer, soil samples collected in May 2004, (mg 100 g^{-1} of soil, according to Hejcman et al., 2010). All analyses were conducted in accordance with standardized methods of the Association of German Agricultural Analytical and Research Institutes (VDLUFA, 1991). Numbers represent mean values of five replications (according to Hejcman et al., 2010).

Treatment abbreviations	P (CAL)	K (CAL)	pH ($CaCl_2$)	Ntot (%)	Ctot (%)	C/N	Mg ($CaCl_2$)
A	1.5	4.3	4.9	0.373	4.9	13.1	13.2
B	0.6	2.5	6.5	0.350	4.2	12.0	19.9
C	0.4	2.3	6.5	0.364	4.4	12.0	19.5
D	31.1	3.2	6.6	0.363	4.3	11.8	19.9
E	22.6	9.4	6.5	0.363	4.4	12.0	20.7
F	22.2	10.5	6.6	0.367	4.5	12.3	20.7

Table 2. Supplied nutrients, alliances and biomass production. Amounts of nutrients (kg ha^{-1}) supplied annually to the treatments from 1941 (according to Schellberg et al., 1999), classification of vegetation into alliances according to Chytrý et al. (2009) and dry matter biomas production (according to Hejcman et al., 2010).

Treatment abbreviations	Applied nutrients (kg ha^{-1})	Nutrient	Alliance	Biomass production (t ha^{-1})
A	unfertilized control	0	*Violion caninae*	2.5
B	Ca=718; Mg=67	Ca	*Polygono-Trisetion*	2.9
C	Ca=752; N=100; Mg=67	Ca/N	*Polygono-Trisetion*	4.9
D	Ca=936; N=100; P=35; Mg=75	Ca/N/P	*Arrhenatherion*	6.5
E	Ca=936; N=100; P=35; K=133; Mg=90	Ca/N/P/KCl	*Arrhenatherion*	8.9
F	Ca=936; N=100; P=35; K=133; Mg=75	Ca/N/P/K_2SO_4	*Arrhenatherion*	9.6

Table 3. GLM analysis. Results of the GLM for the cover of the dominant plant species, functional groups and number of plant species. Df - Degrees of freedom, *F* ratio - *F* statistics for the test of particular analysis, *p*-value- obtained probability value. Significant results of *p*-values in background shading.

	Tested variable	Treatment (Df 5)	Year (Df 9)	Treatment*Year (Df 45)
Number of all species	*F*-ratio	12.25	13.63	2.23
	p-value	<0.001	<0.001	<0.001
Number of species > 1%	*F*-ratio	17.82	11.63	3.74
	p-value	<0.001	<0.001	<0.001
Shannon index H	*F*-ratio	4.3	15.11	3.19
	p-value	0.003	<0.001	<0.001
Simpson index D	*F*-ratio	2.49	14.44	2.25
	p-value	0.045	<0.001	<0.001
Alopecurus pratensis	*F*-ratio	53.24	3.28	0.75
	p-value	<0.001	0.004	0.877
Arrhenatherum elatius	*F*-ratio	124.8	3.4	1
	p-value	<0.001	0.003	0.398
Briza media	*F*-ratio	35.82	2.08	7.51
	p-value	<0.001	0.052	<0.001
Festuca rubra agg.	*F*-ratio	32.36	1.69	5.5
	p-value	<0.001	0.119	<0.001
Galium mollugo	*F*-ratio	118.7	3.3	0.4
	p-value	<0.001	0.004	1
Leucanthemum vulgare	*F*-ratio	69.74	1.46	2.72
	p-value	<0.001	0.192	<0.001
Lotus corniculatus	*F*-ratio	53.73	1.17	1.83
	p-value	<0.001	0.334	0.002
Nardus stricta	*F*-ratio	91.95	0.53	0.45
	p-value	<0.001	0.847	0.999
Plantago lanceolata	*F*-ratio	87.27	5.03	1.14
	p-value	<0.001	<0.001	0.26
Rhinanthus minor	*F*-ratio	28.19	3.52	7.56
	p-value	<0.001	0.002	<0.001
Trifolium pratense	*F*-ratio	17.93	2.28	4.58
	p-value	<0.001	0.034	<0.001
Trisetum flavescens	*F*-ratio	133.3	4.4	1
	p-value	<0.001	<0.001	0.427

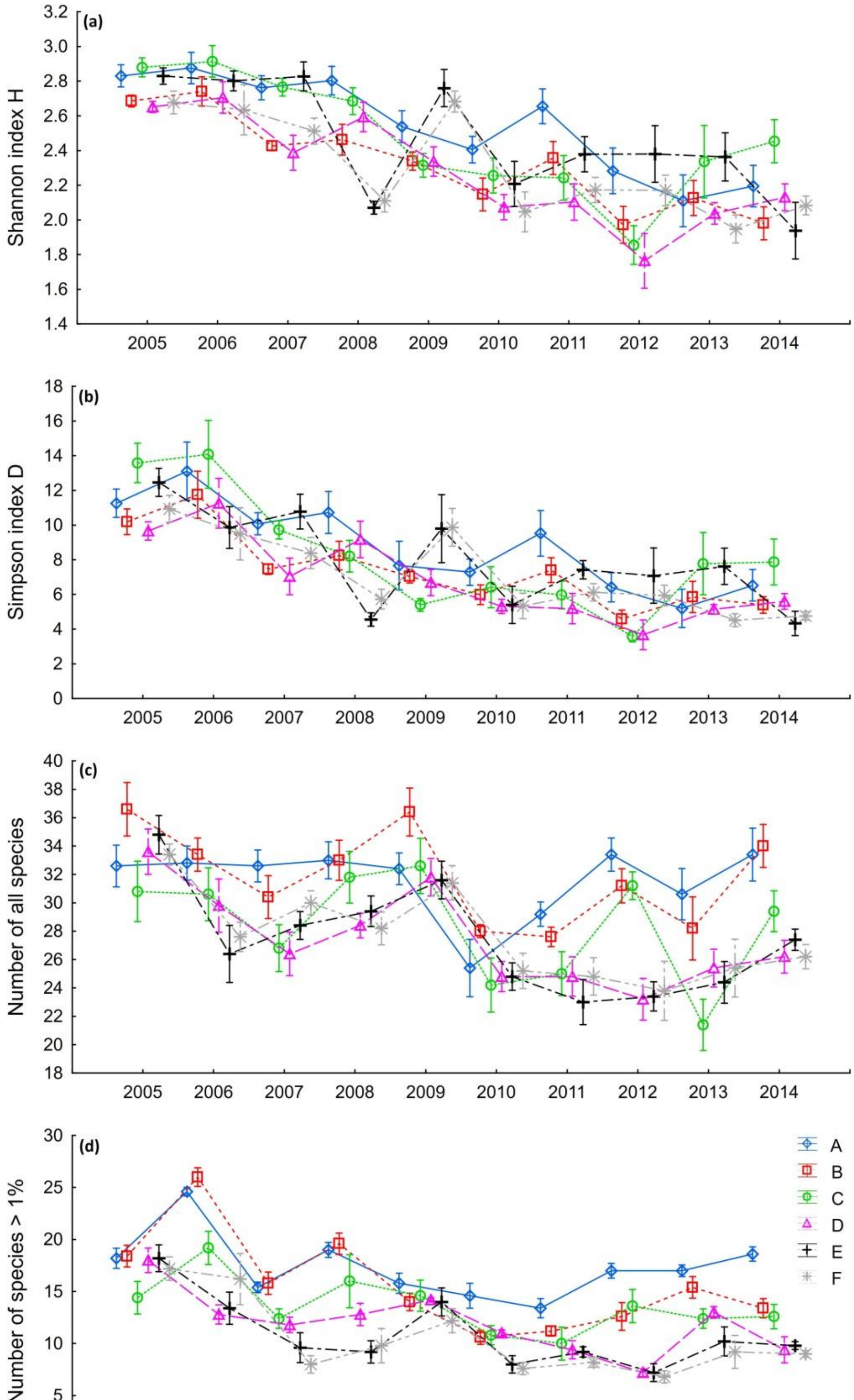


Fig. 1. Plant diversity measurements. (a) Shannon index H, (b) Simpson index D, (c) number of all vascular plant species and (d) number of vascular plant species with cover more than 1% in the treatments over the study period 2005 – 2014. Treatment abbreviations and fertilizer inputs for each treatment are explained in Table 2.

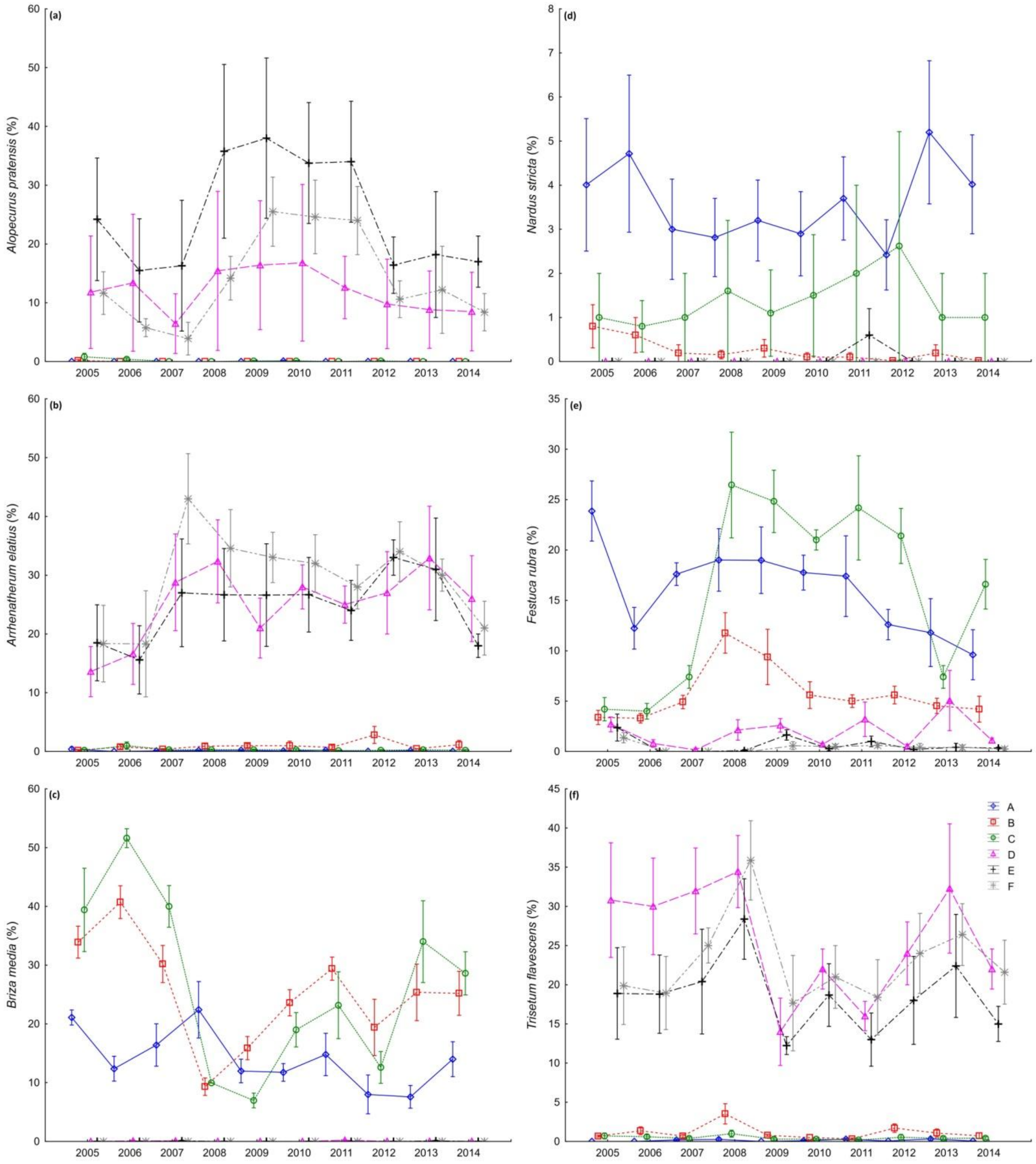


Fig. 2. Dominant grass species. The mean cover (%) of the dominant grass species – (a) *Alopecurus pratensis*, (b) *Arrhenatherum elatius*, (c) *Briza media*, (d) *Nardus stricta*, (e) *Festuca rubra* agg. and (f) *Trisetum flavescens* in the treatments over the study period 2005 – 2014. Treatment abbreviations and fertilizer inputs for each treatment are explained in Table 2.

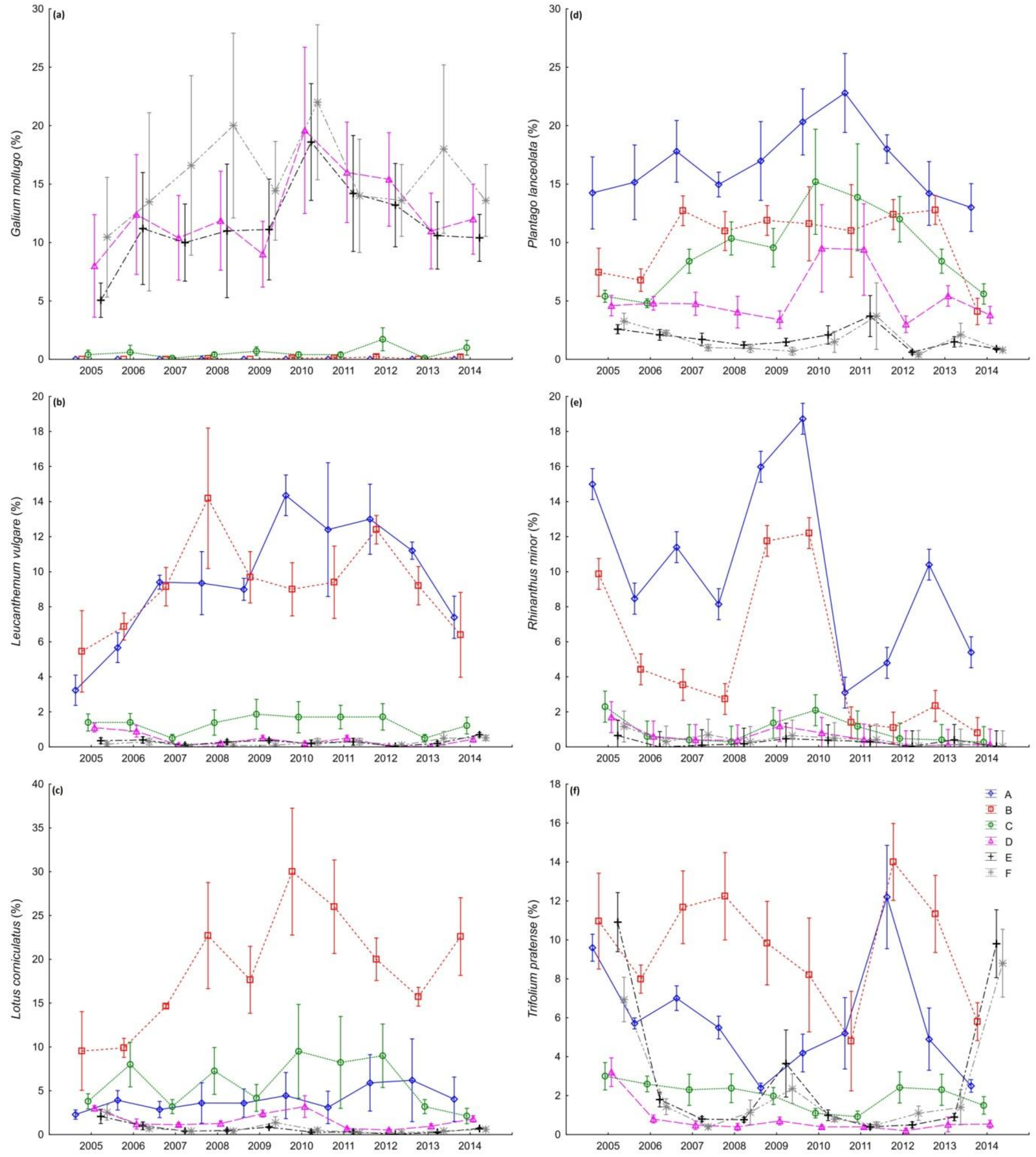


Fig. 3. Dominant forb species. The mean cover (%) of the dominant forb species – (a) *Galium mollugo*, (b) *Leucanthemum vulgare*, (c) *Lotus corniculatus*, (d) *Plantago lanceolata*, (e) *Rhinanthus minor* and (f) *Trifolium pratense* in the treatments over the study period 2005 – 2014. Treatment abbreviations and fertilizer inputs for each treatment are explained in Table 2.

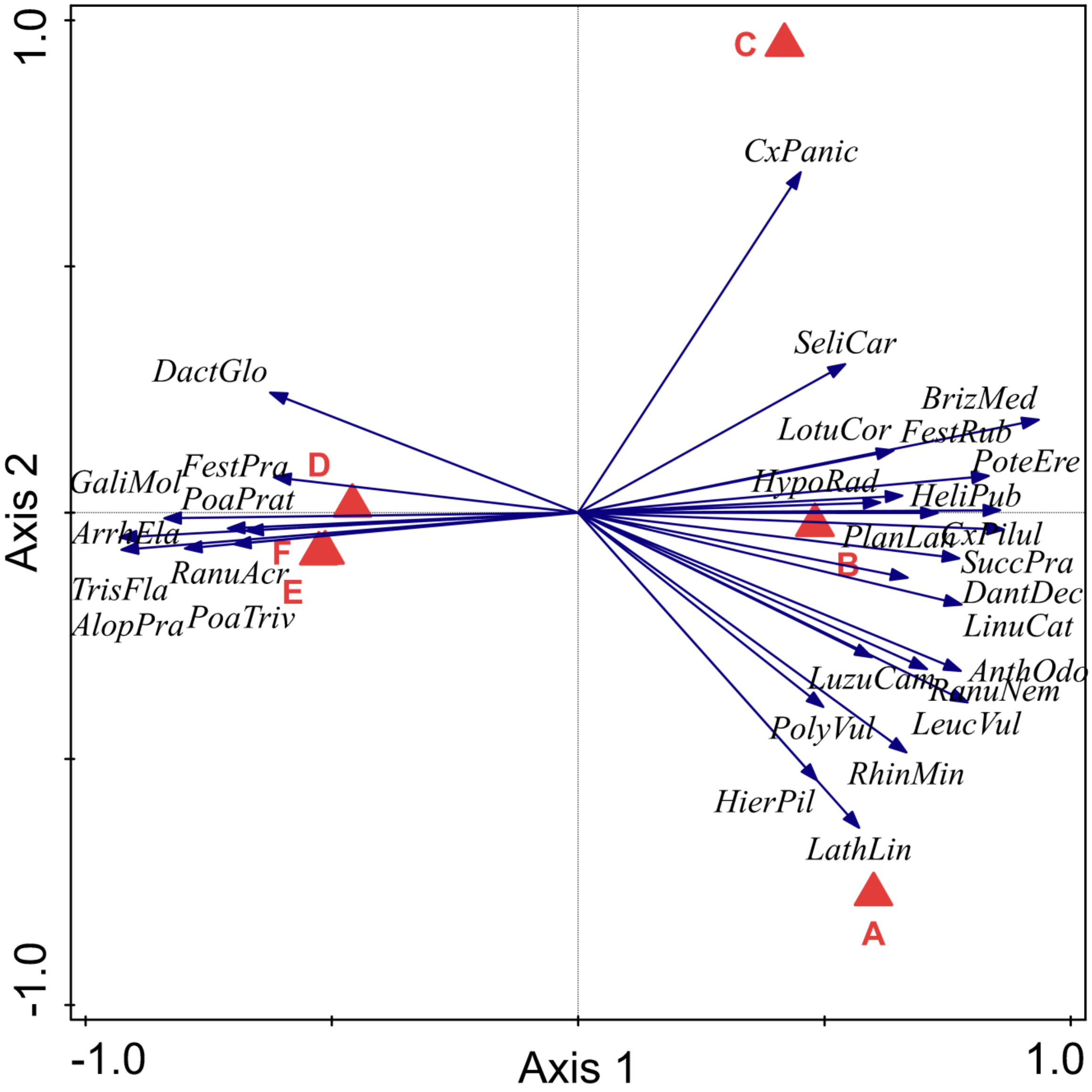
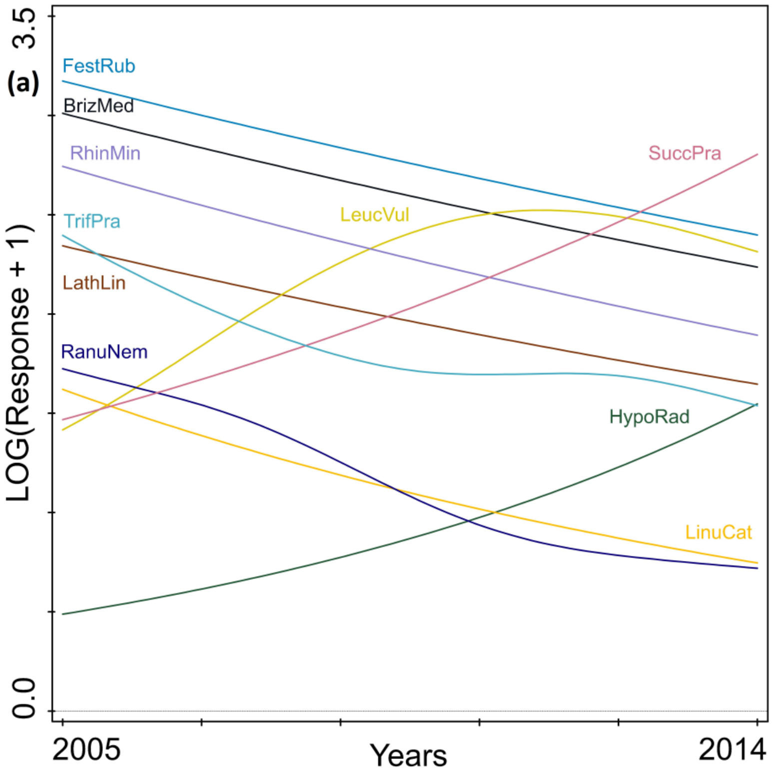


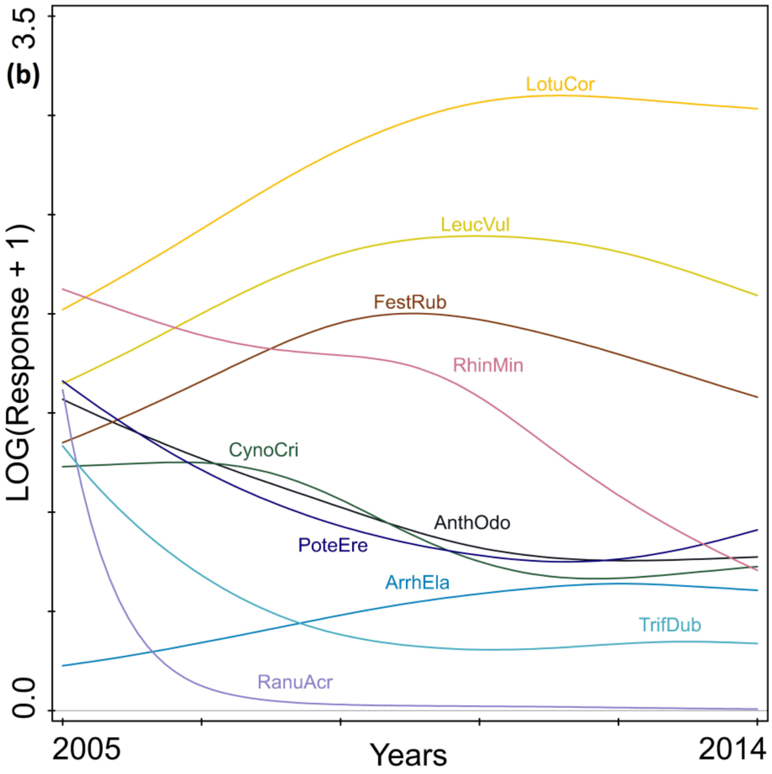
Fig. 4. RDA ordination diagram. Result of RDA analysis of plant species composition. For treatment abbreviations and fertilizer inputs for each treatment see Table 2. For plant species abbreviations see (Appendix S1).

Appendix S1: **Plant species abbreviations.**

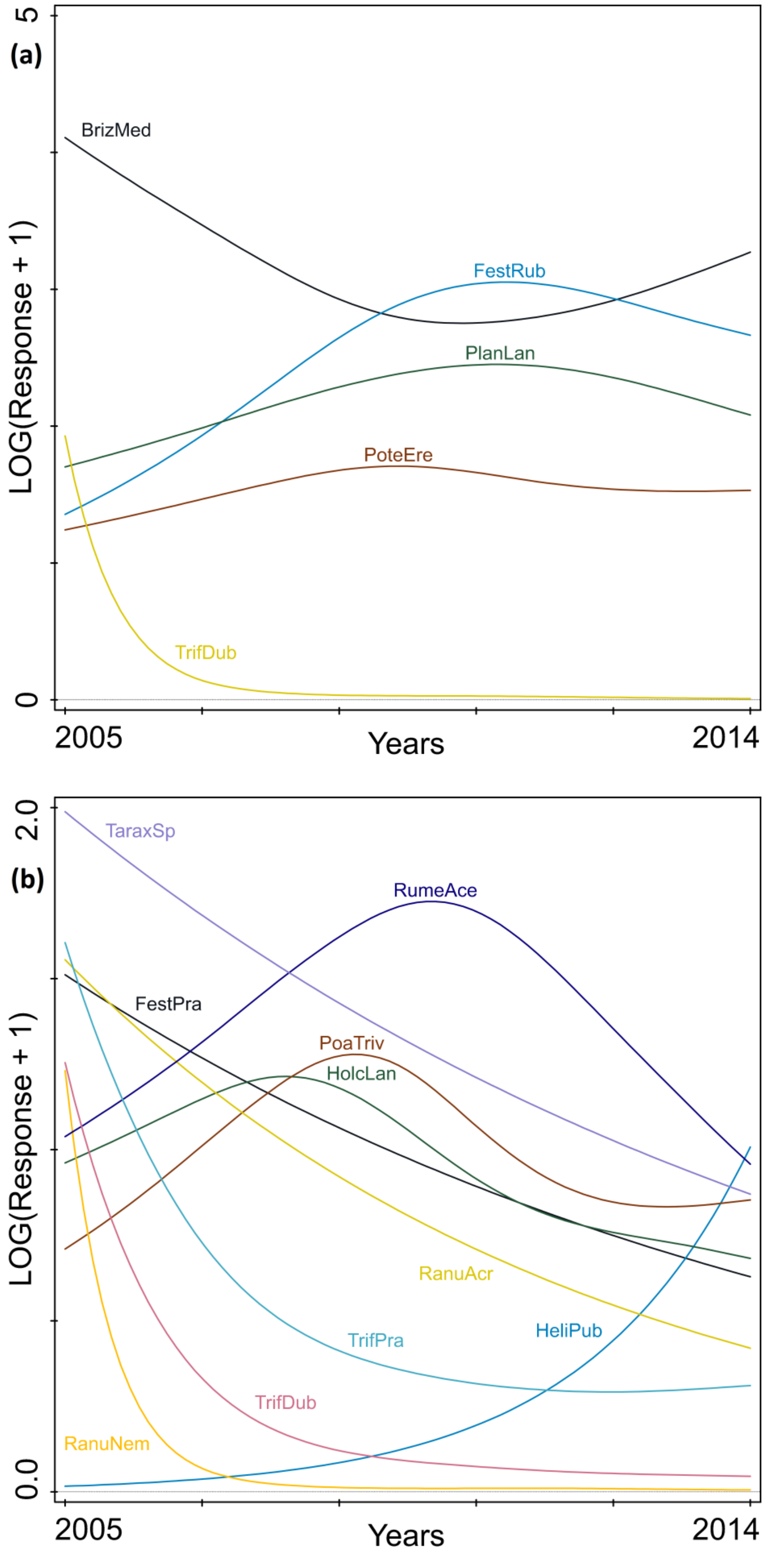
Abbreviations	Species name	Abbreviations	Species name
AgroCap	*Agrostis capillaris*	LinuCat	*Linum catharticum*
AlopPra	*Alopecurus pratensis*	LotuCor	*Lotus corniculatus*
AnthOdo	*Anthoxanthum odoratum*	LuzuCam	*Luzula campestris*
ArrhEla	*Arrhenatherum elatius*	NardStr	*Nardus stricta*
BelPer	*Bellis perennis*	PhlePra	*Phleum pratense*
BrizMed	*Briza media*	PlanLan	*Plantago lanceolata*
CentJac	*Centaurea jacea*	PoaPra	*Poa pratensis*
CrepBie	*Crepis biennis*	PoaTriv	*Poa trivialis*
CxFlac	*Carex flacca*	PolyVul	*Polygala vulgaris*
CxPanic	*Carex panicea*	PopuTre	*Populus tremula*
CxPilul	*Carex pilulifera*	PoteEre	*Potentilla erecta*
CynoCri	*Cynosurus cristatus*	RanuAcr	*Ranunculus acris*
DactGlo	*Dactylis glomerata*	RanuNem	*Ranunculus nemorosus*
DactMac	*Dactylorhiza maculata*	RhinMin	*Rhinanthus minor*
DantDec	*Danthonia decumbens*	RumeAce	*Rumex acetosa*
FestPra	*Festuca pratensis*	SeliCar	*Selinum carvifolia*
FestRub	*Festuca rubra* agg.	SuccPra	*Succisa pratensis*
GaliMol	*Galium mollugo*	TaraxSp.	*Taraxacum sp.*
HeliPub	*Helictotrichon pubescens*	TragPra	*Tragopogon pratensis*
HierPil	*Hieracium pilosella*	TrifDub	*Trifolium dubium*
HolcLan	*Holcus lanatus*	TrifHyb	*Trifolium hybridum*
HypoRad	*Hypochaeris radicata*	TrifPra	*Trifolium pratense*
LathLin	*Lathyrus linifolius*	TrifRep	*Trifolium repens*
LeonAut	*Leontodon autumnalis*	TrisFla	*Trisetum flavescens*
LeucVul	*Leucanthemum vulgare*	ViolCan	*Viola canina*

Appendix S2: Plant species responses (A, B). Plant species covers (Log (response+1) in: a) A treatment and b) B treatment over the study period 2005–2014. For plant species abbreviations see (Appendix S1).

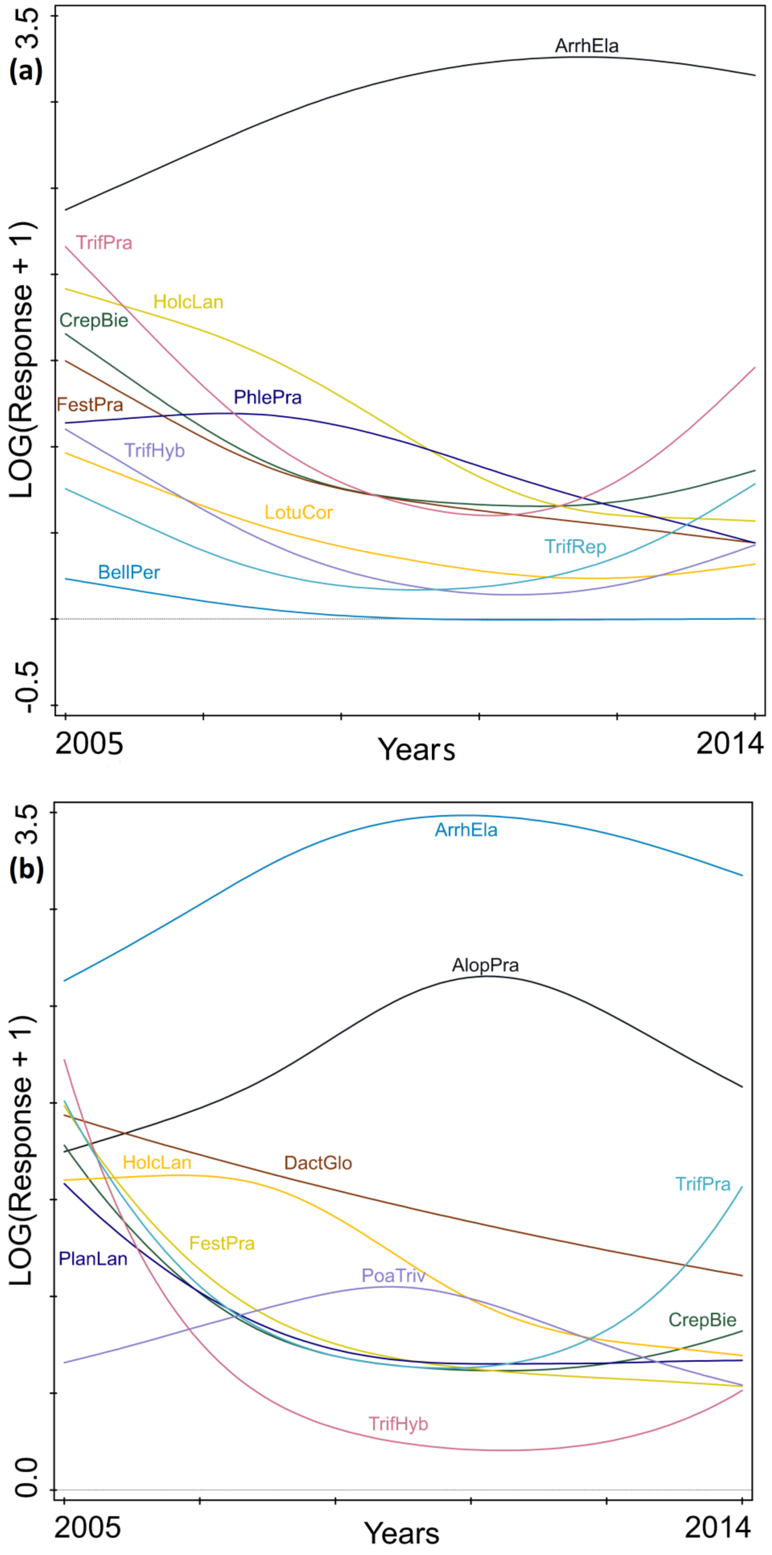




Appendix S3: Plant species responses (C, D). Plant species covers (Log (response+1) in: a) C treatment and b) D treatment over the study period 2005–2014. For plant species abbreviations see (Appendix S1).



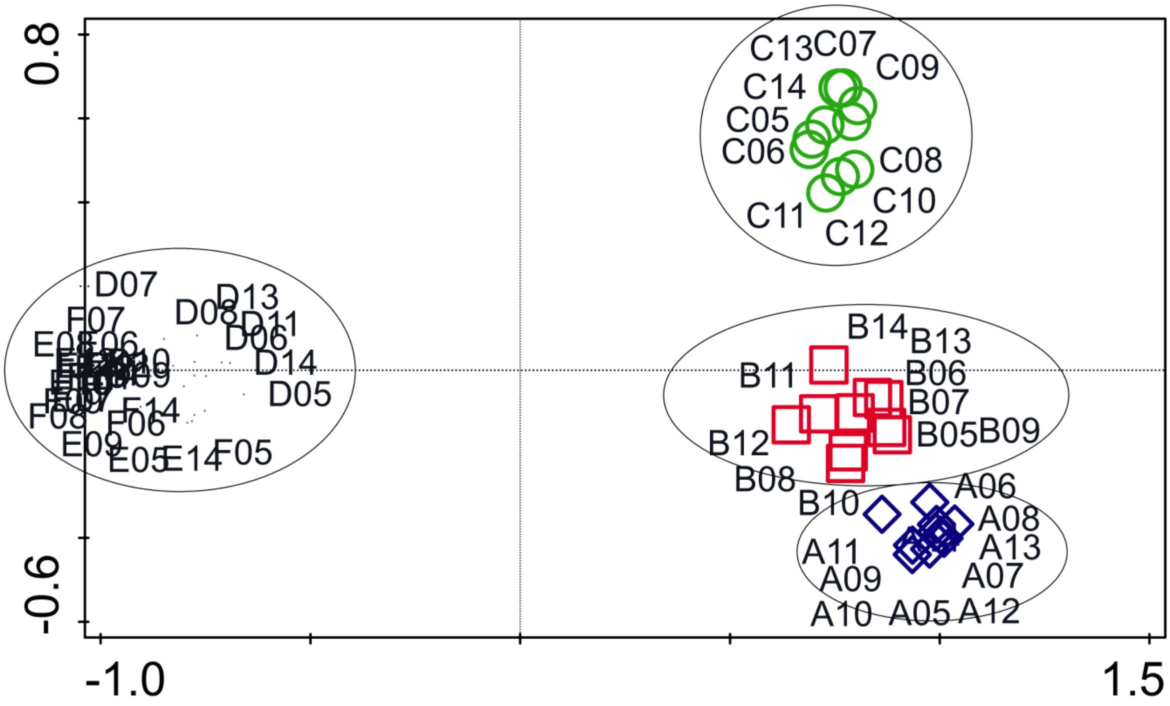
Appendix S4: Plant species responses (E, F). Plant species covers (Log (response+1) in: a) E treatment and b) F treatment over the study period 2005–2014. For plant species abbreviations see (Appendix S1).



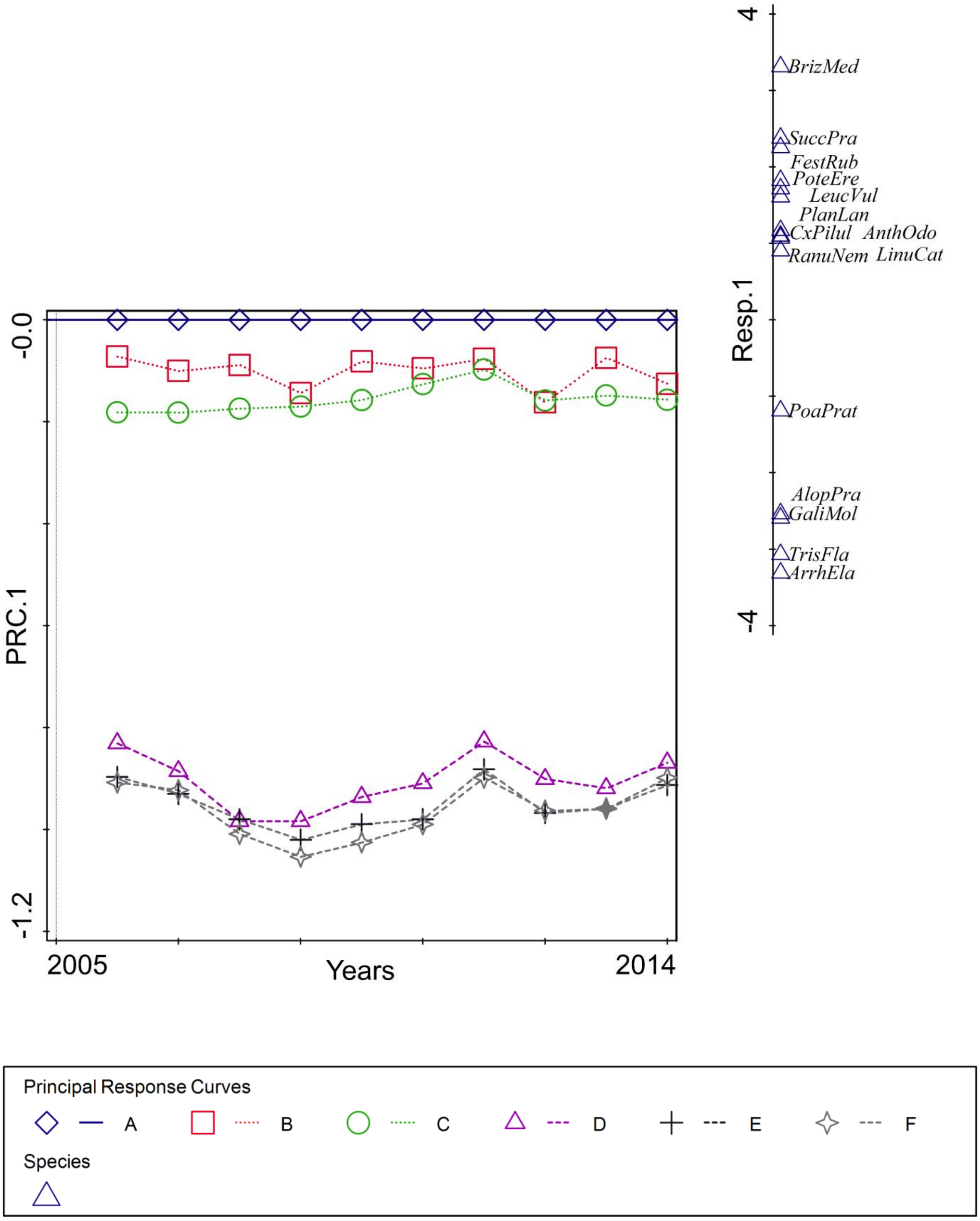
Appendix S5: RDA analyses. Results of RDA analysis of plant species composition in each year of the experiment. (%) expl. var. 1st axis (all axes) - (%) explanatory variability explained by the first (all) ordination axis (measure of explanatory power of the explanatory variables); F-ratio 1st axis (all axes) - F statistics for the first (all) ordination axis for the test of the particular analysis; P-value was for all experimental years < 0.001); Covariables were blocks; Explanatory variables were treatments (A, B, C, D, E, F). Nutrient inputs for treatments A-F are given in Table 2.

Year	(%) expl. var. 1st axis (all axes)	*F*-ratio 1st axis (all axes)	Year	(%) expl. var. 1st axis (all axes)	*F*-ratio 1st axis (all axes)
2005	53.21 (66.73)	26.2 (9.2)	2010	62.46 (75.77)	38.3 (14.4)
2006	54.40 (68.00)	27.4 (9.8)	2011	61.16 (73.49)	36.2 (12.8)
2007	60.19 (74.5)	34.8 (13.4)	2012	60.84 (73.21)	35.7 (12.6)
2008	59.39 (72.14)	33.6 (11.9)	2013	56.21 (71.20)	29.5 (11.4)
2009	59.77 (74.95)	34.2 (13.8)	2014	57.87 (75.39)	31.6 (14.1)

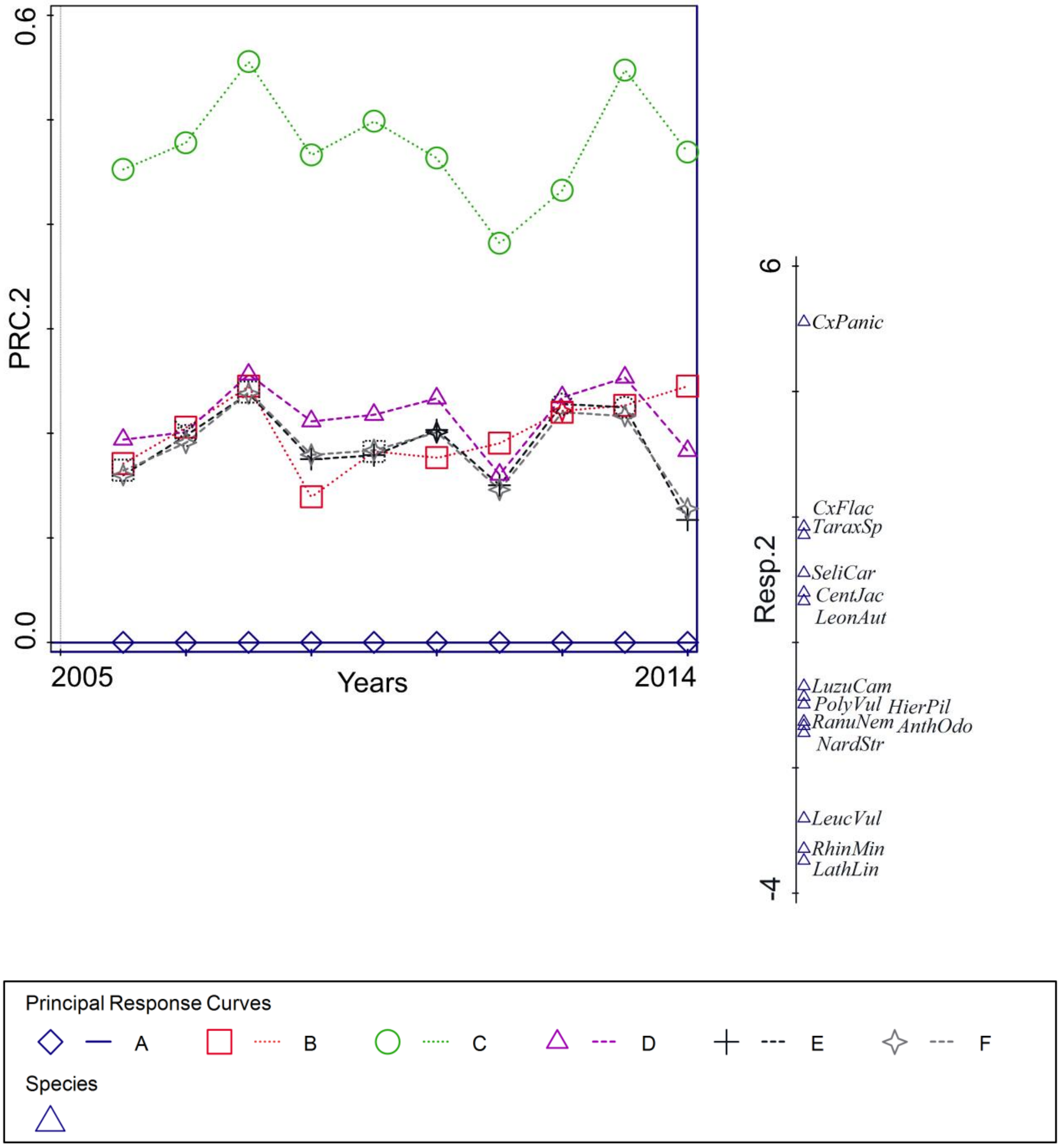
Appendix S6: PCA analysis. Results of PCA analysis for the treatments over the study period 2005 – 2014. Treatments abbreviations according Table 2. Last two numbers are abbreviations for years 05-14 (2005-2014).



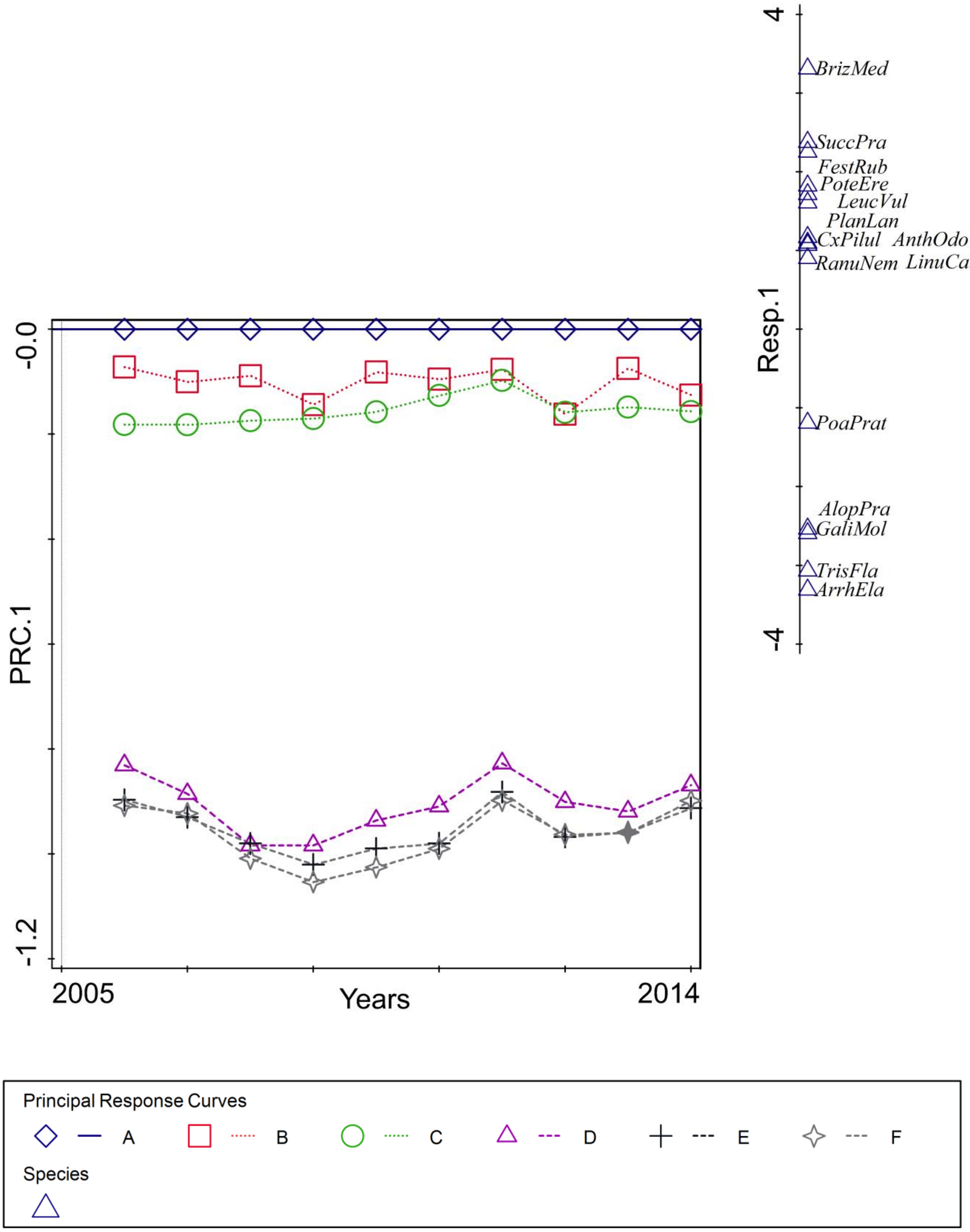
Appendix S7. PRC curve – 1 axis. Result of partial RDA analysis – PRC curve – 1 axis of plant species composition data for the treatments over the study period 2005 – 2014. Reference treatment was control treatment. For treatment abbreviations see Table 2. For plant species abbreviations see (Appendix S1).



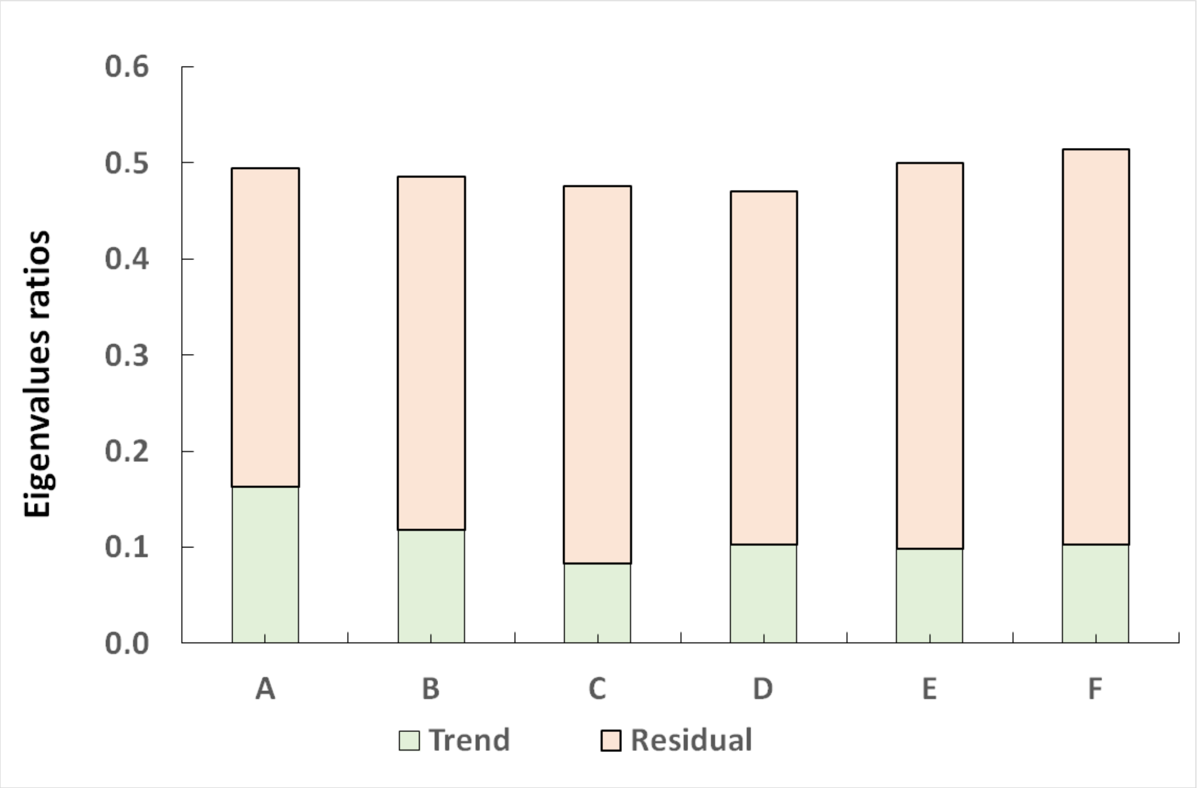
Appendix S8. PRC curve – 2 axis. Result of partial RDA analysis – PRC curve – 2 axis of plant species composition data for the treatments over the study period 2005 – 2014. Reference treatment was control treatment. For treatment abbreviations see Table 2. For plant species abbreviations see (Appendix S1).



Appendix S9. PRC curve – 3 axis. Result of partial RDA analysis – PRC curve – 3 axis of plant species composition data. Reference treatment was control treatment for the treatments over the study period 2005 – 2014. For treatments abbreviations see Table 2. For plant species abbreviations see (Appendix S1).



Appendix S10. Variability decomposition. Temporal variability decomposition of individual treatments (A-F) over the study period 2005–2014. Treatments abbreviations are explained in Table 2.



Chapter IV

The Admont Grassland Experiment: 70 years of fertilizer application and its effects on soil properties, species diversity, plant species composition and yield in an alluvial meadow managed under a three-cut regime

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ABSTRACT

Fertiliser application is a widely used management technique for increasing forage production from agricultural grassland. Fertilisation is also a key driver of changes in soil nutrient status and plant species composition of grassland as shown in many short-term studies. Results from long-term experiments can further improve understanding of plant-soil relationships and help with management recommendations for agricultural and environmental outcomes. We collected data from a long-term experiment on alluvial meadow (Admont Grassland Experiment, Austria; established 1946) with 24 fertilisation treatments managed under a three-cut regime. Soil sampling in autumn 2015 and vegetation sampling in spring 2016 were conducted in seven selected treatments. Combinations of N (nitrogen 80 kg ha^{-1}), P (phosphorus 35 kg ha^{-1}) and K (potassium 100 kg ha^{-1}) were applied annually and compared with a non-fertilised control. Treatments were: Control, N, P, K, NP, NK, PK and NPK fertilisation.

Long-term different fertilisation affected soil pH and nutrient concentrations in the soil and plant species composition, but no significant effects on species richness were found. Short species (< 0.5 m height) prevailed in all treatments regardless of nutrient application, probably as a result of the three-cut defoliation. The dry matter biomass (DMB) yield in the Control was limited by N and P and synergisticly co-limited by N, P and K, and DMB yields of more than 5 t ha^{-1} per year were achieved under nutrient combinations containing P (NP, PK, NPK) without loss of species richness.

Results from the Admont Grassland Experiment show that the tested nutrient combinations significantly increased DMB yield and changed the species composition, but without significant effects on species richness. Long-term biomass yields of more than 5 t ha^{-1} DMB per year can be achieved with any nutrient combination containing P without loss species richness in an alluvial meadow managed under a three-cut regime.

Keywords: Biomass; Cutting; Herbage; Meadow; Nutrients; Species richness

1. Introduction

Grasslands occupy a major part of the utilized agricultural area of the temperate and continental areas of Europe and provide major forage resources for ruminant livestock (Hejcman et al., 2013; Huyghe et al., 2014). Systems of extensive agricultural grassland management, including cutting of forage for indoor feeding and extensive grazing by cattle or sheep over successive years, have led to the development of species-rich permanent grassland communities. During the latter years of the 20th century, agriculture based on traditional (low-input/low-output) management ceased to be economical compared with systems based on high fertilizer use and sown swards containing improved forage varieties (Lepš, 1999). In many areas this has led to agricultural abandonment, and elsewhere to other land-use changes including intensification of grassland management, or conversion to cropping or afforestation (Isselstein et al., 2005; Taube et al., 2014). However, during the same period, there has been an increasing recognition of the potential of grassland, particularly grasslands of diverse botanical composition, to deliver other ecosystem services including biodiversity, mitigation of greenhouse gas emissions, hydrological benefits and cultural benefits to society (Isselstein and Kayser, 2014). The loss of botanically diverse grassland, whether by management intensification or other causes, has environmental implications beyond its changing role as a source of feed production for livestock diversity (Lepš, 1999). In situations where management intensity has increased there are environmental problems linked to fertilizer use including nutrient leaching, groundwater contamination, eutrophication and soil acidification (e.g., Kidd et al., 2017).

Sustainable use of grassland in agriculture requires management systems that can match forage production and utilization with livestock requirements, and which are also consistent with maintaining other ecosystem services including nature conservation (Isselstein and Kayser, 2014). There is a considerable evidence of the effects of different application rates of the main nutrient inputs (N, P, K and their combinations) on both forage production and botanical composition of grassland, based on studies conducted on farms (Hopkins et al., 1986) and especially from field experiments conducted mainly on research stations (e.g. Crawley et al., 2005; Kidd et al., 2017; Titěra et al., 2020). These studies provide essential evidence for informing policy and grassland management decisions, although in most cases experimental data has been obtained from short-term fertilization studies; despite their usefulness they may not allow the separation of inter-annual fluctuations from real trends (Kidd et al., 2017).

Nitrogen (N), whether applied as fertilizer N or as recycled manure containing N, is generally considered to be one of the main factors for increased herbage production from grassland and in reducing plant species richness (Rabotnov, 1977; Clark et al., 2007; Humbert et al., 2016), especially the abundance and contribution of forbs and legumes (Kidd et al., 2017). Sustained addition of even relatively small amounts of N ultimately leads to reduced plant diversity in the long-term (Humbert et al., 2016), although a positive relationship between N-fertilization and plant biodiversity/biomass in N-limited grassland ecosystems has also been found (e.g. Müller et al., 2013; Strecker et al., 2015). Several authors have highlighted the detrimental effect of N fertilization on plant species diversity, particular when P is not the limiting nutrient (Janssens et al., 1998; Hejcman et al., 2007). However, on more botanically diverse grasslands the effects of differential nutrient additions can be more complex. For instance, many endangered and scarce plant species have been reported to persist under phosphorus-limited, rather than nitrogen-limited, conditions (Wassen et al., 2005). Long-term fertilization with N only, on a mountain meadow in Poland, led eventually to considerable reduction in yield and sward height as it allowed greater removal of other nutrients by cutting, although the number of species remained high and similar to treatments not fertilized with nitrogen (Galka et al., 2005).

Phosphorus (P) has also been shown to have a negative influence on the number of plant species in grasslan (Janssens et al., 1998; Oelmann et al., 2009) with the highest number of species found under conditions with below the agricultural optimum of plant available P (5 mg P/100 g dry soil, by Olsen method). In contrast, high K concentrations in the soil were compatible with high values of diversity. This means that the greatest number of species was observed when plant available K was optimal for plant nutrition (20 mg/100 g of dry soil, Olsen method). However, the relationships between soil nutrient concentration levels and plant species diversity and/or plant species composition remain unclear. For example, although Janssens et al. (1998) reported that the highest cover of legumes was in soils with low P concentrations, several other authors (Galka et al., 2005; Hrevušová et al., 2014; Titěra et al., 2020) found the opposite relationship in studies in central European mesophytic meadows. Generally, the application of P has long-term effects and previous P fertilization can be detected in the soil more than 20 years after the last addition (Smits et al., 2008; Pavlů et al., 2011).

Soil pH also affects plant species composition and low pH has been shown to have a negative influence on species richness in a wide range of European temperate grasslands (Gough et al., 2000; Crawley et al., 2005; Stevens et al., 2010; Kidd et al., 2017). Application of lime can greatly improve the agricultural value of acidic soils and results in a more favourable environment for a wider range of plant species in upland grasslands (Hejcman et al., 2007). In contrast, N application can lead to reduced soil pH, although this depends on the type of N fertilizer used. In the long-term Rothamsted Park Grass Experiment in England, soil pH was reduced in plots that received N as ammonium sulphate but not when supplied as sodium nitrate (Crawley et al., 2005). Furthermore, soil pH influences plant availability of many soil nutrients (Ashman and Puri, 2002).

The effects of nutrient addition are further complicated by the method of grassland utilization. Regular long-term cutting with biomass removal in temperate European grasslands has been shown to result in nutrient depletion (Schaffers et al., 1998; Galka et al., 2005; Pavlů et al., 2016; Titěra et al., 2020; Pavlů et al., 2021). This can alter both plant species composition and species richness (Oomes, 1990; Titěra et al., 2020). However, P is very stable in soils (Janssens et al., 1998) and the quantities removed in harvested forage are usually relatively small (10–30 kg ha^{-1} year^{-1}) (Marrs, 1993; Hrevušová et al., 2014). In contrast, K is removed in harvested herbage in much greater quantities (about 200 kg ha^{-1} year^{-1}) (Janssens et al., 1998) provided there is a little delay between cut and hay removal (Schaffers et al., 1998). Moreover, K is more leachable from soil than P, and thus the soil-K concentration decreases quite rapidly if it is no longer applied (Janssens et al., 1998), although this depends on the soil type (Alfaro et al., 2004; Kayser and Isselstein, 2005).

Land-use intensification by fertilizer application increases biomass production and, in most cases, causes loss of biodiversity in grasslands (Allan et al., 2015). Many studies have focused on how fertilization affects the plant species richness-biomass productivity relationship in different type of grasslands. Although the 'hump-shaped' relationship pattern of biomass productivity and species richness is widely reported (Mittelbach et al., 2001), differences in specific site conditions and types of plant communities have led some authors to show there may be a negative relationship between species richness and productivity (Gough et al., 1994; Cornwell and Grubb, 2003; Crawley et al., 2005; Galka et al., 2005). Biomass production is influenced positively not only by soil nutrient availability (Lepš, 1999; Pavlů et al., 2011; Humbert et al., 2016) but also by water availability (Cornwell and Grubb, 2003) and other ecological factors (Rabotnov, 1977; Wellstein et al., 2007).

We therefore have a situation in which, despite a considerable body of evidence from observations and experimentation, the relationships between nutrient supply and species richness/productivity, together with the effects of other environmental influences, indicate this is a highly complex and imperfectly understood process, particularly over the long term. We noted above that much of the evidence in literature is based on results from short-term studies. Long-term experiments, maintained with constant treatments of successive decades, are few and require a commitment of resources that is seldom available. The Admont Grassland Experiment in Austria is one such resource for helping further our understanding of the mechanisms and relationships in alluvial meadows under management with long-term fertilizer application. Established in 1946, and still running, it is one of the oldest well-designed long-term experiments with different fertilizer application treatments in Continental Europe. Despite this long history, this paper is the first presentation of results from this experiment in a scientific journal. Although the vegetation and soil data we present in this paper were collected during one year only we considered that they reflect the stage of the grassland community after 70 years of different fertilizer application. This assumption was based on work by Titěra et al. (2020) whose results (from the Rengen Grassland Experiment, in Germany, established 1941) revealed, that after decades of fertilization some stage of equilibrium of the grassland community was achieved, and their ten-year data showed a similar response of grassland communities to the treatments as those within particular years.

We hypothesized that the 70 years of continuous management of the different treatment plots on the Admont Grassland Experiment would have led to the development of vegetation communities whose species composition and agronomic properties were strongly linked to the treatment and the soil properties of each treatment. Within this context, our aim is to answer the following questions: what is the long-term effect of different treatments of fertilizer application on: i) the soil properties and, ii) vegetation (plant species diversity, plant species composition, dry matter biomass yield, sward height) of an alluvial meadow after 70 years of continuous management?

2. Material and methods

2.1. Study site

The long-term field fertilization experiment was established in 1946 in Admont, province of Styria (Austria), (47°34`52``N and 14°27`04``E; 635 m a.s.l.). The long-term average annual precipitation at this site is 1,227 mm, and long-term mean annual air temperature is 6.8°C. The mean temperature and average precipitation for the years 2015, 2016 and for the period 2007–2016 are presented in Table 1. The parent material at the experimental site is associated with the nearby Enns-river. According to the WRB-system the soil is classified as Gleyic Fluvic Dystric Cambisol with no supplementary qualifiers (IUSS Working Group WRB, 2015). In 1946 the soil characteristics were: pH/$CaCl_2$ = 6.05, P = 23 mg kg^{-1} (extracted by calcium acetate lactate - CAL), K = 323 mg kg^{-1} (extracted by CAL). The indicated the P concentration was classified as very low, whereas the K concentration was classified as high (BMLFUW, 2017). The experimental field had previously been used for cereal experiments in the 1930s and later reverted to an unsown grassland vegetation. In spring 1946 the field was ploughed and resown (24th May) with a grass-clover mixture with the following species and seeding rates: *Arrhenatherum elatius* 10.0 kg ha^{-1}; *Trisetum flavescens* 4.0 kg ha^{-1}; *Festuca pratensis* 20.0 kg ha^{-1}; *Poa pratensis* 4.5 kg ha^{-1}; *Phleum pratense* 4.5 kg ha^{-1}; *Agrostis stolonifera* 1.0 kg ha^{-1}; *Trifolium repens* 3.0 kg ha^{-1}; *Lotus corniculatus* 1.5 kg ha^{-1}. The present dominant species are *Agrostis capillaris, Anthoxanthum odoratum, Trisetum flavescens, Leontodon hispidus, Plantago lanceolata* and *Trifolium pratense*.

2.2. Design of the experiment

The Admont Grassland Experiment with 23 treatments and a non-fertilized control was established in four permanent randomized blocks (replications), using rectangular plots of 2.9 m × 7.1 m each with a buffer zone between plots of 17 cm, and 30 cm between blocks. The detailed experimental design is included as supplementary material (Fig. S1). All treatments were (and are) cut regularly three times a year (around 25th May, 20th June and 30th September, depending on the particular weather and growing conditions). Cutting is carried out with a finger-bar mower, and the height of cut is about 5 cm above the soil surface.

In 2015, eight out of 23 different fertilizer treatments with various combinations of N (nitrogen 80 kg ha^{-1} year^{-1}), P (phosphorus 35 kg ha^{-1} year^{-1}), K (potassium 100 kg ha^{-1} year^{-1}) and a non-fertilized control (Control) were selected for comprehensive botanical survey. These treatments were: (i) unfertilized Control, (ii) N, (iii) P, (iv) K (v) NP, (vi) NK, (vii) PK and (viii) NPK fertilization. Nitrogen was applied as calcium ammonium nitrate ($NH_4NO_3+CaCO_3$; 27% N), phosphorus was supplied in the form of basic slag ($(CaO)_5\ P_2O_5\ SiO_2$) from 1946 to 1997 and after 1998 as superphosphate ($Ca(H_2PO_4)_2$), and potassium was applied by potassium chloride (KCl). Phosphorus and potassium were applied in autumn after the third cut, whereas nitrogen was supplied twice per year: one half applied at the beginning of vegetation in April and one half immediately after the first cut at the end of May.

2.3. Species richness and plant species composition

In May 2016 just before the first cutting date (peak of growing season), the cover of all vascular plant species was recorded visually in each experimental plot using the percentage scale. To avoid edge effects, data were collected from within the inner 12 m^2 (2 m × 6 m) section of each plot.

The nomenclature of the plant species follows Fischer et al. (2008). The species richness was represented by the total number of vascular plant species in the plot, the Shannon index of diversity (H) and species evenness index (J). Shannon index of diversity and species evenness index (Shannon index of diversity divided by the natural logarithm of species richness) were calculated according to Begon et al. (2005). Based on the mean height of vascular plants in the Austrian flora (Fischer et al., 2008) the species were *a priori* categorized into short and tall graminoids, and short and tall forbs. Species with a mean height ≥ 0.5 m were classified as tall, whereas those below this threshold were classified as short. Ratios (tall/short species and graminoids/forbs) were based on percentage cover. Legumes were included in the functional group of forbs.

2.4. Compressed sward height and dry matter biomass yield

Compressed sward heights were measured with a rising plate meter (Correll et al., 2003) before the first cutting, and a total of ten measurements were performed within each experimental plot. To identify dry matter biomass (DMB) yield four herbage sub-samples were taken from randomly located sub-plots each of 50 cm × 25 cm within each experimental plot in each of three cuts. The harvested herbage was dried at 85^{o}C until totally desiccated and the DMB yield was then calculated. The mean of four sub-samples per experimental plot was used for statistical analysis.

2.5. Soil chemical properties

Soil samples were taken in September 2015 using an auger. Ten individual soil cores were taken from the 0 – 10 cm layer from randomly located areas within the inner 12 m^2 (2 m × 6 m) section of each plot after all above-ground plant debris had been removed. Samples were then combined into one representative sample per plot. The soil samples were then air-dried, ground in a mortar and sieved to particles of maximum of 2 mm after removal of any biomass residues and roots. All chemical analyses were performed in an accredited laboratory of the Crop Research Institute in Chomutov. Plant-available P, K, Ca and Mg were extracted by the Mehlich III method (Mehlich, 1984) and then determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Determination of pH ($CaCl_2$) and pH/H_2O was done using a pH meter (Sentron Wellinq, Leek, The Netherlands). Total N (N_{tot}) was analysed by the Kjeldahl method and organic carbon (C_{org}) by conventional oxidation procedure with chromo-sulphuric acid and colorimetry (AOAC, 1984).

2.6. Data analysis

A linear-mixed model (LMM) with block as random factor was used for the evaluation of the effect of treatment on number of plant species, Shannon index of diversity (H), species evenness index (J), cover of mosses (E_0), cover of vascular plant species (E_1), cover of functional groups (tall and short graminoids, tall and short forbs, legumes, ratio of tall/short species, ratio of graminoids/forbs), cover of selected species, compressed sward height, DMB yield and soil properties. If necessary, data were log-transformed to meet LMM assumptions. Benjamini-Hochberg's procedure was applied to control for false-discovery rate (FDR) (Verhoeven et al., 2005). To identify significant differences between individual treatments a post-hoc comparison using Tukey’s HSD test was applied. The relationships between soil and herbage characteristics were analysed by linear regression analysis. All univariate analyses were performed in Statistica 13.1 (Dell Inc., Texas, 2016).

Nutrient limitation of biomass production was quantified by DMB yield and computing the log response ratio (LRR) and critical threshold of LRR at $P= 0.05$, using the approach of Fay et al. (2015).

Redundancy analysis (RDA) in the CANOCO 5 program (ter Braak and Šmilauer, 2012) was used to evaluate multivariate vegetation, and soil and herbage chemical properties data. All plant cover, soil and herbage chemical properties 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 bi-plot ordination diagram was used.

3. Results

3.1. Soil chemical properties

A significant effect of different management treatments was observed for pH/H_2O, pH/$CaCl_2$, N_{tot} and measured concentrations of plant-available P, K, Ca and Mg (Fig. 1). The mean value of pH/H_2O was lowest in the NK treatment (pH 4.58) and highest in the P (5.61) and NP (5.53) treatments (Fig. 1a). There were similar trends for pH/$CaCl_2$ and values were lower than the pH 6.05 obtained in 1946 at the start of the experiment (Fig. 1b). The highest mean values of N_{tot} were found in the NK (3.9 g kg^{-1}) and in the NPK (3.8 g kg^{-1}) treatments, and the lowest value was in the K treatment (3.2 g kg^{-1}) (Fig. 1c). The highest mean values for plant available P were in the P (125.4 mg kg^{-1}) and PK (114.4 mg kg^{-1}) treatments and the lowest values were in the NK (23.1 mg kg^{-1}), N (28.4 mg kg^{-1}), K (32.6 mg kg^{-1}) and Control

(43.6 mg kg^{-1}) treatments (Fig. 1d). The highest mean value for plant available K was revealed in the K treatment (238.8 mg kg^{-1}), and the lowest value was in the NP (56.6 mg kg^{-1}) treatment (Fig. 1e). The highest mean plant available Ca concentrations were in the P (2148 mg kg^{-1}) and NP (2099 mg kg^{-1}) treatments, and the lowest values were in K (309 mg kg^{-1}) and NK (501 mg kg^{-1}) treatments (Fig. 1f). Further, the highest mean concentration of plant available Mg was found in the Control (134.9 mg kg^{-1}) treatment and the lowest concentration was in the NP (51.7 mg kg^{-1}) treatment (Fig. 1g). No significant effect of fertilization on the concentration of C_{org} was detected (Fig. 1h).

There were also some significant correlations between selected sward characteristics, the most abundant plant species and soil parameters (Table 2).

3.2. Plant species richness

Across all the studied plots there were 88 vascular plant species recorded (30 graminoids, 6 legumes, 50 other forbs and 2 seedlings of woody species). The mean cover values of these species are presented as supplementary material (Table S1). There was no significant effect of the different fertilization treatments on the total number of plant species, number of plant species with cover $\leq$ 0.1%, Shannon index of diversity (H) and species evenness index (J) (Fig. 2a).

3.3. Plant species composition

There was a significant effect of treatment on vascular plant (E_1) cover. The highest E_1 cover was in the NPK (98.0%), PK (96.5%) and NP (96.3%) treatments, and the lowest was in the Control (68.8%) treatment (Fig. 2b). The moss layer (E_0) was also significantly influenced by treatment, with the lowest cover of mosses in the NPK (6.3%), NP (7.0%) and PK (8.8%) treatments and the highest in the Control treatment (45.0%). *Rhytidiadelphus squarrosus* was the dominant moss species (Fig. 2b).

Plots of the NPK treatment had the highest proportion contributed by the functional group 'tall graminoids' (38.1%). The contributions of tall graminoids were lowest in the K (3.5%) and N (5.1%) treatments, with a tendency for higher proportions in the treatments with P fertilization (Fig. 2c). The N, NK and NP treatments also showed a tendency to have higher cover values of short graminoids (Fig. 2c). Both tall and short forbs had highest cover values in the PK treatment (23.3 and 61.5%, respectively) whereas the lowest cover values were in the N treatment (1.9 and 20.4%, respectively) (Fig. 2c).

Plots of the PK treatment had the highest mean cover of legumes (46.2%). In contrast, there were low mean cover values for legumes in treatments that received N applications: 0.8, 1.4 and 1.6%, for treatments NP, N and NK respectively (Fig. 2c). The NPK treatment was the only one of the treatments that included N to retain a relatively high cover of legumes (16.3%). The most abundant legumes recorded in the PK treatment tended to be *Trifolium repens, T. pratense* and *L. pratensis* (Fig. 3b).

Short species prevailed in all treatments. The highest ratio of tall/short species was observed in the NPK treatment (0.9); conversely, the N-only treatment showed the lowest ratio of tall/short species (0.1). The ratio of graminoids/forbs was also highest in the N-only treatment (3.5) (Fig. 2d).

The proportion of the sward contributed by *A. capillaris* varied from 36.3% in the N-only treatment to 1.8% in the NPK treatment. Apart from the NPK treatment there was a tendency for *A. capillaris* to be the dominant species in other N-application treatments (N, NK and NP) (Fig. 3a). *Anthoxanthum odoratum* was also an abundant short-grass species but it showed no significant response to fertilization (Table S1). *Trisetum flavescens* was present in relatively high proportions in treatments with P fertilization: for NPK, PK, NP and P its mean cover values were 30.0, 17.8, 16.3 and 12.0%, respectively. The lowest cover values for *T. flavescens* were in treatment K (0.4%) and Control (0.6%). No effect of treatment on cover was found for *F. rubra* agg. (Fig. 3a). The cover of *L. campestris* was highest in treatments N (9.3%), Control (6.3%) and K (5.5%) but was almost absent from the NPK treatment (0.1%) (Fig. 3a). Although *L. hispidus* was among the most abundant species recorded in the experiment, there were no effects of fertilization in relation to its cover (Fig. 3b). Among other forb species, *Achillea millefolium* had a significantly higher cover in the NK treatment (17.0%) than in other treatments (Fig. 3b). The cover of *Taraxacum officinale* agg. was also affected by treatment: it was highest in the NPK (5.1%) treatment and lowest in the K (0.2%) and Control (0.2%) treatments (Fig. 3b). *Alchemilla* spp. and *P. lanceolata* were not affected by fertilization (Table S1).

Based on the RDA analysis the fertilizer treatments explained 25.5% ($P < 0.001$) of variability on the first axis and 57.1% ($P < 0.001$) on all axes. Four groups of treatments with similar plant species composition were distinguished on the ordination diagram (Fig. 4, Fig. S2). The first group was created by P and PK treatments, the second by NP and NPK treatments, the third by N and NK treatments and the fourth by Control and K treatments. The first group was mainly connected with legumes (*T. pratense, T. repens, L. pratensis*) but also with *L. hispidus.* The second group was related to *P. pratensis, A. podagraria, R. acris, T.*

officinale agg., *Veronica chamaedrys, T. flavescens, B. perennis* and *P. lanceolata.* The position of *F. rubra* agg. in the ordination diagram was between the second and the third group. The third group was created predominantly by short graminoids (*A. capillaris, L. campestris* and *Carex* species), *Ajuga reptans* and *Hypochaeris radicata,* whereas *A. millefolium, Leucanthemum vulgaris* and mosses were connected with the fourth group.

3.4. Dry matter biomass yield and compressed sward height

The DMB yields from the first cut, as well as total DMB yield (sum of all 3 cuts), were significantly influenced by treatment (Fig. 5ab). The lowest DMB yield from the first cut was found in the K treatment (0.86 t ha^{-1}) and the highest was in the NP treatment (2.69 t ha^{-1}). The highest annual total DMB yields were recorded in the NPK (6.33 t ha^{-1}) and NP (5.72 t ha^{-1}) treatments, whereas the lowest yields were in the K (1.94 t ha^{-1}) and Control (2.10 t ha^{-1}) treatments.

The LRR was in the following order: K (-0.80) < N (0.40) < P (0.45) < NK (0.67) < PK (0.90) < NP (1.00) < NPK (1.10). The K treatment was the only one that did not exceed the threshold LLR (0.10) and provided evidence of nutrient limitation for N and P and co-limitation for NK, PK, NP and NPK.

Different fertilizer application treatments significantly influenced compressed sward height before the first cut (Fig. 5c). The lowest heights were recorded in the K and Control treatments with mean values 5.3 and 6.1 cm, respectively. On the other hand, the highest compressed sward height was in the NPK treatment with mean value 16.5 cm.

There were some significant correlations related to sward characteristics (Table 3).

4. Discussion

4.1. Soil chemical properties

In the P-only fertilisation treatment the P was supplied as basic slag from 1946 to 1997 and from 1998 as superphosphate. Both of these phosphate fertilisers also contain calcium which is likely to have increased soil Ca concentration and also increased the soil pH. The low soil pH in the K treatment where KCl was applied cannot be explained easily but may have been caused by displacement of Ca^{2+} ions from sorption complex in this treatment (Du et al., 2010; Tkaczyk et al., 2020). This was also reflected in the low soil Ca concentrations in the K and NK treatments.

Mineral-N fertilisers have been shown to lead to soil acidification (Galka et al., 2005; Honsová et al., 2007; Humbert et al., 2016), although when applied as calcium ammonium nitrate this effect is reduced as $CaCO_3$ contributes to the pH buffering capacity of soils (Bolan et al., 2003). As a consequence, the N-only treatment (ammonium nitrate) in the present study showed soil pH values either slightly more acidic (Fig. 1a) or similar (Fig. 1b) to the control. The other N-containing treatments reflect the presence of the other chemical compounds present in the fertiliser (NK had the lowest soil pH/H_2O) values due to KCl; NP had high soil pH/H_2O values due to Ca compounds in the phosphate fertiliser; Fig. 1a, b).

In addition, there was also a decline in soil pH /$CaCl_2$ recorded in the control treatment over the duration of the experiment since 1946. This effect was independent of fertiliser use and is likely to have been caused by atmospheric deposition of nitrogen and sulphur in the second half of the last century (Silvertown et al., 2006).

The highest soil K concentration in the K-only fertilisation (Fig. 1e) can be linked to low biomass yield (Fig. 5b) in this treatment, and likely to have been a result of surplus K fertiliser that was not taken up by the plants, and therefore not removed with the harvested herbage. In the treatments where K was applied in combination of other nutrients (NK, PK and NPK treatments) the DMB yield was increased relative to the control (Fig. 5b). The increased herbage yield in the treatments with combinations of nutrients resulted in more K being removed from the soil and thus soil K concentration was reduced.

Soil P and K concentrations in the control treatment remained sufficient to meet fertiliser recommendations for grassland, including those of the Czech Agriculture Department recommendations for grassland management (Anonymous, 2009). This was despite the long-term biomass removal without any additional P and K inputs. In the case of P, this element is very stable in soils (Janssens et al., 1998) and the quantities removed in herbage biomass are typically small (2- 4 kg P/ kg DM) (Hopkins et al., 1994; Marrs, 1993; Hrevušová et al., 2014). In the case of K, the mineral-rich alluvial clay soils on this site are able to release considerable amount of K (Schellberg et al., 1999; Hejcman et al., 2010) and thus maintain a sufficient K level in the soil despite the high amount of K removed with harvested herbage (Alfaro et al., 2003).

4.2. Plant species richness

Many previous studies have shown that long-term fertiliser applications lead to reduced plant species richness in grasslands (e.g. Silvertown, 1980; Schellberg et al., 1999; Hejcman et al., 2007; Kidd et al., 2017; Titěra et al., 2020). However, among the treatments examined in the Admont experiment we did not detect any significant effects of fertilisation on species richness. It is also unlikely that weather conditions before the vegetation data collection (autumn 2015 and spring 2016) would have influenced species richness and botanical composition.

Although there was no direct effect of fertiliser applications on the total number of species in the Admont Grassland Experiment, there was a positive relationship between the total number of species and soil pH/H_2O, the value of which was significantly influenced by the type of fertiliser used. A similar positive relationship has also been reported in other research (Silvertown, 1980; Gough et al., 2000; Crawley et al., 2005; Hejcman et al., 2014; Kidd et al., 2017). The explanation of negative influence of acidic soils on the number of species was described by Palpurina et al. (2007 and citations therein). The number of species in the sward can also be constrained by high phytotoxicity (high concentrations of Al^{3+}, Fe^{2+} and Mn^{2+}) as well as by nutrient limitation (deficiency of Ca^{2+}, Mg^{2+}, K^{+}) in acidic conditions (Palpurina et al., 2007).

In the Admont Grassland Experiment higher soil pH was associated with P fertilisation as the $CaCO_3$ present in superphosphate reduced soil acidity. Although high concentrations of extractable P in grassland soils are widely considered to be an important cause of reduced plant species richness (Janssens et al., 1998) the simultaneous increase in soil pH from the $CaCO_3$ in superphosphate in the Admont Grassland Experiment probably reduced the otherwise detrimental effect of P inputs on species richness. Nevertheless, reductions in the number of species following P fertilisation have been found to occur in studies conducted under several different conditions. Examples include Critchley et al. (2002) (a survey of soils of temperate lowland grasslands in the UK), Hejcman et al. (2014) (Steinach Grassland Experiment in Germany, on alluvial meadows, established in 1993) and Titěra et al. (2020) (Rengen Grassland Experiment in Germany, on upland meadow, established in 1942). In contrast, Kidd et al. (2017) reported that the highest number of species was observed under a P-only fertilisation treatment in the Palace Leas Hay Meadow experiment (a lowland grassland experiment, established in 1897 in the UK). In the Admont Grassland Experiment, this P treatment of 60 kg P ha^{-1} $year^{-1}$ was applied as basic slag and later as triple superphosphate, and the soil pH/H_2O was 4.9 and P concentration in the soil (Olsen method: Murphy and Riley, 1962) was 27.95 mg kg^{-1}. It seems that in the case of the Palace Leas Hay Meadow a potential for negative effect of P fertilisation on species richness was suppressed by less acidic soil conditions in the plots receiving P fertilisation and also by very low P availability. For comparison, in the Admont Grassland Experiment, where no effect of P fertilisation on species richness was revealed, soil pH and P concentration in the soil were higher than at Palace Leas Hay Meadow, and therefore the different responses to P fertilisation may be linked to differences in soil conditions.

In the Admont Grassland Experiment there was also no effect of N fertilisation on species richness. The potential for fertiliser N application to have negative effects on species richness is likely to have been reduced by the low availability of P in the N-only and NK treatments, as observed in several other studies (e.g. Janssens et al., 1998; Hejcman et al., 2007; Titěra et al., 2020). In an international 45-site study of nutrient-addition experiments (Harpole et al., 2016), it was revealed that greater loss of diversity was associated with higher P and K concentrations in the soil, but not with higher N supply.

4.3. Plant species composition

In the nitrogen-only fertilisation treatment there was a shift in sward composition towards dominance by graminoid species. This is consistent with most results from long-term experiments (Silvertown et al., 2006; Honsová et al., 2007; Kidd et al., 2017). Although N fertilisation is generally considered to support tall graminoids (Hejcman et al., 2014), the predominance of short graminoids over tall ones in this treatment was probably caused by an imbalance of nutrients together with the relatively high frequency of cutting.

The suppression of legumes in treatments with N application (N, NP, NK) contrasts with the increased proportion of graminoids, and was likely caused by the negative effect of higher soil N concentration on the competitive abilities of legumes within the grassland community (Kramberger et al., 2015) and also on symbiotic relationships between legumes and root nodule bacteria (Velich, 1986; Honsová et al., 2007). On the other hand, legumes (especially *T. repens*, *T. pratense*, *L. pratensis*) were most supported by the fertilisation treatments of PK, NPK and K. Similar findings were shown previously by Velich (1986), Honsová et al. (2007), Pavlů et al. (2012), Kidd et al. (2017), Zarzycki and Kopeć (2020).

In the treatments where P was supplied in combination with other nutrients, the higher inputs of nutrients led to an increase in the cover of vascular plant species as well as an increase in sward height and DMB yield. This would have increased the competitive pressure from vascular plant species and, together with lower light availability in the moss layer, resulted in a decline of moss cover. This indirect negative effect of fertilisation on moss cover has also been detected in other experiments (Hejcman et al., 2007; Kidd et al., 2017).

Trisetum flavescens was the only tall grass species that was tolerant of the three-cut frequency. The higher cover of *T. flavescens* in all the treatments with P application could be connected with its high tolerance to a wide range of fertiliser levels as well as to the cutting frequency (Grime et al., 1988). The positive relationship between *T. flavescens* and soil P concentration may be linked to the considerable amount of Ca contained in the P fertilisers, which also generally supports occurrence of *T. flavescens* (Grime et al., 1988). In contrast, low availability of P in the treatments without P application had a tendency to restrict the persistence and dominance of highly productive and tall growing grass species such as *T. flavescens*, as also described by Hejcman et al. (2007).

Agrostis capillaris showed a tendency to be the most abundant of the short grass species especially in treatments with N application (except the NPK treatment). It usually shows better growth in swards on soils with low P soil status than the more rapidly growing tall grasses, against which it less competitive (Hejcman et al., 2014). This was also confirmed in the Admont Grassland Experiment.

Festuca rubra is known for its plasticity to different frequencies of defoliation (Grime et al., 1988; Pavlů et al., 2007, 2011, 2012; Gaisler et al., 2013) and fertiliser application (Grime et al., 1988; Galka et al., 2005; Honsová et al., 2007; Pavlů et al., 2012; Hejcman et al., 2014; Kidd et al., 2017). Its abundance was found to be low in all treatments in the Admont Grassland Experiment. This could be explained by the local site conditions (Gleyic Fluvic Dystric Cambisol with no supplementary qualifiers) in combination with higher cutting frequency that was applied in all treatments. *Leontodon hispidus* was the most frequent non-legume forb in most treatments of the Admont Grassland Experiment. Grime et al. (1988) describes *L. hispidus* as a species of unproductive grasslands, but at Admont its occurrence was highly variable and could not be related to fertiliser-treatment differences. Therefore, it seems that cutting frequency, which reduced the competitiveness of other species, was a stronger factor than soil nutrient status. A positive correlation of *P. lanceolata* and a negative *A. millefolium* relationship, in terms of their cover values and soil Ca concentration, as well as soil pH, in the Admont Grassland Experiment is consistent with the ecology of these species as described by Grime et al. (1988). They described *P. lanceolata* as a species of soils with higher pH, and *A. millefolium* as a species of acidic soils.

4.4. DMB yield and compressed sward height

In the K treatment the total DMB yield was similar to the Control treatment, which suggests that there was no K limitation in the Control treatment. In the P and N treatments, however, the total DMB yields were increased relative to the Control treatment by an average of 57% (LRR = 0.45) and 49% (LRR = 0.40), which indicates there was single P and N limitation of biomass production. Although biomass production was not limited by K (LRR = -0.08), all combination of nutrients with K pointed to there being a K co-limitation, and combined applications of NK and PK increased DMB yields by an average 95% (LRR = 0.67) and 146% (LRR = 0.90) over the Control treatment. Nevertheless, a total DMB yield of over 5 t ha^{-1} $year^{-1}$ was revealed in all nutrient combinations with P. The combined applications in PK, NP and NPK treatments increased biomass production by an average of 146% (LRR = 0.90), 172% (LRR = 1.00) and 200% (LRR = 1.1) compared to the Control treatment, respectively. As response to multiple-nutrient treatments was greater than the sum of the responses to each nutrient added individually, all types of co-limitation revealed in the Admont Grassland Experiment were synergistic. This finding is consistent with Fay et al. (2015) who report there is a synergistic co-limitation of N, P and K in grasslands worldwide.

There is a widely accepted opinion, supported by many authors, about the negative influence that high biomass productivity and sward height exert on plant species richness (Silvertown, 1980; Critchley et al., 2002; Galka et al., 2005; Clark et al., 2007; Honsová et al., 2007; Humbert et al., 2016; Zarzycki and Kopeć, 2020). The findings we report in this study are not fully consistent with these results. We did not detect any relationships between the total number of species or DMB yield of the first cut and sward height, and this applied to all treatments. Nevertheless, the negative effect of sward height on the occurrence of species with cover values of less than or equal to 0.1% in the Admont Grassland Experiment (i.e. predominantly very short species) is important for species diversity evaluation as these species contributed around half of the species richness.

We propose two reasons for explaining why short plant species dominated in all treatments in the Admont Grassland Experiment where three-cut frequency was applied. First, the effect of cutting is more damaging for tall species than short ones (Pavlů et al., 2011; Gaisler et al., 2019) because it removes a greater proportion of the above ground biomass of tall plants. Secondly, a frequent cutting regime resulted in reduction of vegetation shading and light exclusion, and thus short species are not disadvantaged by the presence of taller and more vigorously growing plants (Pavlů et al., 2011; Pavlů et al., 2016).

5. Conclusion

The results from the alluvial meadow of the Admont Grassland Experiment showed that the long-term different fertilisation treatments affected soil pH and nutrient concentrations in the soil, and also the plant species composition. However, no significant effects on species richness characteristics were revealed. Short species (< 0.5 m height) prevailed in all treatments regardless of nutrient application, and this outcome was probably linked to the common management of a three-cut defoliation.

Total DMB yield was limited by N and P and synergisticly co-limited by N, P and K. The experiment revealed that a long-term biomass yield of more than 5 t ha^{-1} DMB per year can be achieved with any nutrient combination containing P without loss of species richness.

In contrast to our findings reported here for grassland under a three-cut defoliation, other long-term experiments in Europe that have focused on grassland fertilisation have been based on either single cut or two cuts per year, a system that is likely to lead to reduced light penetration in the sward. Therefore, the Admont Grassland Experiment may be regarded as unique among long-term grassland experiments as it allows the study of long-term changes in soil and plant characteristics of fertilised agricultural grassland under a three-cut regime, a management system more representative of current agricultural grassland practice in Europe.

CRediT authorship contribution statement

Lenka Pavlů: Investigation, Conceptualization, Methodology, Writing - original draft. **Erich M. Poetsch**: Conceptualization, Methodology, Writing - review & editing. **Pavlů V. Vilém**: Investigation, Formal analysis, Visualization, Writing - original draft. **Jan Titěra**: Investigation, Formal analysis. **Michal Hejcman**: Investigation, Formal analysis. **Jan Gaisler**: Investigation, Visualization. **Alan Hopkins**: Writing - review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at: https://doi.org/10.1016/j.scitotenv.2021.152081

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List of Appendices

Fig. S1 Aerial view of the Admont Grassland Experiment (© Google 2015) with the design of the experiment and the dose of fertilizers for individual treatment.

Fig. S2. Ordination diagram presenting the results of the RDA analysis showing samples of individual treatments.

Table S1 The mean covers (in %) of vascular plant species for treatments (four replicates each) recorded in May 2016.

Table 1
Mean temperature and average precipitation for the year 2015, 2016 and period 2007–2016 (meteorological station at Hall)

	Mean temperature (°C)			Mean precipitation (mm)		
Month/Year	2015	2016	2007–16	2015	2016	2007–16
January	-0.8	-1.9	-2.0	126.5	128.1	95.3
February	-0.5	3.0	-0.1	46.0	111.6	65.3
March	4.3	4.6	4.4	74.3	37.5	62.8
April	8.3	9.4	9.3	92.8	59.9	61.8
May	13.3	12.9	13.2	149.1	180.6	142.7
June	17.3	17.5	16.8	103.2	204.0	170.1
July	20.8	19.3	18.8	171.5	262.9	183.3
August	20.2	18.1	18.4	65.1	188.6	148.4
September	13.0	15.8	14.0	124.9	127.9	129.8
October	9.2	8.7	9.1	73.9	108.3	94.2
November	5.2	2.7	3.8	48.9	52.5	59.8
December	-0.4	-1.3	-1.1	25.8	75.1	63.6
Mean	**9.2**	**9.1**	**8.7**	**1102.0**	**1537.0**	**1277.0**

Table 2
Correlation (r) between selected sward characteristics, plant species and soil parameters. Abbreviation: TNPS – total number of plant species, NPS≤0.1 – number of plant species ≤ 0.1%, DMB Yield 1st cut – dry matter biomass yield at the 1st cut, Total DMB Yield – total dry matter biomass yield as a sum of all 3 cuts, CSH – compressed sward height; P, K, Ca, Mg – plant available nutrients in the soil. Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). P – value = corresponding probability value.

Variables	pH/H_2O	P	K	Ca	Mg
TNPS	0.39*	0.19	-0.15	0.29	-0.15
NPS≤0.1	0.01	-0.20	0.11	-0.15	-0.17
DMB Yield 1st cut	0.22	0.35*	-0.39*	0.46**	-0.33
Total DMB yield	0.24	0.44*	-0.31	0.46**	-0.27
Graminoids					
Agrostis capillaris	-0.32	-0.46**	-0.26	-0.18	-0.44*
Anthoxanthum odoratum	-0.08	-0.21	-0.27	0.01	-0.31
Festuca rubra agg.	0.20	-0.18	-0.38*	0.23	-0.42*
Luzula campestris	-0.28	-0.39*	-0.14	-0.28	-0.17
Poa pratensis	0.63***	0.34	-0.53**	0.66***	-0.40*
Trisetum flavescens	0.41*	0.58***	-0.20	0.51**	-0.09
Forbs					
Achillea millefolium	-0.60***	-0.46**	0.35	-0.54**	0.08

Lathyrus pratensis	0.02	0.44*	0.01	0.11	0.24
Leontodon hispidus	-0.06	0.30	0.40*	-0.03	0.56***
Plantago lanceolata	0.47**	0.31	-0.28	0.54**	-0.23
Taraxacum officinale agg.	0.28	0.22	-0.24	0.33	-0.24
Trifolium pratense	0.11	0.40*	0.34	0.02	0.48**
Trifolium repens	-0.19	0.34	0.23	-0.15	0.35

Table 3
Correlation (r) between selected sward characteristics. Abbreviation: E_1 – cover of vascular plant species, E_0 – cover of mosses, TNPS - total number of plant species, NPS≤0.1 – number of plant species ≤ 0.1%, CSH – compressed sward height, DMB Yield 1st cut – dry matter biomass yield at the 1st cut. Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). P – value = corresponding probability value.

Variable	E_1	E_0	TNPS	NPS≤0.1	CSH
E_0	-0.84 ***				
TNPS	0.01	0.017			
NPS≤0.1	-0.38*	0.38*	0.59***		
CSH	0.84***	-0.73***	0.1	-0.37*	
DMB Yield 1st cut	0.85***	-0.72***	0.07	-0.33	0.90***

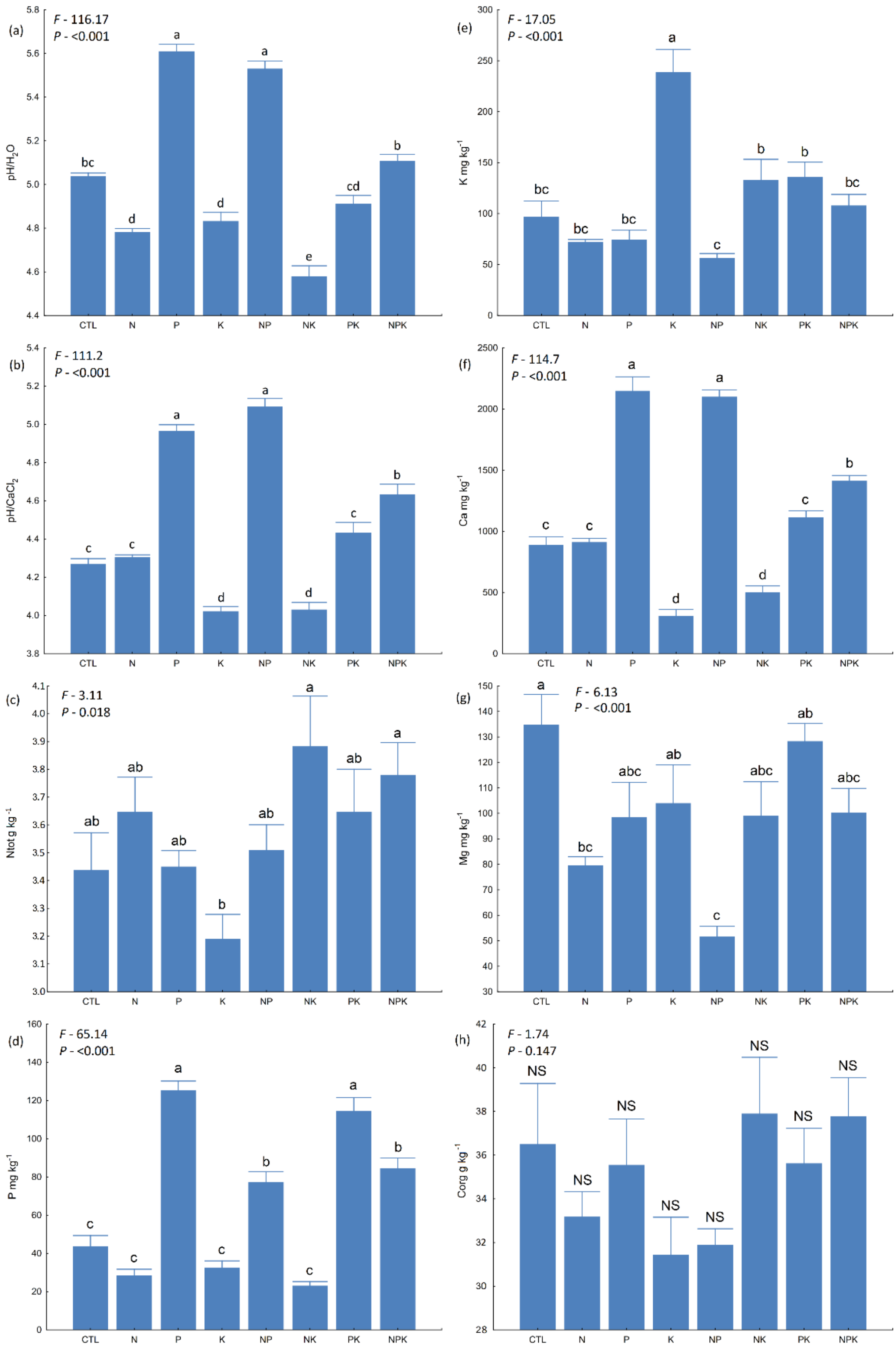

Fig. 1. Effect of fertilizer treatment on soil chemical properties: a) pH/H_2O, b) pH/$CaCl_2$, c) N_{tot}, d) P, e) K, f) Ca, g) Mg, h) C_{org}. Treatment abbreviations: CTL – non-fertilized control, N – N fertilization, P – P fertilization, K – K fertilization, NK – NK fertilization, NP – NP fertilization, PK – PK fertilization, NPK – NPK fertilization. In cases of significant differences obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction, the post-hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments, which are indicated by different small letters. Error bars represent standard error of the mean.

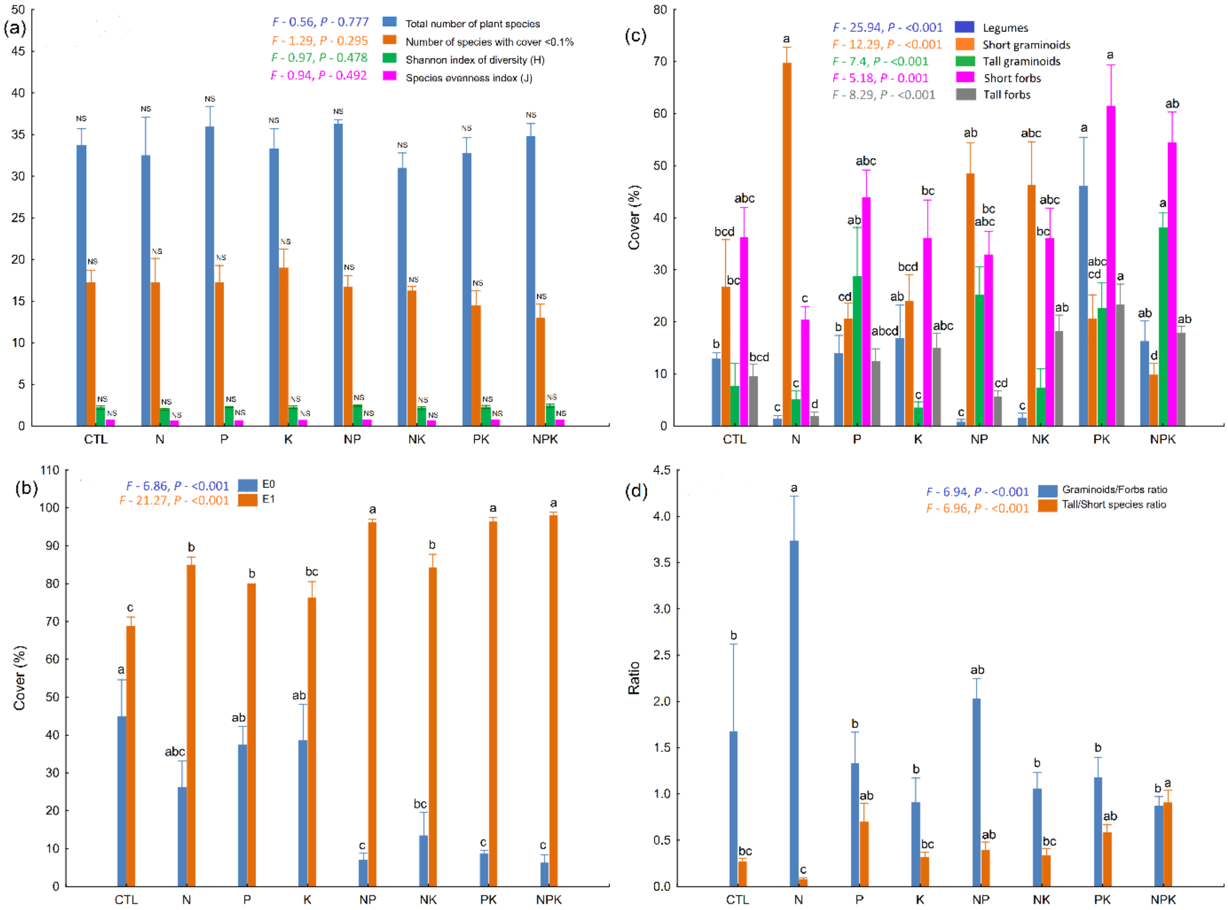

Fig. 2. Effect of fertilizer treatment on: a) species richness, b) cover (%) of E_0 (mosses) and E_1 (cover of vascular plant species), c) cover (%) of functional groups, d) ratios of graminoids/forbs and tall/short species. Treatment abbreviations are given in Fig. 1. In cases of significant differences obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction, the post-hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments, which are indicated by different small letters. Error bars represent standard error of the mean.

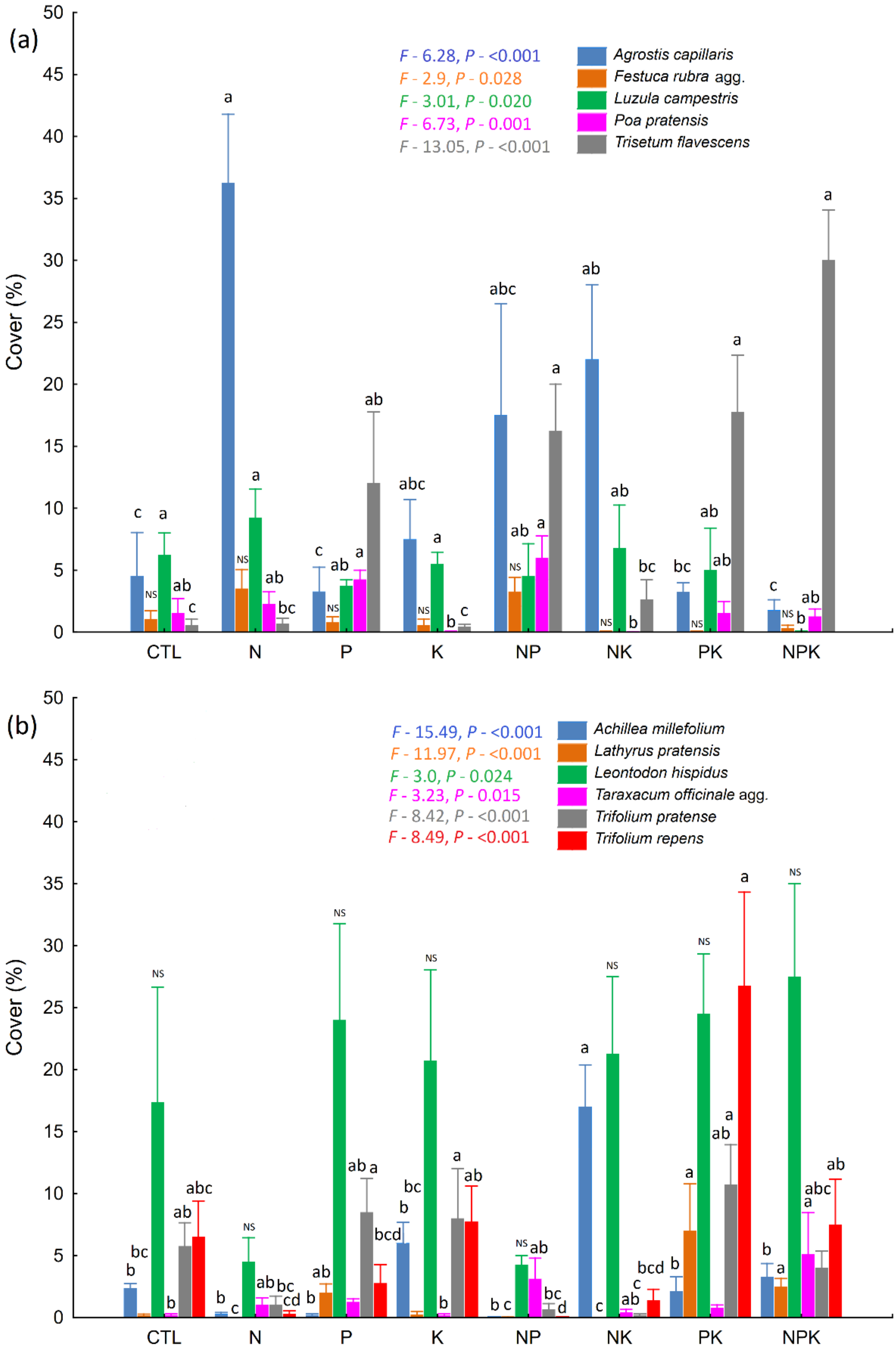

Fig 3. Effect of fertilizer treatment on cover (%) of the most abundant vascular plant species: a) graminoids, b) forbs. Treatment abbreviations are given in Fig. 1. In cases of significant differences obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction, the post-hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments, which are indicated by different small letters. Error bars represent standard error of the mean.

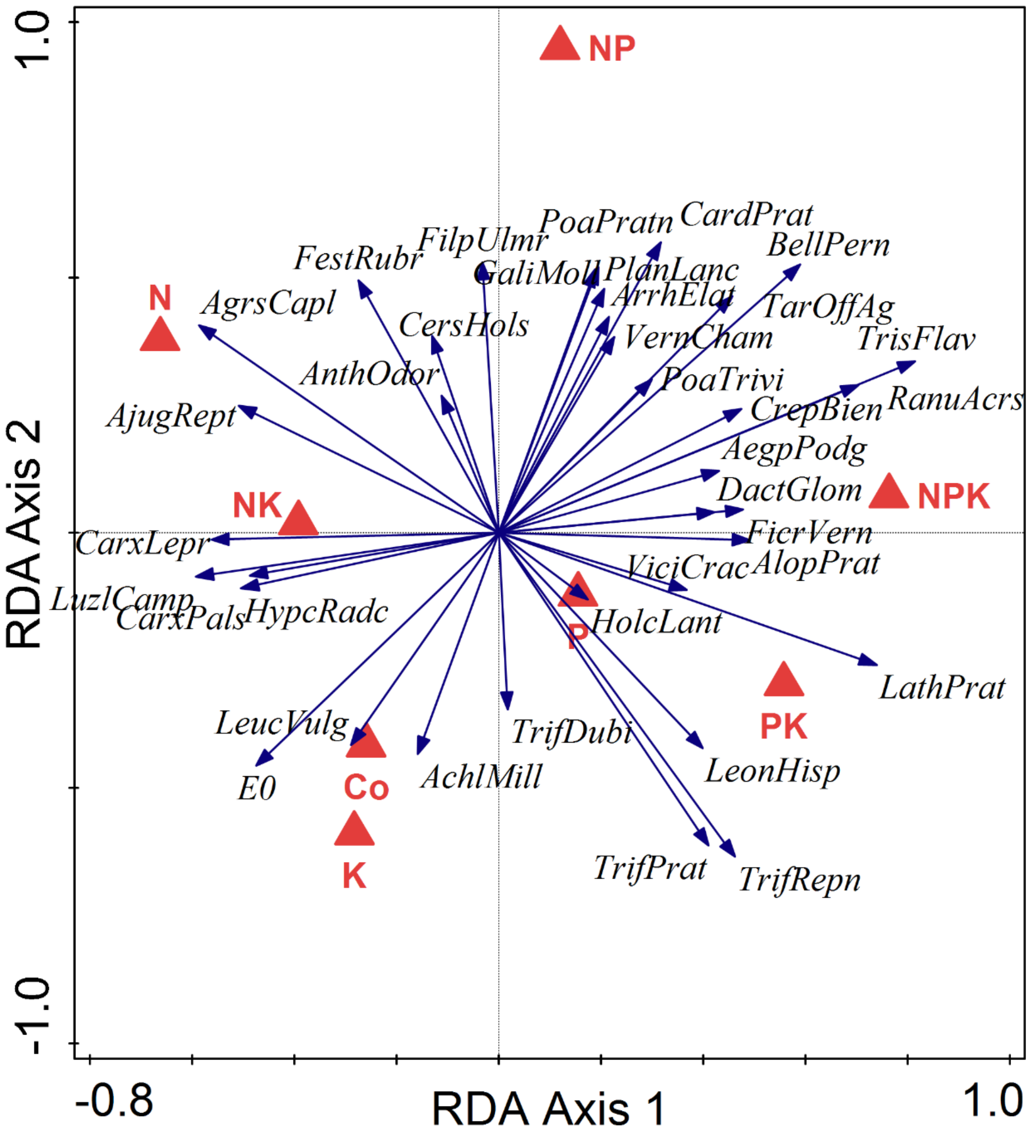

Fig. 4. Ordination diagram presenting the results of the RDA analysis showing changes in plant species composition, with treatments used as predictors. Treatment abbreviations are given in Fig. 1. Species abbreviations: *AchlMill* = *Achillea millefolium*, *AegpPodg* = *Aegopodium podagraria*, *AnthOdor* = *Anthoxanthum odoratum*, *AgrsCapl* = *Agrostis capillaris*, *AjugRept* = *Ajuga reptans*, *AlopPrat* = *Alopecurus pratensis*, *ArrhElat* = *Arrhenatherum elatius*, *BellPern* = *Bellis perennis*, *CardPrat* = *Cardamine pratensis*, *CarxLepr* = *Carex leporina*, *CarxPals* = *Carex pallescens*, *CrepBien* = *Crepis biennis*, *DactGlom* = *Dactylis glomerata*, *E0* = mosses, *FestRubr* = *Festuca rubra* agg., *FicrVern* = *Ficaria verna*, *FilpUlmr* = *Filipendula ulmaria*, *GaliMoll* = *Galium mollugo*, *HolcLant* = *Holcus lanatus*, *HypcRadc* = *Hypochaeris radicata*, *LathPrat* = *Lathyrus pratensis*, *LeonHisp* = *Leontodon hispidus*, *LeucVulg* = *Leucanthemum vulgare*, *LuzlCamp* = *Luzula campestris*, *PlanLanc* = *Plantago lanceolata*, *PoaPratn* = *Poa pratensis*, *PoaTrivi* = *Poa trivialis*, *RanuAcrs* = *Ranunculus acris*, *StelGram* = *Stellaria graminea*, *TarOffag* = *Taraxacum officinale* agg., *TrifDubi* = *Trifolium dubium*, *TrifPrat* = *Trifolium pratense*, *TrifRepn* = *Trifolium repens*, *TrisFlav* = *Trisetum flavescens*, *VernArvn* = *Veronica arvensis*, *VernCham* = *Veronica chamaedris*, *ViciCrac* = *Vica cracca*.

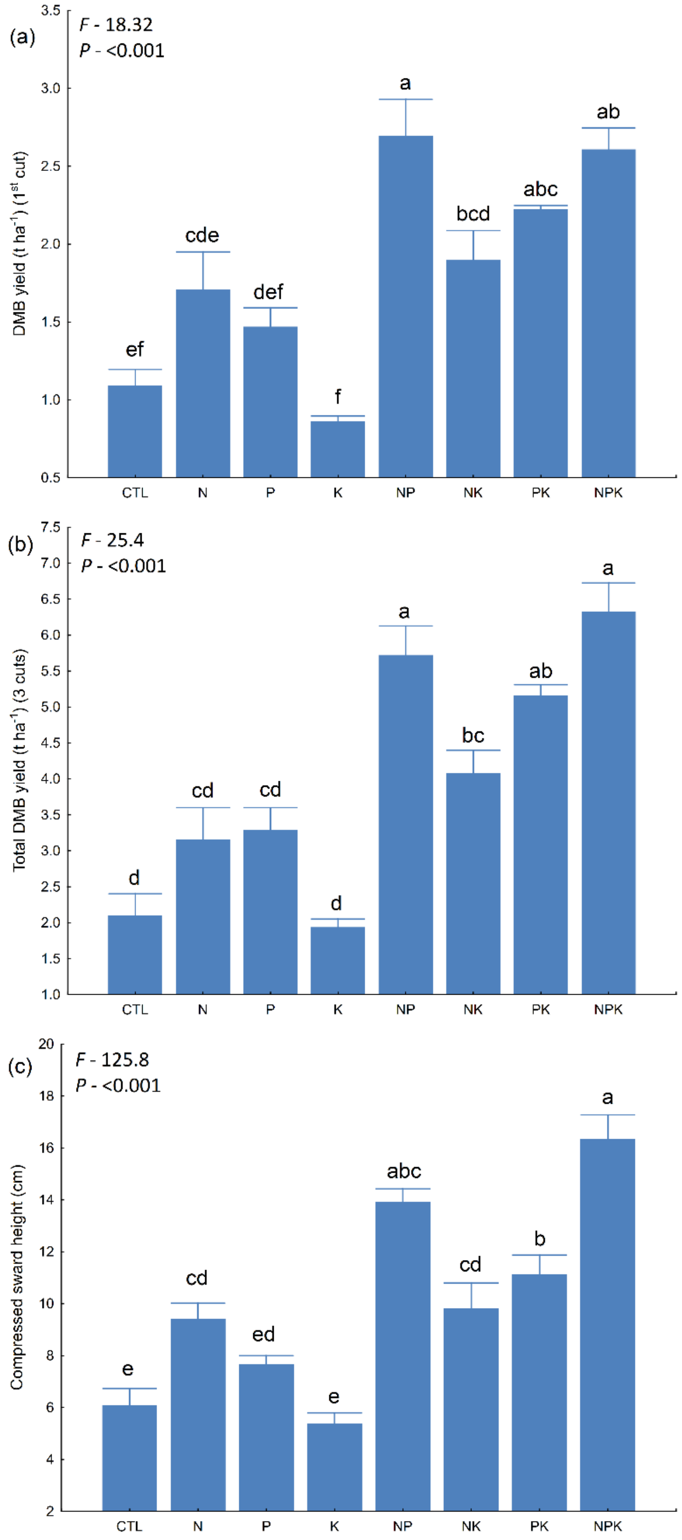

Fig. 5. Effect of fertilizer treatment on a) dry matter biomass (DMB) yield before the 1st cut, b) total DMB yield, c) compressed sward height. Treatment abbreviations are given in Fig. 1. In cases of significant differences ($P < 0.05$) obtained by linear mixed-effects modelling, the post hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments, which are indicated by different small letters. Error bars represent standard error of the mean.

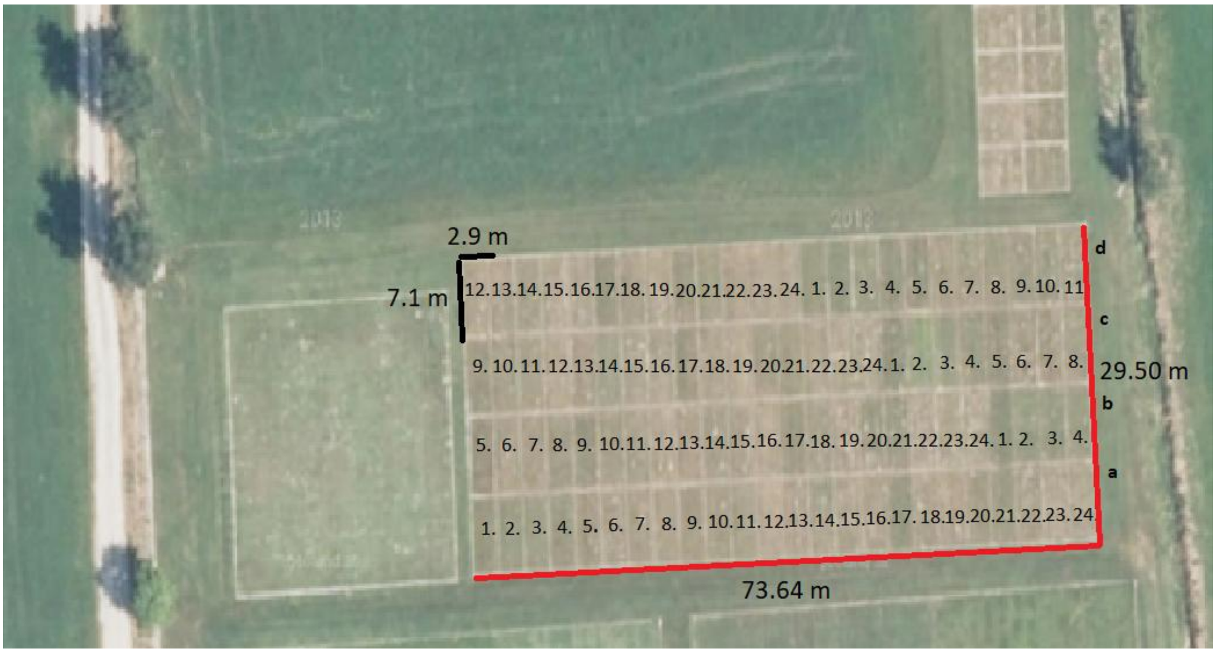

Fig. S1 Aerial view of the Admont Grassland Experiment (© Google 2015) with the design of the experiment and the dose of fertilizers for individual treatment.

Number of treatment	Dose of fertilizers
1	non (unfertilized)
2	923 Ca kg ha^{-1} every third year
3	35 P kg ha^{-1} per year
4	100 K kg ha^{-1} per year
5	80 N kg ha^{-1} (40 + 40) per year
6	100 K + 80 N kg ha^{-1} per year
7	35 P + 80 N kg ha^{-1} per year
8	35 P + 100 K kg ha^{-1} per year
9	17.5 P + 50 K kg ha^{-1} per year
10	17.5 P + 50 K + 40 N kg ha^{-1} per year
11	35 P + 100 K + 40 N kg ha^{-1} per year
12	35 P + 100 K + 80 N kg ha^{-1} per year
13	17.5 P + 50 K + 80 N kg ha^{-1} per year
14	35 P + 100 K kg ha^{-1} per year and 923 Ca every third year
15	35 P + 100 K + 80 N kg ha^{-1} per year and 923 Ca every third year
16	15 t ha^{-1} stable manure every year

17	15 t ha^{-1} stable manure every third year
18	15 t ha^{-1} stable manure one year/ 35 P + 100 K kg ha^{-1} second year / 35 P + 100 K kg ha^{-1} third year
19	15 t ha^{-1} stable manure first year / 35 P + 100 K + 80 N kg ha^{-1} second year / 35 P + 100 K + 80 N kg ha^{-1} third year
20	40 t ha^{-1} slurry (20 + 20) every year
21	35 P kg ha^{-1} one year / 40 t ha^{-1} slurry second year
22	15 t ha^{-1} stable manure one year/ 40 t ha^{-1} slurry second year
23	35 P + 100 K + 923 Ca kg ha^{-1} first year / 15 t ha^{-1} stable manure second year/ 35 P + 100 K + 80 N kg ha^{-1} third year / 40 t ha^{-1} t slurry fourth year
24	35 P + 100 K + 923 Ca kg ha^{-1} first year / 15 t ha^{-1} stable manure + 17.5 P + 50 K kg ha^{-1} second year/ 17.5 P + 50 K + 40 N kg ha^{-1} third year

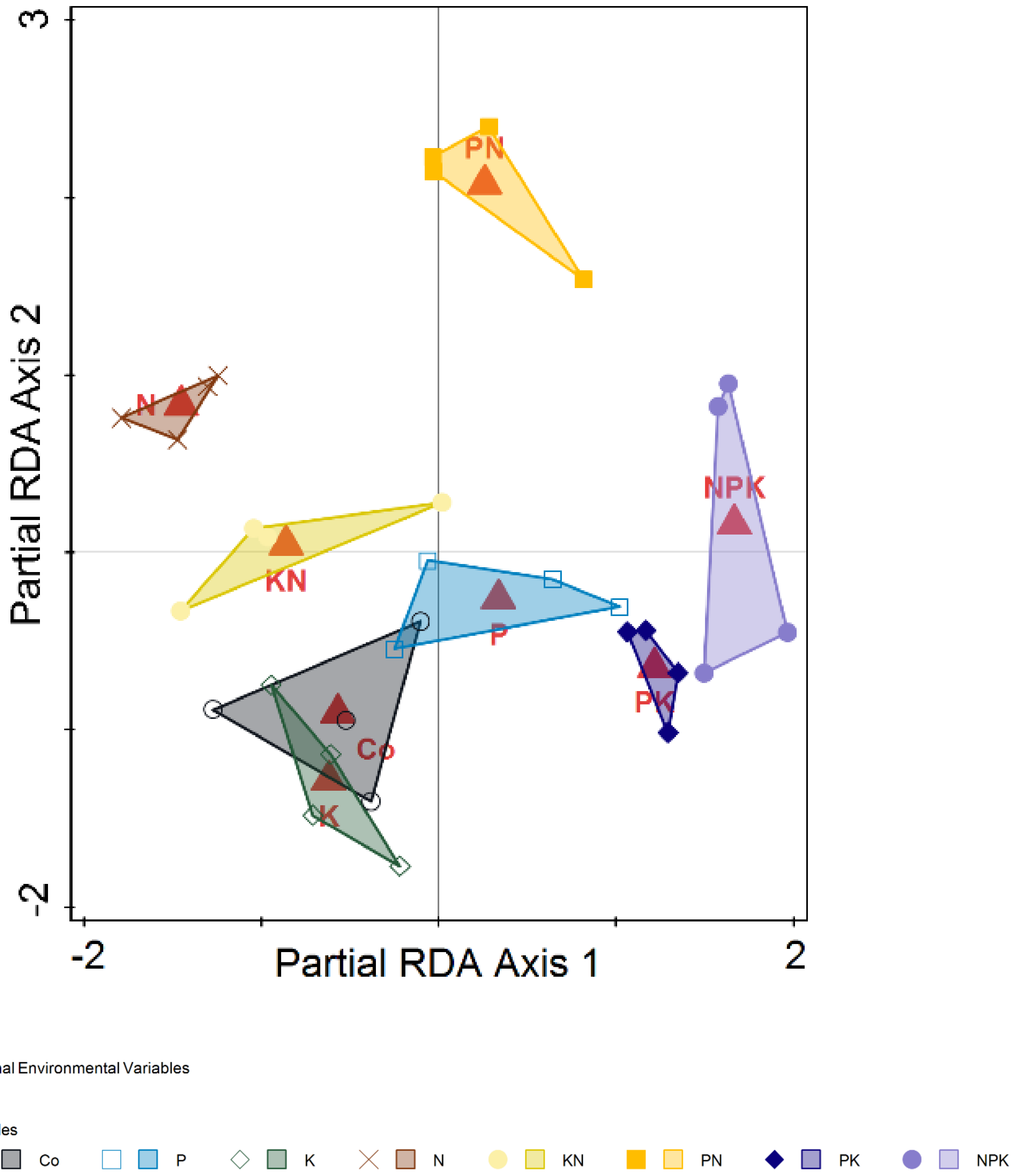

Fig. S2. Ordination diagram presenting the results of the RDA analysis showing samples of individual treatments. Treatment abbreviations are given in Fig. 1.

Table S1 The mean covers (in %) of vascular plant species for treatments (four replicates each) recorded in May 2016. Treatment abbreviations: CTL – non-fertilized control, N – N fertilization, P – P fertilization, K – K fertilization, KN – KN fertilization, PN – PN fertilization, PK – PK fertilization, NPK – NPK fertilization.

Species	Treatment							
	CTL	P	K	N	KN	PN	PK	NPK
Graminoids								
Agrostis capillaris	4.53	3.28	7.50	36.25	22.00	17.50	3.25	1.78
Agrostis stolonifera	0.00	0.00	3.50	0.00	0.00	0.00	0.00	0.00
Alopecurus pratensis	0.03	0.03	0.00	0.00	0.53	0.08	0.80	2.05
Anthoxanthum odoratum	13.00	8.75	7.00	20.00	17.25	20.50	10.75	6.75
Arrhenatherum elatius	0.00	0.00	0.00	0.03	1.05	2.03	0.28	0.80
Avenula pubescens	0.00	1.28	0.00	0.00	0.00	0.03	0.03	0.05
Brachypodium pinnatum	5.00	12.50	0.75	0.00	0.00	0.00	0.00	0.00
Briza media	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00
Bromus erectus	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Carex flacca	0.25	0.00	0.00	0.28	0.00	0.00	0.00	0.00
Carex flava	0.03	0.05	0.03	0.03	0.00	0.00	0.00	0.00
Carex hirta	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Carex leporina	0.63	0.00	0.18	0.88	0.13	0.00	0.03	0.00
Carex pallescens	0.50	0.03	0.30	0.78	0.18	0.00	0.00	0.00
Carex panicea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carex pilulifera	0.05	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Carex sylvatica	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00
Dactylis glomerata	0.80	1.03	1.53	0.33	2.63	2.28	3.25	3.25
Deschampsia cespitosa	0.03	0.00	0.03	0.08	0.20	0.53	0.05	0.05
Elymus repens	0.00	0.00	0.03	0.05	0.13	0.08	0.03	0.25
Festuca arundinacea	0.00	0.00	0.00	0.25	0.00	0.00	0.25	0.00
Festuca pratensis	0.15	0.30	0.00	0.05	0.03	0.20	0.00	0.28
Festuca rubra agg.	1.05	0.80	0.58	3.53	0.08	3.28	0.08	0.33
Holcus lanatus	0.03	0.78	0.20	0.08	0.08	0.00	0.05	0.50
Juncus sp.	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00
Luzula campestris	6.25	3.75	5.50	9.25	6.78	4.53	5.03	0.05
Poa annua	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Poa pratensis	1.55	4.25	0.05	2.28	0.03	6.00	1.55	1.28
Poa trivialis	0.00	0.00	0.00	0.00	0.00	0.53	0.05	0.53
Trisetum flavescens	0.58	12.03	0.43	0.68	2.65	16.25	17.75	30.00
Legumes								
Lathyrus pratensis	0.15	2.00	0.25	0.00	0.00	0.03	7.00	2.50
Trifolium dubium	0.50	0.25	0.90	0.05	0.03	0.00	0.28	0.25
Trifolium pratense	5.75	8.50	8.00	1.05	0.20	0.68	10.75	4.00
Trifolium repens	6.50	2.78	7.75	0.30	1.40	0.10	26.75	7.50
Vicia cracca	0.08	0.53	0.03	0.00	0.00	0.00	1.25	1.03
Vicia sepium	0.00	0.00	0.00	0.00	0.00	0.00	0.15	1.03
Forbs								

Aegopodium podagraria	0.03	0.00	0.00	0.03	0.55	0.15	0.03	2.28
Achillea millefolium	2.38	0.20	6.00	0.28	17.00	0.10	2.15	3.28
Ajuga reptans	0.75	0.55	0.08	3.75	2.53	0.55	0.08	0.25
Alchemilla sp.	2.50	1.25	0.33	1.15	0.60	1.38	0.33	2.03
Anemone nemorosa	0.00	0.13	0.28	0.03	1.25	0.13	0.00	0.00
Bellis perennis	0.10	0.68	0.10	0.43	0.33	4.25	2.00	1.75
Campanula patula	0.15	0.05	0.10	0.05	0.05	0.05	0.08	0.05
Cardamine pratensis	0.10	0.10	0.08	0.05	0.08	2.28	0.43	0.43
Centaurea pseudophrygia	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00
Cerastium holosteoides	0.08	0.18	0.13	0.55	0.58	0.80	0.20	0.10
Cirsium oleraceum	0.00	0.05	0.00	0.00	0.00	0.00	0.15	0.00
Crepis biennis	0.00	0.05	0.00	0.00	0.00	0.28	0.03	0.75
Dactylorhiza sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Euonymus europaeus juv.	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Euphrasia sp.	0.03	0.03	0.05	0.03	0.05	0.03	0.03	0.00
Ficaria verna	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.13
Filipendula ulmaria	0.03	0.01	0.00	0.13	0.00	0.65	0.00	0.00
Galium mollugo	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.18
Galium palustre	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
Galium uliginosum	0.03	0.13	0.03	0.03	0.00	0.08	0.00	0.00
Glechoma hederacea	0.05	0.03	0.00	0.03	0.05	0.05	0.05	0.20
Hieracium subg. *Pilosella*	1.00	3.75	0.00	0.00	0.13	0.00	0.00	0.00
Hypericum maculatum	0.05	0.08	0.08	0.08	0.03	0.05	0.00	0.03
Hypochoeris radicata	2.08	0.30	2.78	2.78	2.78	0.10	0.28	0.05
Leontodon hispidus	17.38	24.00	20.75	4.50	21.25	4.25	24.50	27.50
Leucanthemum vulgare	0.15	0.00	0.30	0.00	0.13	0.03	0.03	0.00
Listera ovata	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Lychnis flos-cuculi	0.00	0.03	0.00	0.00	0.00	0.00	0.03	0.03
Lysimachia nummularia	0.03	0.00	0.03	0.03	0.03	0.05	0.08	0.28
Mentha arvensis	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00
Myosotis arvensis	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.03
Pimpinella major	0.03	0.08	0.05	0.05	0.00	0.05	0.15	0.03
Plantago lanceolata	1.88	7.28	1.63	3.00	3.50	10.50	3.00	4.50
Platanthera sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polygonum bistorta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Primula sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Prunella vulgaris	0.33	0.43	0.33	1.75	0.80	1.05	1.28	0.18
Prunus padus juv.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ranunculus acris	0.68	0.83	0.33	0.13	0.33	2.75	1.75	3.75
Ranunculus repens	0.00	0.00	0.00	0.03	0.00	0.03	0.00	0.00
Rumex acetosa	0.10	0.08	0.10	0.08	0.08	0.33	0.08	0.08
Rumex acetosella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sagina procumbens	0.03	0.03	0.00	0.00	0.00	0.00	0.03	0.00
Scorzoneroides autumnalis	0.00	0.00	0.05	0.00	0.00	0.03	0.00	0.00
Stellaria graminea	0.08	0.05	0.08	0.08	0.08	0.08	0.03	0.00
Symphytum officinale	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Taraxacum officinale agg.	0.20	1.25	0.18	1.05	0.43	3.13	0.78	5.13
Tragopogon sp.	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00

Veronica arvensis	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.03
Veronica chamaedrys	2.25	0.53	0.03	0.68	0.05	3.78	1.05	1.03
Veronica officinalis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Veronica serpyllifolia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Chapter V

Effect of different management measures on *Rumex obtusifolius* occurrence in a mountain grasslands

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Abstract

Rumex obtusifolius infestation is a serious problem in gathering places of livestock in mountain grasslands. In order to control this weed a long term experiment was established in the Nízké Tatry Mts (Slovakia) in 2004. The treatments were: i) unmanaged control, ii) cutting twice per year, iii) herbicide application (glyphosate) and grass/forbs mixture reseeding in the first experimental year followed by cutting twice a year in all subsequent experimental years. Cover of plant species (%) were visually estimated in the experimental plots every year (2004 -2011) in late June. The results showed that one-time herbicide application with consequent grass/forbs mixture reseeding reduced the cover of *R. obtusifolius* to 3 % after the first experimental year. Consequently, this cover was further decreased by cutting twice a year and even led to *R. obtusifolius* disappearance in 2008, which remained until the end of the experiment. Cutting twice a year led to a gradual reduction of *R. obtusifolius* from 77 % to 3 % during the experimental time, but it did not lead to its complete eradication. On contrary, unmanaged plots supported *R. obtusifolius* and its high cover about 75% maintained during all experimental years. This long-term experiment showed that single-use herbicide application with subsequent grass/forb mixture reseeding and cutting twice a year in the following years can completely suppress *R. obtusifolius* and establish grasslands with desirable species composition. However, the long term cutting twice per year seems to be also efficient to control *R. obtusifolius*, especially on sites where herbicide application is prohibited.

Keywords: dock weed, gathering places of livestock, reseeding, glyphosate, botanical composition

Introduction

R. obtusifolius belongs to the most problematic perennial weeds in the world. In grasslands, *R. obtusifolius* frequently grows on highly productive, nutrient-rich soils independent of cutting frequency (Strnad *et al*., 2012). It is also a dominant plant species in abandoned former resting places of sheep or cattle in the mountains. For its control, usually biological, mechanical, cultural or combined methods are used, but its total eradication is rarely successful (Zaller, 2004). The success of control management depends on *R. obtusifolius* dominancy, soil nutrient concentrations, source of seeds and previous management. In order to find applicable ways of *R. obtusifolius* control and to support species rich mountain grasslands suitable for forage production, a long-term experiment was established in the Nízké Tatry Mts (Slovakia).

Materials and methods

An experiment was established in 2004 in the National Park Nízké Tatry (Slovakia, 48° 51' 22" N, 19° 14' 57" E). The experimental site was 1,140 m above sea level. Mean annual precipitation is 800 mm and the average temperature during vegetation season (IV. – IX.) was 8°C. The terrain of the experiment showed an east exposure with a slope of 8 – 10°. Geological subsoil was created by porphyric granodiorite, granite and quartzite. Soil with roughly 5 pH-value is classified as cambisol, subtype modal and variety acidic. In 2004, the mean nutrient concentrations in the soil were 6.44 g kg $^{-1}$ Ntot, 260 mg kg $^{-1}$ plant available P and 890 mg kg $^{-1}$ plant available K. *R. obtusifolius* and *Urtica dioica* were the dominant vascular plant species in the experimental area. The experiment was arranged in three randomised blocks with three treatments and a single plot size of 15 m^2. The applied treatments were: U - unmanaged control; 2C - cutting twice per year only; H - herbicide application (Roundup Biaktiv-glyphosate) and subsequent grass/forbs mixture reseeding (after 3 weeks) with 18 autochthonous plants (*Dactylis glomerata* L.*, Festuca pratensis* Huds., *Phleum pratense* L., *Poa pratensis* L., *Festuca rubra* L., *Trisetum flavescens* (L.) P. Beauv, *Trifolium repens* L., *Trifolium pratense* L., *Lotus corniculatus* L., *Plantago lanceolata* L., *Achillea millefolium* L., *Carum carvi* L, *Taraxacum officinale* Weber, *Alchemilla vulgaris* L., *Daucus carota* L., *Acetosa pratensis* Mill., *Leucanthemum vulgare* Lam., *Prunella vulgaris* L.) in the first experimental year and then cutting twice per year in all following years. The percentage of covers of all vascular plant species were visually estimated in each experimental plot every year (2004 – 2011) at the end of June. Repeated measures ANOVA was used to analyse effect of time, treatment and time × treatment on *R. obtusifolius* cover. Redundancy analysis (RDA) in the CANOCO 5.0 program (ter Braak and Šmilauer, 2012) was used to evaluate multivariate vegetation data (2004 – 2011).

Results and discussion

The cover of *R. obtusifolius* was significantly (*P* = < 0.001) affected by treatment, year and treatment*year interaction according to the repeated measures ANOVA. The highest cover of *R. obtusifolius* was observed in the U treatment during all experimental years (Fig. 1a) and remained relatively stable (about 75%) during the total course of the experiment. This was caused by nutrient enrichment (N, P, K) in former cattle resting places. A gradual decrease of *R. obtusifolius* from 77% at the beginning of the experiment to 3% in the last year of the experiment was recorded in the 2C treatment. This is in contrast with the study of Hann *et al*. (2012), which indicated that *R. obtusifolius* is well adapted to cutting management. But other studies (Hann *et al.*, 2012; Hejcman *et al*., 2012) showed, that *R. obtusifolius* also thrive on unmanaged grasslands with higher nutrient availability, as occurred in our experiment. In the H treatment the cover of *R. obtusifolius* also sharply decreased from 76% to 3% after the first year of the experiment. Consequent cutting twice per year in the following experimental years resulted in a further reduction of *R. obtusifolius*. From the year 2008, on a total eradication of *R. obtusifolius* was revealed and diverse grassland was established (Fig. 1b). Therefore H treatment seems to be a reliable method for *R. obtusifolius* eradication, but use of herbicides is limited in protected areas. RDA analyse revealed that the species-rich grassland was more effectively established by means of the H treatment, where the number of plant species was the highest (Fig. 1b). Results of this long-term experiment showed, that both treatments 2C and H could in a long-term perspective be sufficient to control *R. obtusifolius* in mountains grasslands.

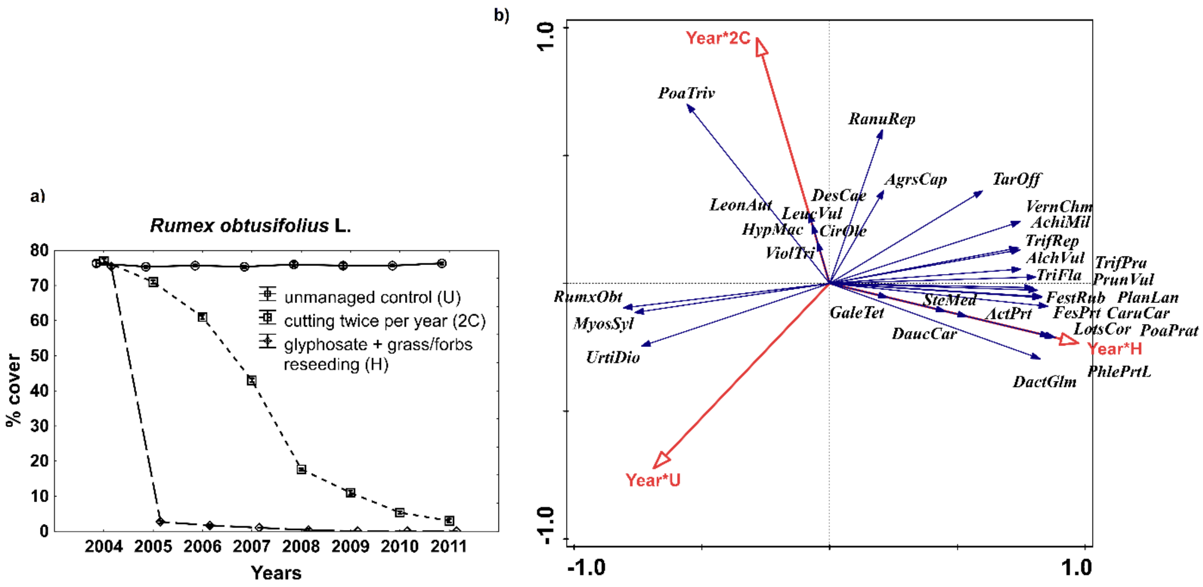

Figure 1. a) Changes in the cover (%) of *R. obtusifolius* during 2004 – 2011; b) Ordination diagram is showing the result of RDA analysis of vegetation data. For the treatment abbreviations see Materials and methods section. Species abbreviations are based on the first three or four letters for genus and species name. RDA analysis explained 63.96 % variability on the first ordination axis (*F-value = 60.3, P = < 0.001*).

Conclusions

This study shows that *R. obtusifolius* is very well established in mountain nutrient-rich unmanaged grasslands, especially in abandoned former cattle resting places. The herbicide application in combination with reseeding of grass/forb mixtures can completely suppress *R. obtusifolius* and establish species rich-grasslands with desirable species composition suitable for forage production. However, due to possible environmental risks the using of glyphosate is highly debatable. Therefore long-term cutting twice per year seems to be more suitable and environmentally friendly management for *R. obtusifolius* controlling in the mountain area.

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Chapter VI

What is a suitable management for *Typha latifolia* control in wet meadows?

Managements for *Typha latifolia* control

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Abstract

Questions: *Typha latifolia* causes serious problems in wet meadows by overgrowing and suppressing other native plants. To find suitable management for *T. latifolia* control, we addressed the following question: What are the effects of long-term cutting at different frequencies (once or twice per year and no management) and biomass removal on cover and other characteristics of *T. latifolia*, and on sward productivity and plant species composition?

Location: Malá Strana nature reserve, situated in the Jizerské hory Mountains, Czechia.

Methods: A long-term experiment arranged in a randomised block design with three blocks was established in 2005. Data were collected from five treatments: unmanaged control; cutting once a year in June without biomass removal and with biomass removal; cutting twice per year in June and August without biomass removal and with biomass removal. Percentage cover of *T. latifolia* and other vascular plant species was visually estimated and *T. latifolia* characteristics (tiller density, height, dry matter biomass (DMB) yield and litter), sward height and DMB yield were measured during 2005-2018 at the end of June.

Results and discussion: Regular cutting once or twice per year regardless of cut biomass removal led to reductions in tiller density, height, litter and DMB yield of *T. latifolia*. Biomass removal had only a slight tendency to affect *T. latifolia* characteristics. The higher rate of cutting intensity significantly decreased the mean *T. latifolia* cover, litter and DMB yield. Cutting once or twice per year regardless of biomass removal led to the successive changes in plant species composition but had no effect on the number of plant species and evenness.

Conclusions: Cutting at least once per year without biomass removal seems to be sufficient to achieve a decrease in cover, DMB yield and litter of *T. latifolia* plants, and thereby maintain the wet-meadow vegetation without loss of species richness and also preventing the overgrowth of shrubs and trees.

Keywords: biomass removal, cutting frequency, dry matter biomass yield, Ellenberg indicator values, functional groups, plant species composition, species diversity, sward characteristics, *Typha latifolia* characteristics, wet meadow

1. INTRODUCTION

Typha spp. are common species in wetland ecosystems, particularly in freshwater habitats, and occur in the many parts of the world (Sale & Wetzel, 1983). This genus has a cosmopolitan distribution and includes species which can behave aggressively and may suppress other native species in their natural communities (Apfelbaum, 1985). Bansal et al. (2019) summarized the extensive knowledge regarding the ecological characteristics of *Typha* spp. and noted that the species can have considerable impacts on local fauna and flora, and also affect biogeochemical cycling, wetland hydrology and overall wetland functioning. Many other authors have mentioned the large impacts that *Typha* spp. have on natural wetland communities (Ball, 1990; Lishawa et al., 2017; Sharma & Kushwaha, 1990), but few of them have examined the changes in the characteristics of *T. latifolia* species by long-term experimentation with different managements. Important characteristics of grassland vegetation such as cover and tiller density of individual species, as well as dry matter biomass yield, are usually associated with investigations of grassland performance (Mannetje & Jones, 2000) and assessments of these characteristics can also be used to determine the fitness of *T. latifolia*.

Although *Typha latifolia* is a native species in Europe and North America, it may become a problematic weed species in many wetland habitats and can often significantly suppress the original vegetation (Bansal et al., 2019; Clevering & Toorn, 2000; Lishawa et al., 2017; Murkin & Ward, 1980). Reduction or total suppression of *T. latifolia* helps to increase habitat heterogeneity, the cover of other plant species (Apfelbaum, 1985) and species diversity (Lishawa et al., 2017). The harvested biomass of *T. latifolia* could also be used as a sustainable feedstock for renewable energy or for bioremediation (Bansal et al., 2019; Garver et al., 1988; Maddison et al., 2009).

Typha latifolia tolerates a broad range of climatic conditions and soils that remain wet or saturated by water over a major part of the growing season (Grace & Harisson, 1986). It has high growth rate (Sale & Wetzel, 1983), especially under conditions with favourable nutrient supply. Further, special photosynthetic acclimation to flooded conditions (Li et al., 2004) and high proliferation by vegetative growth (Sale & Wetzel, 1983) enable *T. latifolia* to out-compete other vascular plant species in many wet habitats.

Management approaches based on either cutting or herbicide application are considered appropriate for control of *Typha* spp., although there are other potentially suitable methods including flooding, burning and physical removal (Apfelbaum, 1985). Harvesting during the summer is most detrimental for *T. latifolia* plants, because they have only a short period to translocate nutrients and non-structural carbohydrates from the shoots to the belowground organs. These belowground reserves are necessary especially for the initial growth of new shoots in the following spring (Maddison et al., 2009), and to enable the plants to overcome disturbance (Toet et al., 2005). If the plants are cut below the water surface their ability to supply air to the rhizomes in the deep-water declines (Grace, 1989; Murkin & Ward, 1980). Other benefits of *T. latifolia* control by cutting include restricting plant growth to the immature phases and reducing transpiration rates (Martin et al., 2003). Cutting of shoots three times over the growing season is considered to be an optimal management technique for *Typha* spp. reduction (Apfelbaum, 1985; Sharma & Kushwaha, 1990).

The cut biomass of wet meadows should be removed to prevent it affecting the growth of other species present, the impact of which varies according to the thickness of the mown layer and the rate of biomass decomposition.The decomposition rate of *T. latifolia* is affected, as in other plants, by lignin concentrations (Murphy et al., 1998) and C:N ratio of litter (Lee & Bukaveckas, 2002), C:N:P content in plant detritus (Enríquez et al., 1993), pH (Batty & Younger, 2007) and by site-specific climatic conditions (Murphy et al., 1998). These include temperature and drought effects (Liski et al., 2003). The decomposition process also depends on microorganisms (bacteria, fungi) (Álvarez & Bécares, 2006) and surface water and sediment nutrient concentrations (Lee & Bukaveckas, 2002), all of which have indirect impacts on the plant growth.

The wet meadows in Central Europe provide a wide range of ecosystem services including plant and animal biodiversity, water retention and carbon storage; however, their area and species richness have declined rapidly during the last fifty years. There are two contrasting management regimes that negatively affect the diversity of wet meadows: i) management intensification as a result of land drainage, increased fertilization and increased mowing frequency (Wesche et al., 2012); and ii) land abandonment (Swacha et al., 2018). As a result of these two contrasting but damaging developments, wet meadows are now among the most threatened grassland types (Krause et al., 2015; Wesche et al., 2012).

Although many previous studies have addressed the effects of regular cutting management on *T. latifolia* species when growing in deep water in different waterlogged areas, none of them have considered the long-term effect of cutting on this species when it is growing in wet meadows with shallow water supply. To address this important knowledge gap, a long-term experiment was established in the wet meadow alliance *Calthion* with *T. latifolia* dominance.

In the present paper we have focused on the following questions: (i) What is the effect of long-term cutting at different frequencies (once or twice per year, relative to no cutting management) on *T. latifolia* characteristics (cover, number and height of tillers, dry matter biomass (DMB) yield and litter), and on sward characteristics of wet meadow (species diversity, sward height, DMB yield, plant species composition and functional groups)? and (ii) Is there any effect of biomass removal after cutting on *T. latifolia* characteristics and on sward characteristics of wet meadow?

2. METHODS

2.1 Study site

The experiment was established in the Malá Strana nature reserve in the Jizerské hory Mts. (Czechia, 50°45'52"N, 15°12'19"E; 722 m a.s.l.). The site has a mean annual precipitation 1373 mm. Average annual temperature is 4.4 °C (Vesecký, 1961). Data for average monthly precipitation and temperature (meteorological station in Bedřichov, 5 km from study site) during the study period are presented in Appendix S1. The bedrock is a porphyritic, medium granular granite and granodiorite (Chaloupský et al., 1989). The soil cover comprises cambisol, pseudogley and gley (Tomášek, 1995). In the layer 0-20 cm the soil pH/H_2O is 5.7 and soil organic-C content is 740 000 mg kg^{-1}. The concentrations of plant available nutrients using the Mehlich III method (Mehlich, 1984) were P: 35 mg kg^{-1}, K: 601 mg kg^{-1}, Ca: 5128 mg kg^{-1} and Mg: 628 mg kg^{-1} of soil, respectively. The average amount of N_{tot} is 22 456 mg kg^{-1}.

The vegetation of the experimental grassland is classified as *Calthion* and *Filipendulion* (Chytrý et al., 2007). At the start of the experiment the dominant vascular plant species were *T. latifolia*, *Filipendula ulmaria* and *Carex nigra*.

2.2 Experimental design

The experiment was established in three completely randomized blocks in 2005 (Appendix S2). The area of each plot was 16 m^2 (4 m × 4 m) with a buffer zone between plots of 0.5 m. The following treatments were applied: unmanaged control (U), one cut per year in June without biomass removal (1N), one cut per year in June with biomass removal (1R), two cuts per year in June and in August without biomass removal (2N), two cuts per year in June and August with biomass removal (2R).

2.3 Data collection

2.3.1 Number and height of *Typha latifolia* tillers

The number of *T. latifolia* tillers was recorded in each experimental plot before the first cutting throughout the study period of 2009-2018, and the height of all presented *T. latifolia* tillers was measured in a 30 cm wide strip diagonally in each experimental plot before the first cutting and also through study period of 2008-2017.

2.3.2 Dry matter biomass yield and litter of *Typha latifolia*, dry matter biomass yield and sward height

Herbage samples were taken in the years 2008-2017. Dry matter biomass yield of the first cut was measured in four sub-plots each with dimensions of 50 cm × 50 cm, randomly placed within each experimental plot. Cutting was made to a stubble height of 3 cm. To avoid any residual effect of herbage collection from the previous year in the unmanaged treatment, the biomass sampling site was outside of permanent plots in the surroundings and differed every year. The harvested herbage was sorted into *T. latifolia* herbage and litter, and other herbage. Separated fractions were then dried at 60°C until totally desiccated and dry matter biomass yield (t ha^{-1}) of all subsampled groups was then determined. The mean of four sub-samples per experimental plot was used for statistical analyses.

Compressed sward height (cm) without *T. latifolia* was measured using a rising plate meter (Castle, 1976). A total of ten measurements in each experimental plot were performed before vegetation sampling in each vegetation season in the study period 2010-2018.

2.3.3 Species diversity and botanical composition

The percentage cover of all vascular plant species was visually estimated in each plot. Data were collected in June of 2005-2011, 2013 and 2017. Nomenclature of vascular plant species follows the regional flora (Kaplan et al., 2019). In accordance with the mean height of vascular plants as listed in the regional flora (Kaplan et al., 2019), all plant species recorded in the experiment were a priori categorized into five main functional groups: legumes, short graminoids (mean height < 0.5 m), tall graminoids (mean height ≥ 0.5 m, and excluding *T. latifolia*) and similarly short and tall forbs. (For classification of individual plant species into plant functional groups see Appendix S4). Number of species was calculated from the data of abundance. Buzas-Gibson's evenness was calculated as $E = e^{H'} / S$, where H' is Shannon index of diversity and S is number of species.

2.3.4 Ellenberg indicator values

Ellenberg indicator values (EIV) for soil moisture and nutrients were weighted for each plot by cover of each vascular plant species and the mean of the indicator values was calculated for each treatment (Ellenberg et al., 1992).

2.4 Data analysis

Two sets of general linear models (GLMs) were used to analyse the cover of *T. latifolia*, number of *T. latifolia* tillers, height of *T. latifolia* in transect (cm), *T. latifolia* DMB yield (t ha^{-1}), *T. latifolia* litter (kg ha^{-1}), compressed sward height (cm), DMB yield (t ha^{-1}), number of all vascular plant species, evenness index, main plant functional groups and EIV through the study period. All variables derived from species-cover data were analysed without the data from the first three years (2005-2007) in order to account for the delayed response of vegetation to the management treatments.

First set of models included all five treatments in a single factor. Blocks and years were used as random factors; all interactions were included in the model to estimate appropriate residual mean square and d.f. (Satterthwaite, 1946). If necessary, data were log-transformed to meet GLM assumptions. Benjamini-Hochberg's procedure was applied to control for false-discovery rate (Verhoeven et al., 2005). Further, to identify significant differences between individual treatments, a post-hoc comparison using Tukey's HSD test was applied. The second set of models excluded the U treatment, thereby enabling to test for separate effects of intensity, biomass removal and intensity*biomass removal interaction, with all other settings being same as in the first set. The relationships between DMB yield and number of species and sward height and number of species were analysed by linear regression analysis. For all univariate statistical analyses, software STATISTICA 13.2 was used (Dell Inc., 2019).

The effect of treatment on the community composition was analysed using partial redundancy analysis (RDA) in CANOCO 5 (ter Braak & Šmilauer, 2012). The effect of treatment was used as an explanatory variable, while years and blocks were used as covariates. Species cover (%) data were logarithmically transformed [$x' = \log_{10} (10*x + 1)$]. The data of the first three years were excluded from the analysis to deal with possibility of a delayed response of the vegetation to the managements applied at the beginning of the experiment.

All previous analyses filtered out the effect of time to focus on the part of the effect of treatment which is consistent across years, excluding the initial years. To disclose the temporal dynamics, we supplemented the main analyses by plots of means and standard errors of selected parameters of *T. latifolia*, parameters of the sward, and abundance of selected dominant species in each year including the initial years. To illustrate the temporal dynamics in the whole community relative to the unmanaged control treatment, we present the diagram of principal response curves (PRC). In PRC, the interaction of treatment*year was used as explanatory variable and year as covariate. Monte Carlo permutation test with 4999 permutations in hierarchical design was used with whole-plot permutations freely exchangeable and no split plot permutations.

3. RESULTS

3.1 *Typha latifolia* characteristics

All characteristics of *T. latifolia* were significantly affected by treatment. The highest mean cover of *T. latifolia* was 37.2 ± 2.7% (mean ± standard error) in the U and 25.8 ± 2.1% in the 1N treatment and the lowest in both treatments cut twice per year (6.7 ± 0.6% in the 2R; 8.0 ± 0.8% in the 2N) (Table 1), where its cover decreased over time (Figure 1). The higher intensity of cutting significantly reduced the mean cover of *T. latifolia* (22.8 ± 1.5% in the 1 cut; 7.3 ± 0.5% in the 2 cut) (Appendix S3).

The highest mean number of *T. latifolia* tillers was 186.3 ± 13.1 in the U treatment and the lowest was 66.7 ± 4.2 in the 2R treatment. The highest mean height of *T. latifolia* in the transects was 110.5 ± 3.4 cm in the U treatment and the lowest was in the treatments cut twice per year (81.1 ± 4.4 cm in the 2N; 84.2 ± 3.3 cm in the 2R) (Table 1; Figure 2ab).

The mean *T. latifolia* DMB yield and *T. latifolia* litter were the highest (1.85 ± 0.19 t ha^{-1}; 738.4 ± 111.3kg ha^{-1}) in the U treatment, respectively (Table 1; Figure 2cd). The higher cutting intensity resulted in significantly decreased mean DMB yield of *T. latifolia* (0.63 ± 0.04 t ha^{-1} in the 1 cut; 0.33 ± 0.04 t ha^{-1} in the 2 cut) and in *T. latifolia* litter (93.2 ± 7.8 kg ha^{-1} in the 1 cut; 10.5 ± 2.3 kg ha^{-1} in the 2 cut) (Appendix S3).

3.2 Sward characteristics

Treatment significantly affected sward height (cm) and DMB yield (t ha^{-1}), where U had significantly higher values than other treatment levels; however, these did not differ from each other (Table 1; Figure 3ab).

Overall, 61 plant species were identified in the experiment during the study period 2005-2017. This total comprised 15 tall graminoids, 11 short graminoids, 11 tall forbs, 23 short forbs and one legume (Appendix S4). No effect of the treatment on number of all species, evenness index, and cover of functional groups was found (Table 1; Appendix S5ab). The development of selected dominant plant species throughout the study period 2005-2017 also differed among species (Appendix S6 and S7). There was a significant negative correlation between DMB yield and number of all species (R = -0.23; P value = 0.033), but the correlation between sward height and number of all species was not significant (R = −0.21; P value = 0.103).

In RDA based on the vegetation data without observations from the first three years of the study period (2008-2017), the effect of the treatments on plant species composition explained 16.7% of the variability (*F* value = 6.5, *P* value = 0.023) on all axes (Figure 4). Plant species correlated with the treatments cut twice per year (2R, 2N) were *Anthoxanthum odoratum, Briza media, C. nigra* and *Nardus stricta.* Plant species correlated with the treatments cut once per year (1R, 1N) were *Eriophorum angustifolium*, *Lychnis flos-cuculi* and *Mentha arvensis*. *Alopecurus pratensis, Bistorta officinalis*, *Myosotis nemorosa* and *Scirpus sylvaticus* were typical species associated with the U treatment (Figure 4).

The first axis of the PRC diagram showed that the effect of interaction of treatment and year was statistically significant (*F* value = 0.4, *P* value = 0.014). Principal response curves showed differences between the U treatment and all of the cut treatments (1N, 1R, 2N, 2R) in relation to plant species composition. The PRC diagram showed association of *T. latifolia*, *B. officinalis, M. nemorosa* and *S. sylvaticus* with the U treatment. *Caltha palustris, Carex canescens, E. angustifolium* and *M. arvensis* were associated with the cutting treatments (1N, 1R, 2N, 2R) (Figure 5).

No effect of the treatments on tall graminoids without *Typha latifolia*, tall forbs, short graminoids, short forbs and EIV for moisture was found (Table 1; Figure 6a-d; Appendix S8a). However, an effect of the treatment on EIV for nutrients was found: the highest mean EIV for nutrients was 5.25 ± 0.08 in the U treatment and the lowest was in both of the treatments cut twice per year (3.55 ± 0.12 in the 2R, 3.82 ± 0.14 in the 2N) (Table 1; Appendix, S8b).

4. DISCUSSION

4.1 *Typha latifolia* characteristics

Typha latifolia is a native species in Europe and North America, but its presence may be regarded as nuisance plant in many wetland areas because its growth significantly suppresses other plants in the natural vegetation (Bansal et al., 2019; Clevering & Toorn, 2000). This is particularly relevant in the context of management of nature reserves and other protected areas.

Cutting is generally considered an important method of *T. latifolia* reduction (Apfelbaum, 1985; Lishawa et al., 2017; Murkin & Ward, 1980) and frequent cutting (two or three times per vegetation season) was suggested by Apfelbaum (1985) and Sale & Wetzel (1983) as the optimal management for successful *T. latifolia* control. However, most previous studies dealing with the control of *T. latifolia* (Ball, 1990; Lishawa et al., 2017; Murkin & Ward, 1980) have been conducted over short-term periods and in wetland habitats, but not in wet meadows. The results of our study confirmed the findings of previous short-term studies, that cutting is a suitable management for *T. latifolia* control; however, in terms of intensity of cutting our results are not so straightforward. Thus, although higher frequency of cutting significantly reduced *T. latifolia* cover and its DMB and litter yield, the number and height of its tillers remained unaffected. A possible explanation for this could be that there are changes in *T. latifolia* morphology under the different cutting frequencies, for example in the number of leaves or thickness of plant stems, as well differences in stem or leaves tissue density. In research on another wetland species, *Phragmites australis*, an effect of cutting on morphological traits was also detected (Asaeda et al., 2003).

There was only a slight tendency of removal of cut biomass to affect the characteristics of *T. latifolia* (apart from an effect on *T. latifolia* plant height). The moisture and temperature conditions were favourable (Murphy et al., 1998, Enríquez et al., 1993; Liski et al., 2003) for supporting easy decomposition of aboveground biomass (including biomass of *T. latifolia*). Therefore, undecomposed residues of aboveground cut biomass did not accumulate on the surface for sufficient time to adversely affect plant growth.

4.2 Sward characteristics

The results from our experiment confirm findings from previous research on mezophilous meadows that cutting reduces the height and yield of the sward in the next year

(Pavlů et al., 2013; Kassahun et al., 2021); nevertheless, higher cutting intensity did not further reduce either the height or yield of the first cut in our experiment. Moreover, the results of this experiment did not show the often-observed negative relationship between the number of species and yield/sward height of the first cut (Humbert et al., 2016; Silvertown, 1980; Zarzycki & Kopeć, 2020) caused by competition for light. This was probably due to the wet and oligotrophic conditions of this site which did not allow the formation of a dense sward and thus, even in the unmanaged treatment, light conditions in the sward were still sufficient to support species diversity. Further, almost all species occurring in the vicinity are represented in the experimental plots, and thus none of the treatments applied here can be found to substantially increase plant species diversity.

Because of the relatively low density of plants of *T. latifolia*, the light conditions in the sward were also sufficient to support short and mostly competitively weak species. Nevertheless, according to the RDA ordination diagram, three groups of plant species were promoted by different cutting intensities that differed from each other in their plant species composition. This was not only a result of light availability (Borer et al., 2014; Jensen & Meyer, 2001) but also as a direct response of individual species to the cutting management, as tall species are damaged by defoliation more than short ones (Gaisler et al., 2019; Pavlů et al., 2022). Biomass removal after cutting, compared with leaving cut biomass on the sward surface, had no effect on plant species composition and this was probably because of rapid decomposition processes of the cut biomass on the sward surface under the environmental conditions of the site.

The dominance of *E. angustifolium* in the group of one-cut treatments (1R, 1N) confirmed that management regimes that are either too intensive, such as multiple defoliation (Marrs et al., 1988), or on the other hand too extensive (i.e., abandonment) are unfavourable for the growth of this species.

The dominance of two *Carex* species in the group of two-cut treatments (2R, 2N) could be explained by the positive effect of cutting on the cover of *C. nigra* (Jungerius et al., 1995; Sienkiewicz-Paderewska et al., 2020) and *C. canescens*. Further, preference of *C. palustris* in this group could be caused by a tendency of this species to become frequent in unshaded places at higher altitudes (Grime et al., 1988).

The cover of the highly productive *F. ulmaria* (Merunková & Chytrý, 2012) decreased in in plots with cutting management in our experiment. This is a typical response of this species to defoliation (Čop et al., 2009; George et al., 2021; Tatarenko et al., 2020). Moreover, in unmanaged grassland, the existence of a persistent litter layer does not prevent this species from overgrowing (Grime et al., 1988), as was also observed in the unmanaged plots in our experiment. *Bistorta officinalis* is also a typical species of abandoned grassland. It is able to suppress the surrounding vegetation by its above-ground cover of leaves, large amount of litter and depletes nitrogen from the soil (Pecháčková & Krahulec, 1995) which was confirmed by its higher presence in unmanaged plots in our experiment.

Juncus filiformis is a species which prefers sufficient availability of light (Poptscheva et al., 2009; Schrautzer et al., 2013) and although it is a shade-intolerant species, in our experiment it was also present at a high cover in the unmanaged treatment. Its occurrence in abandoned plots was probably positively influenced by the absence of cutting and at the same time by quite low density of *T. latifolia*, even in abandoned plots, which enabled light to penetrate into the lower layers of the sward.

A decrease in the EIV for nutrients with increasing intensity of defoliation is a typical response of plant communities to long-term mowing (Sand- Jensen et al., 2019). This reflects a tendency to reduce the cover of tall nutrient-demanding plant species (both forbs and graminoids without *T. latifolia*), and a tendency, as found in our experiment, to support short graminoids with increasing defoliation intensity.

4.3 Management implications

Practical management should not be focused only on reduction of *T. latifolia*, but it should also take into account the requirements for nature conservation in terms of the target state of the whole plant community and the rarity and endangered status of individual plant species in the region. As the application of a higher frequency of cutting did not show a straightforward effect on *T. latifolia* control, the response of the whole community to the cutting intensity appears to be a very important factor in the choice of appropriate management.

Another factor that is important for plant species composition is biomass removal. The results from the present experiment led us to suggest that biomass removal does not necessarily have a significant effect on vegetation under certain conditions. It seems that wet edaphic conditions, as in our experiment, can probably accelerate the decomposition of unremoved cut biomass, and thereby preserve conditions of sufficient light penetration required for vegetation growth. Moreover, the issue of biomass removal is also closely linked to the soil nutrient status, as regular and long-term removal of biomass can result in oligotrophication (Titěra et al., 2020; Hejcman et al., 2014).

In wet meadows where there is a dominancy of *T. latifolia* we consider that extensive management (with cutting once per year without biomass removal) can be sufficient to maintain the plant species community in a desirable state. Furthermore, as a management technique, this is a relatively low-cost option compared with multiple cutting and biomass removal. Nevertheless, there are some types of plant species community that require a more intensive management regime (Apfelbaum 1985; Sale & Wetzel 1983), mostly in places with greater concentrations of nutrients in the soil. In these conditions it may be appropriate to apply different management regimes in one locality and thereby create a diverse vegetation structure.

5. CONCLUSION

Regular cutting has been shown to be a suitable management for *T. latifolia* reduction; however, in this long-term experiment *T. latifolia* characteristics did not show a straightforward relationship with higher cutting intensity. Biomass removal had only a slight tendency to affect *T. latifolia* characteristics. Defoliation management cutting once or twice per year, regardless of whether or not the cut biomass was removed, led to the successive changes in plant species composition but had no effect on the number of plant species and species evenness index.

In light of these results, we suggest that cutting at least once per year in summer without biomass removal seems to be sufficient for control of *T. latifolia* in wet meadows and to protect the meadow vegetation from overgrowth by shrubs and trees. This extensive management is a relatively low-cost solution compared with annual multiple cutting and biomass removal. The results have relevance for the development and implementation of wet meadow nature conservation and sustainable management strategies for meadows with *T. latifolia* dominance.

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AUTHOR CONTRIBUTIONS

LP and VP designed the study and established the experiment. VP, LP and JT collected the data. PB performed statistical analysis. JT, LP and VP wrote the paper. All authors discussed the results and commented on the manuscript.

DATA AVAILABILITY STATEMENT

Data are accessible on request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Weather conditions at the meteorological station in Bedřichov.

Appendix S2: Design of the experiment.

Appendix S3: Results of the two sets of general linear models (GLM) of *Typha latifolia* characteristics and sward characteristics.

Appendix S4: Assignment of plant species to functional groups.

Appendix S5: The mean number of all plant species and species evenness index in the years 2005–2017.

Appendix S6: The mean cover (%) of dominant graminoids species in the years 2005–2017.

Appendix S7: The mean cover (%) of dominant forb species in the years 2005–2017.

Appendix S8: The mean Ellenberg indication values in the years 2005–2017**.**

Appendix S9: Photos of experimental site: a) Photo of experimental site, b) Photo of unmanaged treatment, c) Photo of experimental site after cutting.

TABLE 1 Results of the general linear models (GLM) of the *T. latifolia* characteristics and sward characteristics. *P* value – obtained probability value (see *F* and *df* values in Appendix S3). Significant *P* values after controlling for a table-wise Benjamini-Hochberg's false-discovery rate are shaded. Applied treatments were: **U** (unmanaged control); **1N** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2N** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal). Numbers in columns of treatments represent mean ± standard error of the mean of three blocks of all years excluding the first three years of the experiment. Significant differences (*P* value < 0.05) between treatment levels according to the Tukey's post hoc test are indicated by different lower-case letters.

	P value	U	1N	1R	2N	2R
Typha latifolia						
Cover of *T. latifolia* (%)	<0.001	37.2 ± 2.7^{a}	25.8 ± 2.1^{a}	19.7 ± 1.8^{ab}	8.0 ± 0.8^{bc}	6.7 ± 0.6^{c}
Number of *T. latifolia* tillers	0.022	186.3 ± 13.1^{a}	177.4 ± 16.3^{ab}	130.1 ± 6.8^{ab}	78.7 ± 5.7^{ab}	66.7 ± 4.2^{b}
Height of *T. latifolia* in transect (cm)	0.022	110.5 ± 3.4^{a}	91.0 ± 2.1^{ab}	87.2 ± 1.9^{ab}	81.1 ± 4.4^{b}	84.2 ± 3.3^{b}
T. latifolia DMB (t ha^{-1})	<0.001	1.85 ± 0.19^{a}	0.70 ± 0.08^{b}	0.56 ± 0.06^{bc}	0.38 ± 0.09^{bc}	0.27 ± 0.04^{c}
T. latifolia litter (kg ha^{-1})	<0.001	738.4 ± 111.3^{a}	115.4 ± 17.3^{b}	71.0 ± 12.0^{bc}	14.4 ± 4.7^{c}	6.6 ± 1.5^{c}
Sward						
Sward height (cm)	0.001	22.1 ± 0.8^{a}	17.3 ± 0.7^{b}	16.0 ± 0.7^{b}	15.4 ± 0.4^{b}	15.8 ± 0.6^{b}
DMB yield (t ha^{-1})	0.001	3.89 ± 0.30^{a}	1.89 ± 0.12^{b}	1.70 ± 0.14^{b}	1.38 ± 0.10^{b}	1.43 ± 0.08^{b}
Number of all species	0.52	25.6 ± 0.6	28.8 ± 0.7	29.9 ± 1.0	29.1 ± 0.6	28.6 ± 0.9
Evenness index	0.955	0.35 ± 0.02	0.35 ± 0.01	0.35 ± 0.01	0.36 ± 0.02	0.32 ± 0.03
Tall graminoids (%)	0.146	46.2 ± 1.7	26.9 ± 2.3	21.3 ± 1.9	13.4 ± 1.5	8.0 ± 1.0
Tall forbs (%)	0.342	32.6 ± 3.9	16.4 ± 1.8	16.9 ± 1.7	16.7 ± 3.3	11.8 ± 2.7
Short graminoids (%)	0.045	33.2 ± 2.4	45.7 ± 2.7	46.7 ± 3.8	53.6 ± 3.8	59.8 ± 3.2
Short forbs (%)	0.502	18.8 ± 3.0	26.2 ± 3.5	16.1 ± 2.4	17.6 ± 2.3	14.7 ± 2.1
EIV moisture	0.113	8.46 ± 0.07	8.45 ± 0.04	8.49 ± 0.07	7.90 ± 0.11	8.09 ± 0.08
EIV nutrients	0.003	5.25 ± 0.08^{a}	4.93 ± 0.09^{ab}	4.68 ± 0.11^{ab}	3.82 ± 0.14^{bc}	3.55 ± 0.12^{c}

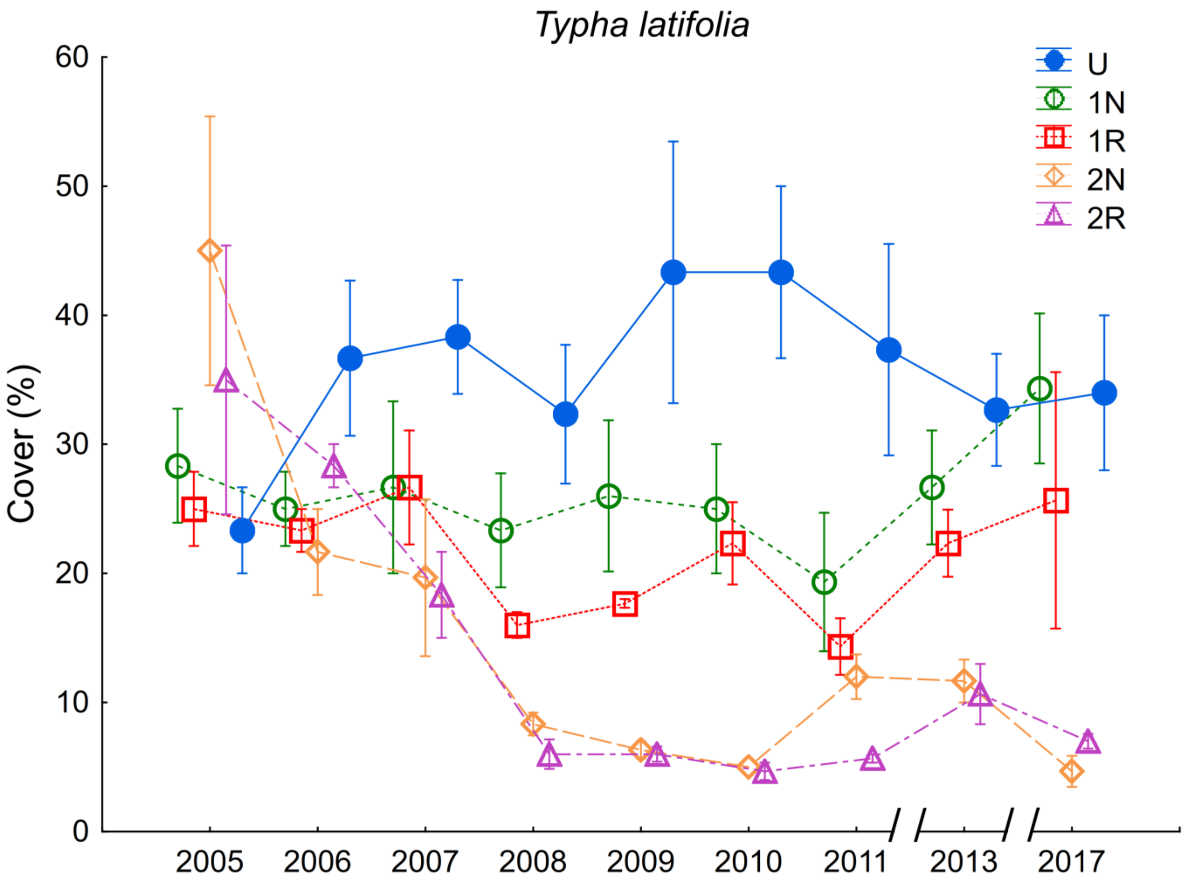


FIGURE 1 The mean cover (%) of dominant plant species *T. latifolia* in the years 2005–2017. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1N** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2N** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).

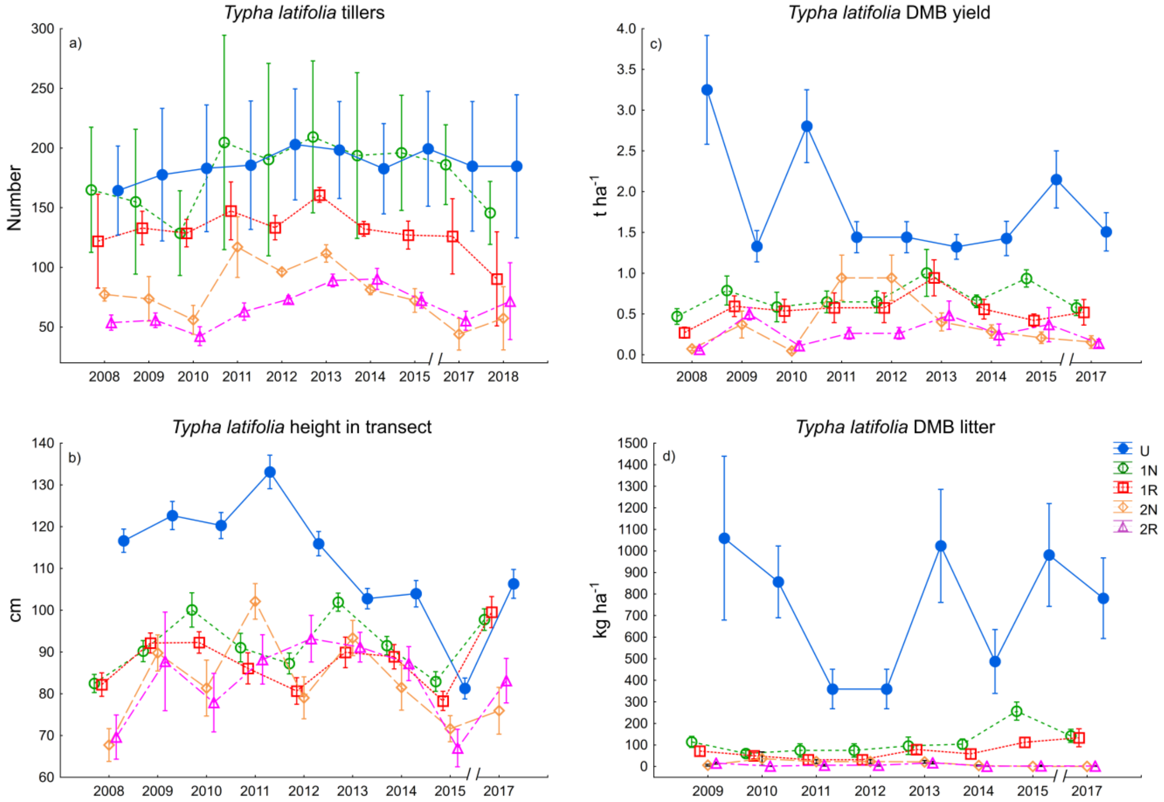


FIGURE 2 The mean of *T. latifolia* characteristics in the years 2008–2018. (a) number of *T. latifolia* tillers (b) height of *T. latifolia* in transect (c) *T. latifolia* DMB yield and (d) *T. latifolia* litter in investigated treatments. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1N** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2N** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).

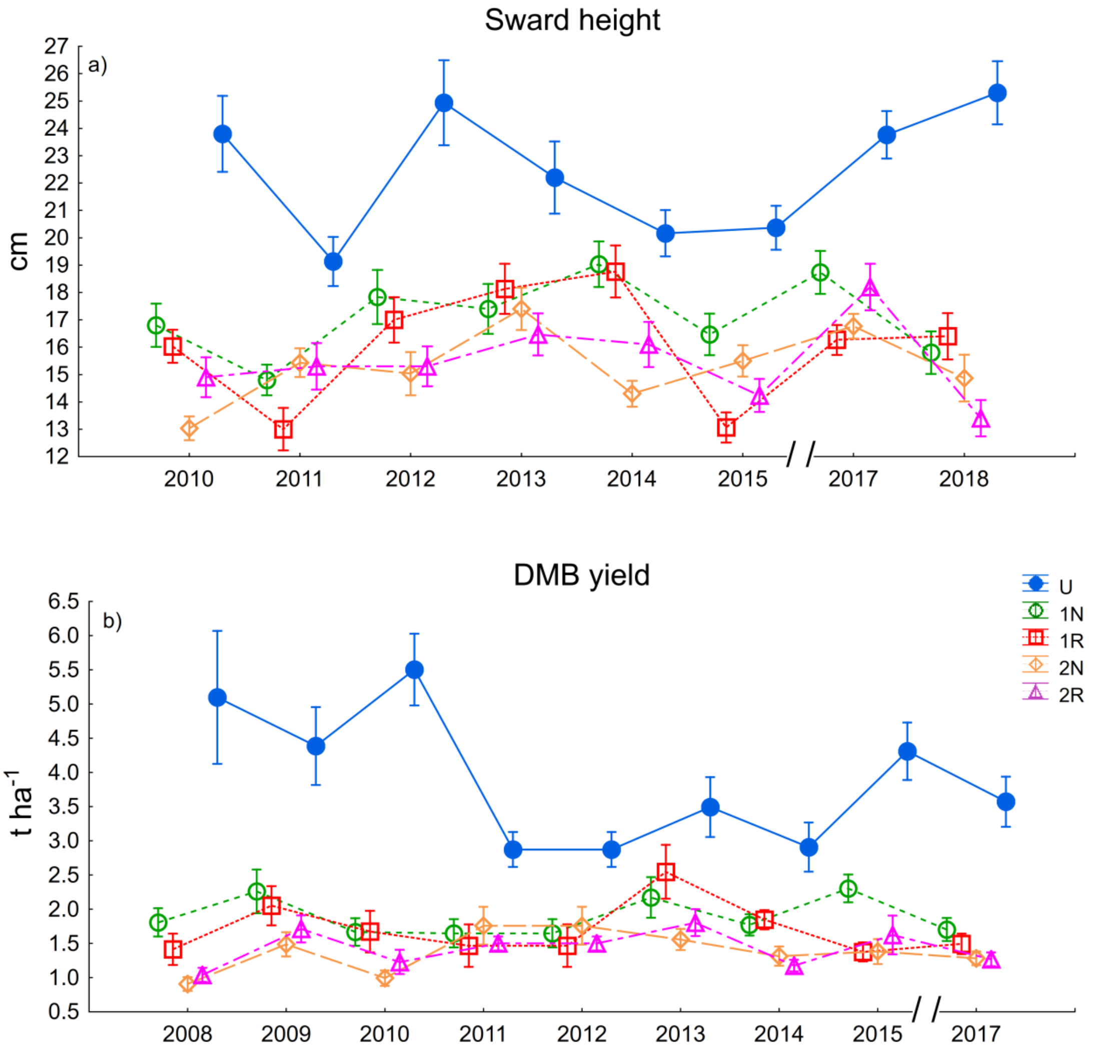


FIGURE 3 The mean of sward height and dry matter biomass (DMB) yield in the years 2008–2018. (a) sward height and (b) DMB yield in investigated treatments. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1N** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2N** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).

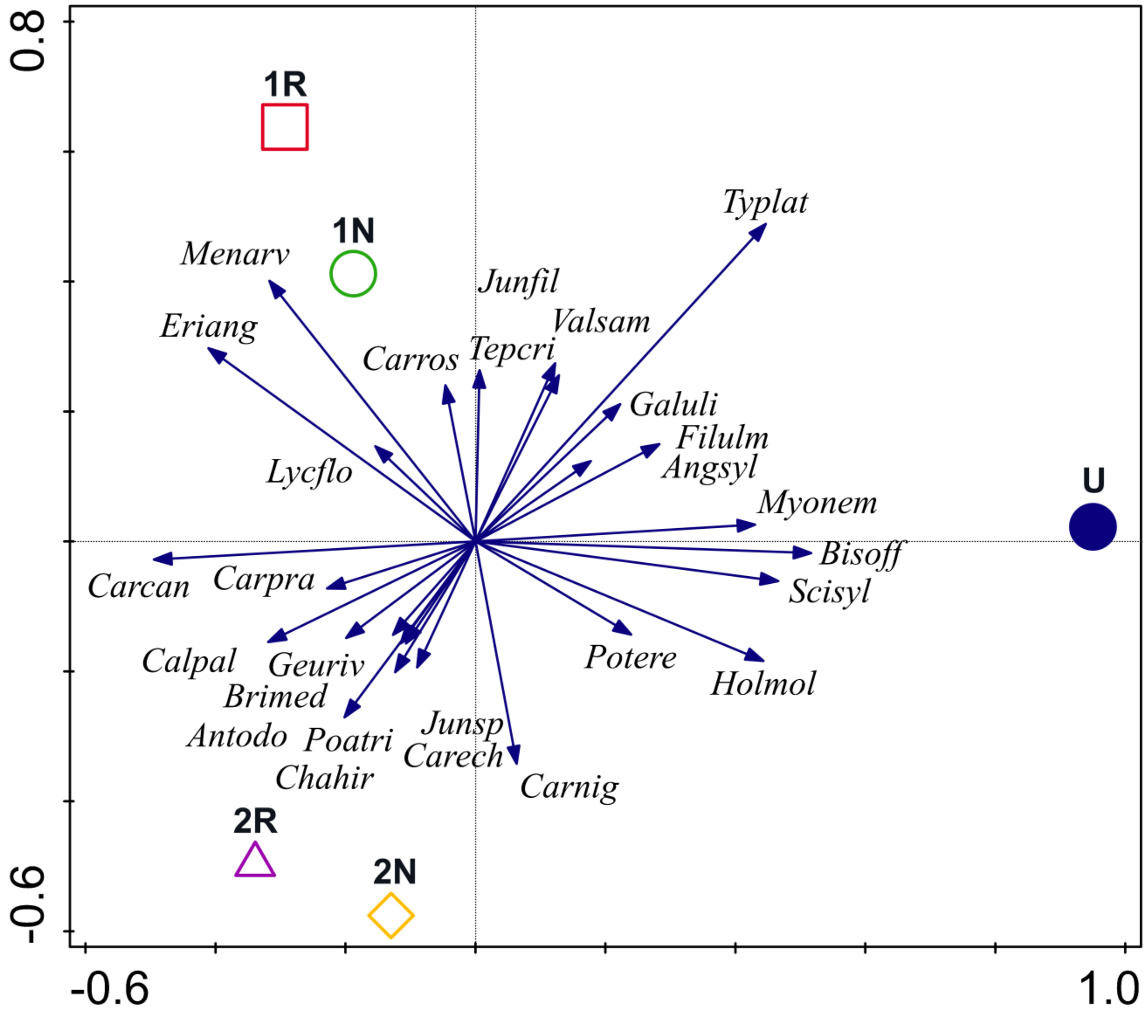


FIGURE 4 Redundancy analysis (RDA) ordination diagram. Results of RDA analysis of plant species composition data collected in the years 2005-2017. The 25 best fitting species are shown. Applied treatments were: **U** (unmanaged control); **1N** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2N** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal). Species abbreviations are based on the first three-letter of genera and the three-letter of species name: *Angsyl* = *Angelica sylvestris*, *Antodo* = *Anthoxanthum odoratum*, *Bisoff* = *Bistorta officinalis*, *Brimed* = *Briza media*, *Calpal* = *Caltha palustris*, *Carcan* = *Carex canescens*, *Carech* = *Carex echinata*, *Carnig* = *Carex nigra*, *Carpra* = *Cardamine pratensis*, *Carros* = *Carex rostrata*, *Chahir* = *Chaerophyllum hirsutum*, *Eriang* = *Eriophorum angustifolium*, *Filulm* = *Filipendula ulmaria*, *Galiuli* = *Galium uliginosum*, *Geuriv* = *Geum rivulare*, *Holmol* = *Holcus mollis*, *Junfil* = *Juncus filiformis*, *Junsp* = *Juncus* sp., *Lycflo* = *Lychnis flos-cuculi*, *Menarv* = *Mentha arvensis*, *Myonem* = *Myosotis nemorosa*, *Poatri* = *Poa trivialis*, *Potere* = *Potentilla erecta*, *Scisyl* = *Scirpus sylvaticus*, *Tepcri* = *Tephroseris crispa*, *Typlat* = *Typha latifolia*, *Valsam* = *Valeriana excelsa* subsp. *sambucifolia*. The effect of the treatments on plant species composition explained 10.0% of the variability and 15.0% of the variability on the first and second axis, respectively.

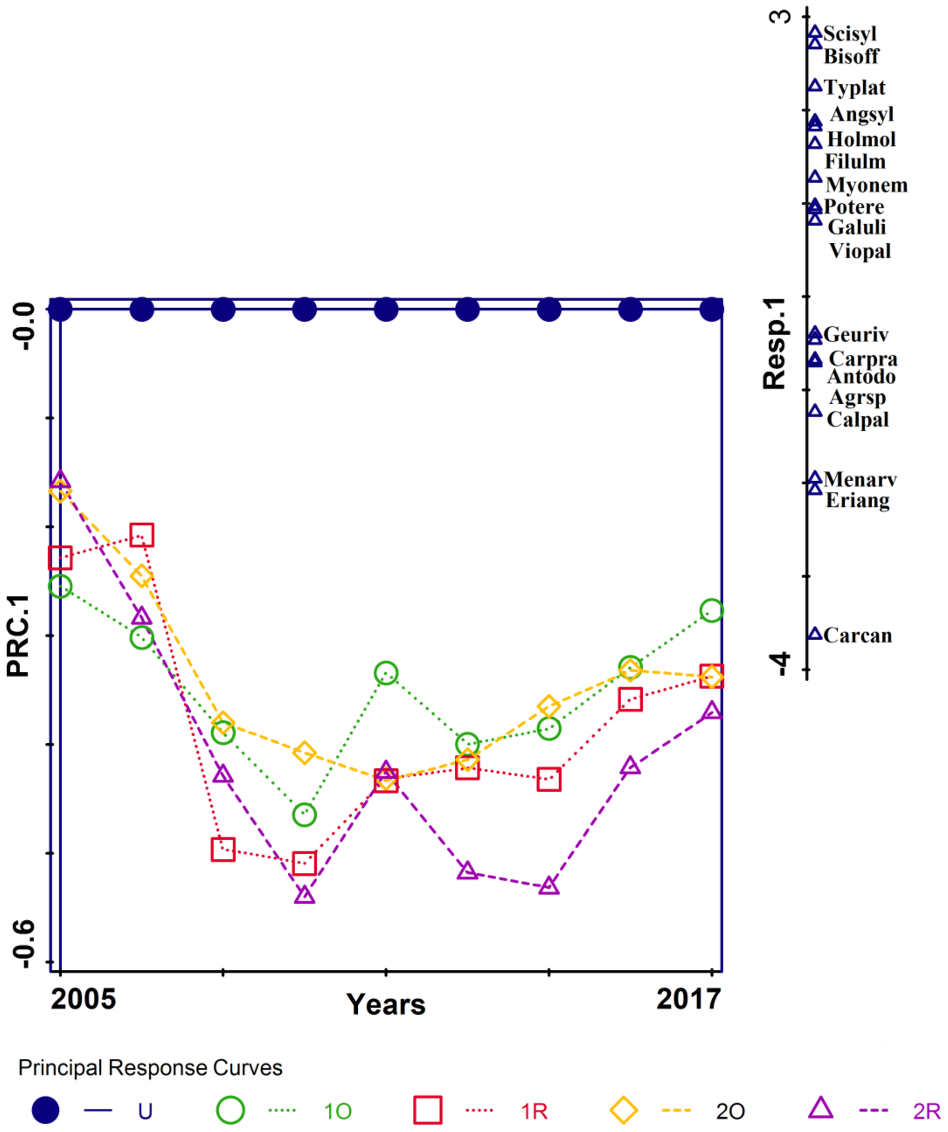


FIGURE 5 Principal response curves (PRC). Result of partial redundancy analysis (RDA) – PRC– 1st axis of plant species composition data collected in the years 2005-2017. Applied treatments were: **U** (unmanaged control); **1N** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2N** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal). Species abbreviations are based on the first three letters of genera and the three letters of species name: Agrsp = *Agrostis* sp., Angsyl = *Angelica sylvestris,* Antodo = *Anthoxanthum odoratum,* Bisoff = *Bistorta officinalis*, Calpal = *Caltha palustris,* Carcan = *Carex canescens*, Carpra = *Cardamine pratensis,* Eriang = *Eriophorum angustifolium*, Filulm = *Filipendula ulmaria*, Galuli = *Galium uliginosum*, Geuriv = *Geum rivulare*, Holmol = *Holcus mollis,* Menarv = *Mentha arvensis*, Myonem = *Myosotis nemorosa*, Potere = *Potentilla erecta*, Scisyl = *Scirpus sylvaticus*, Typlat = *Typha latifolia*, Viopal = *Viola palustris*

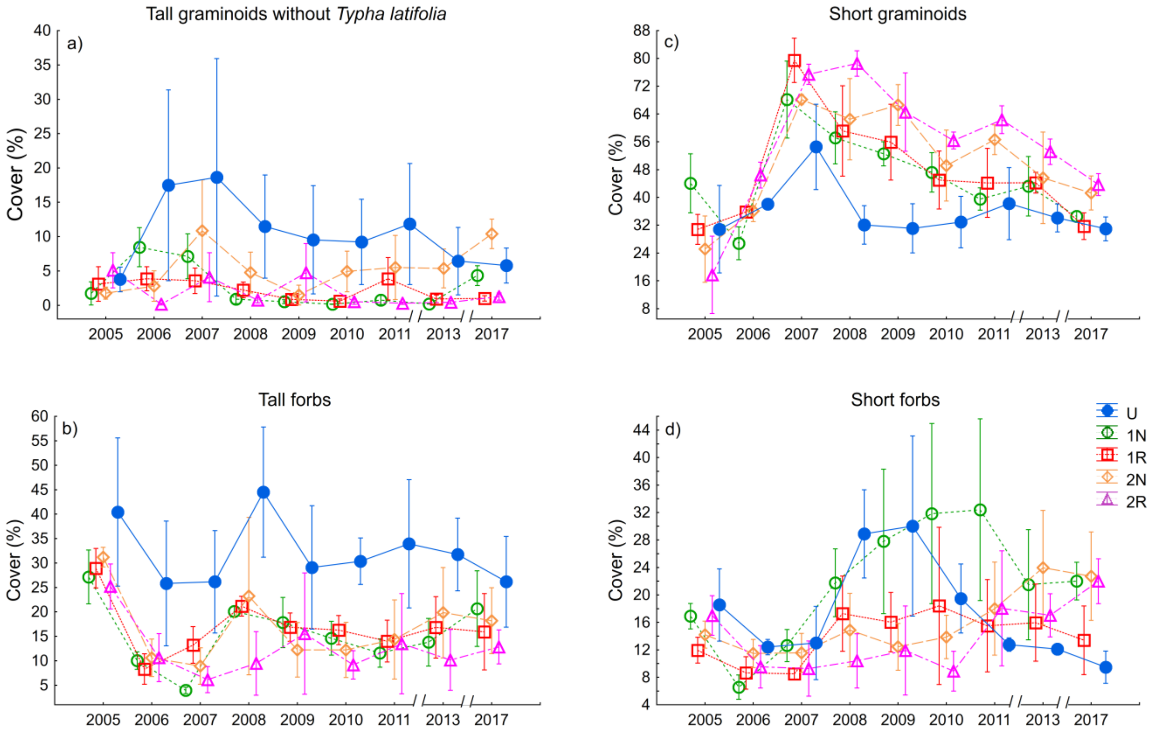


FIGURE 6 The mean cover (%) of main functional groups in the years 2005-2017. (a) tall graminoids without *Typha latifolia*, (b) tall forbs, (c) short graminoids and (d) short forbs in investigated treatments. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1N** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2N** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).

Supporting information to the paper

Titěra, J. et al. What is a suitable management for *Typha latifolia* control in wet meadows? *Applied Vegetation Science*.

Appendix S1: Weather condition of meteorological station in Bedřichov

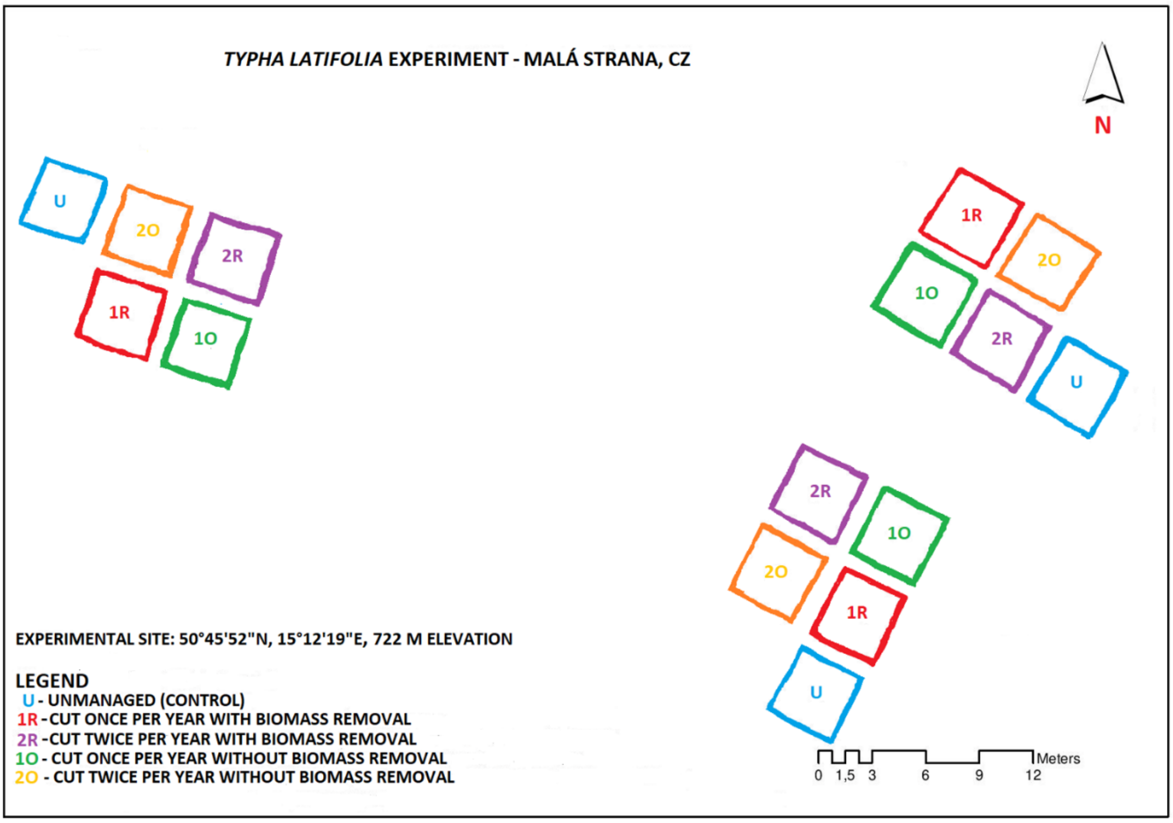
a) Mean temperature for the year 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018 and period 2005–2018

	Mean temperature (°C)														
Month/year	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2005-2018**
January	-2.7	-6.3	0.4	-0.9	-5.6	-7.1	-3.0	-3.1	-4.2	-1.4	-1.6	-3.6	-6.4	-0.6	-3.3
February	-5.7	-5.4	-0.2	0.1	-3.4	-3.8	-4.9	-7.3	-3.9	0.2	-2.5	-0.3	-0.8	-6.5	-3.2
March	-2.6	-2.9	2.3	-0.2	0.2	-0.2	1.0	1.7	-3.9	3.4	1.2	0.0	2.3	-2.4	0.0
April	5.4	3.9	6.9	4.3	7.6	4.6	7.3	5.1	4.0	6.9	4.3	4.0	3.4	9.1	5.5
May	9.6	9.4	11.5	10.7	10.0	8.3	10.4	11.8	9.4	9.3	8.9	10.7	10.5	13.5	10.3
June	12.4	13.9	14.8	14.2	11.6	13.4	13.8	13.3	13.1	12.7	12.3	14.0	14.4	14.1	13.4
July	15.0	18.8	14.6	14.6	14.6	17.0	13.2	15.3	15.8	16.4	16.1	15.1	14.9	16.3	15.6
August	12.8	12.2	14.5	14.0	15.1	14.1	14.8	14.8	14.7	13.1	18.5	13.4	15.0	17.1	14.6
September	11.9	13.5	8.6	9.1	11.8	8.6	11.8	10.6	9.1	11.9	10.3	12.9	9.2	11.5	10.8
October	7.7	8.1	4.8	5.8	4.1	4.0	6.1	5.0	7.6	8.4	6.1	5.1	7.2	7.9	6.3
November	-0.1	3.4	-0.9	2.5	3.8	2.4	2.1	2.9	1.9	4.2	3.8	0.2	1.3	2.7	2.2
December	-3.2	0.7	-2.6	-1.6	-3.3	-7.4	-0.5	-3.4	-0.2	-0.6	2.1	-2.1	-1.6	-1.1	-1.8
Mean	**5.1**	**5.8**	**6.3**	**6.1**	**5.6**	**4.5**	**6.1**	**5.6**	**5.3**	**7.1**	**6.7**	**5.8**	**5.8**	**6.9**	**5.9**

b) Total precipitation for the year 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018 and mean precipitation in period 2005–2018

	Mean precipitation														
Month/year	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2005-2018**
January	167.1	46.2	134.1	135.0	70.5	60.6	89.1	210.3	163.8	34.7	127.0	94.0	92.4	93.0	**108.4**
February	110.1	92.4	111.6	45.6	144.6	38.6	18.3	127.0	78.2	8.5	19.1	94.8	63.5	10.4	**68.8**
March	133.8	97.0	59.9	97.5	144.7	113.6	39.3	56.0	49.2	84.3	70.5	62.9	86.9	73.3	**83.5**
April	30.4	68.4	4.6	75.4	4.4	37.6	49.5	59.1	43.6	82.7	42.3	58.6	131.0	47.4	**52.5**
May	137.1	105.2	76.5	33.2	183.6	174.4	68.6	48.9	127.0	148.0	53.2	56.4	92.0	42.8	**96.2**
June	120.9	87.2	91.3	61.8	114.6	110.0	99.3	96.3	315.9	61.0	98.8	179.8	132.2	91.2	**118.6**
July	190.3	33.5	104.6	130.4	124.6	152.7	461.6	226.9	139.9	190.6	53.4	172.8	200.9	53.1	**159.7**
August	152.8	392.2	98.6	137.2	91.5	473.2	126.7	125.3	87.4	69.8	46.1	66.2	117.4	41.9	**144.7**
September	139.9	43.4	171.0	43.6	40.4	215.5	64.0	38.0	201.6	123.9	57.8	83.8	112.2	49.3	**98.9**
October	35.9	91.5	43.0	114.9	188.8	20.3	84.5	41.1	52.8	90.4	58.1	152.0	212.0	58.7	**88.9**
November	72.2	126.8	173.7	111.1	40.9	124.2	1.0	98.4	75.1	12.7	201.0	73.5	104.5	18.7	**88.1**
December	150.9	89.5	77.5	92.4	99.6	121.3	162.3	77.7	85.3	80.6	42.6	104.2	113.1	197.3	**106.7**
In total	**1441.4**	**1273.3**	**1146.4**	**1078.1**	**1248.2**	**1642.0**	**1264.2**	**1205.0**	**1419.8**	**987.2**	**869.9**	**1199.0**	**1458.1**	**777.1**	**1215.0**

Appendix S2: Design of the experiment.



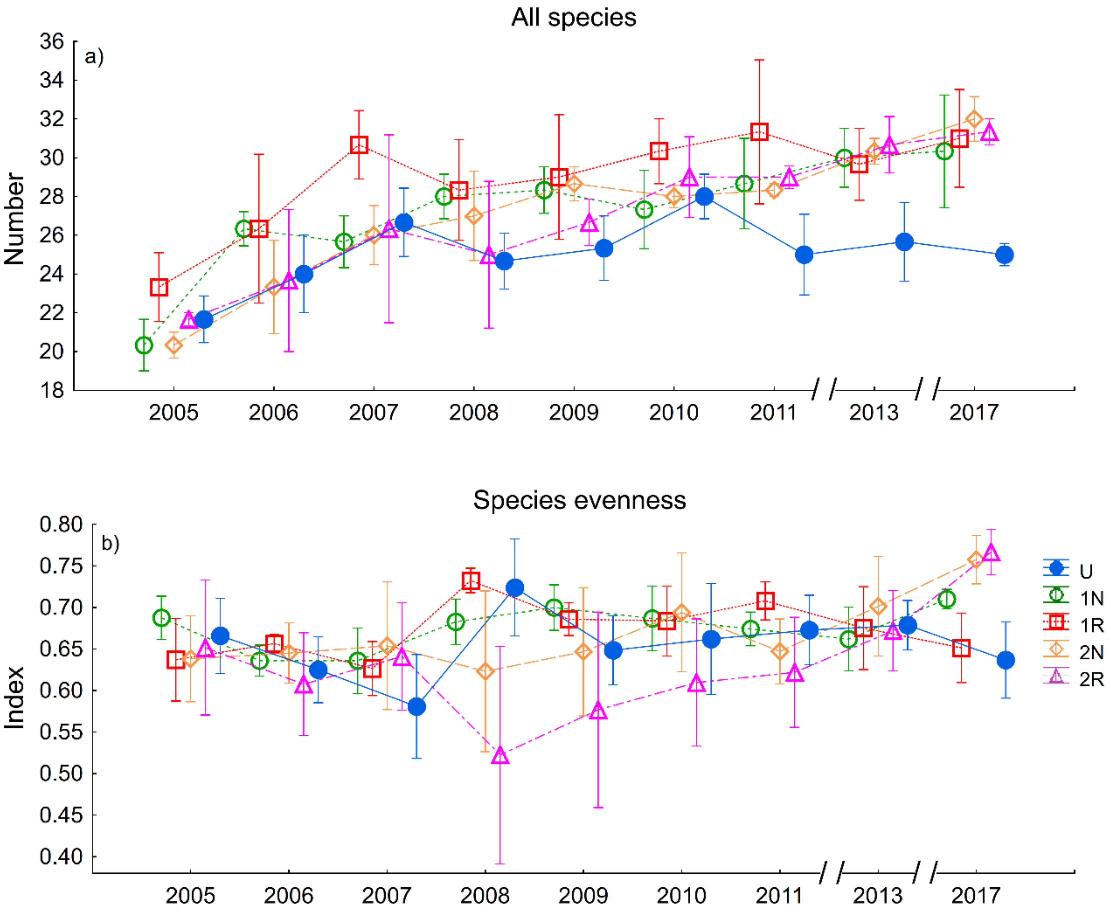
Appendix S3: Results of the two sets of general linear models (GLM) of *Typha latifolia* characteristics and sward characteristics. First set of GLM models tested the effect of all five treatments in a single factor, second set excluded unmanaged treatment and tested the effects of cutting intensity, biomass removal and their interaction. *F* value - *F* statistics for the test of particular variable, *Df. den*. - denominator degrees of freedom (numerator *df*. = 4 for the 5-levels treatment variable, and 1 for each of the intensity, biomass removal and intensity*biomass removal variables), *P* value – obtained probability value. All tests of biomass removal and intensity*biomass removal were not significant (p>0.05) so the respective columns are not presented. Significant *P* values after table-wise Benjamini-Hochberg's FDR correction are in background shading.

		Effect of treatment			Effect of intensity		
Response variable	Transformation	*F* value	*Df. den.*	*P* value	*F* value	*Df. den.*	*P* value
Typha latifolia							
Cover of *Typha latifolia* (%)	$\log_{10}(X+10)$	16.3	11.4	<0.001	85.7	2.0	0.012
Number of *T. latifolia* tillers	$\log_{10}(X+100)$	5.4	7.7	0.022	38.4	1.0	0.099
Height of *T. latifolia* in transect (cm)	$\log_{10}(X+100)$	4.8	9.4	0.022	4.0	2.4	0.163
T. latifolia DMB (t ha^{-1})	$\log_{10}(X+0.1)$	13	14.9	<0.001	30.5	4.5	0.004
T. latifolia litter (kg ha^{-1})	$\log_{10}(X+10)$	23.9	10.9	<0.001	31.1	5.5	0.002
Sward							
Sward height (cm)	$\log_{10}(X+10)$	9.9	10.6	0.001	2.9	4.5	0.156
DMB yield (t ha^{-1})	$\log_{10}(X+0.1)$	11.2	9.8	0.001	1.7	1.9	0.326
Number of all species	none	0.9	8.3	0.520	0.0	2.0	0.864
Evenness	none	0.2	10.3	0.955	0.0	2.3	0.845
Tall graminoids (%)	$\log_{10}(10*X+1)$	2.2	9.3	0.146	0.4	2.0	0.594
Tall forbs (%)	$\log_{10}(X+10)$	1.3	8.1	0.342	0.2	2.0	0.721
Short graminoids (%)	none	4.1	7.5	0.045	5.7	1.6	0.166
Short forbs (%)	$\log_{10}(X+10)$	0.9	10.3	0.502	0.6	2.1	0.527
EIV moisture	asin(sqrt(X))	2.7	7.6	0.113	16.3	1.6	0.081
EIV nutrients	asin(sqrt(X))	9.9	8.2	0.003	19.5	1.7	0.062

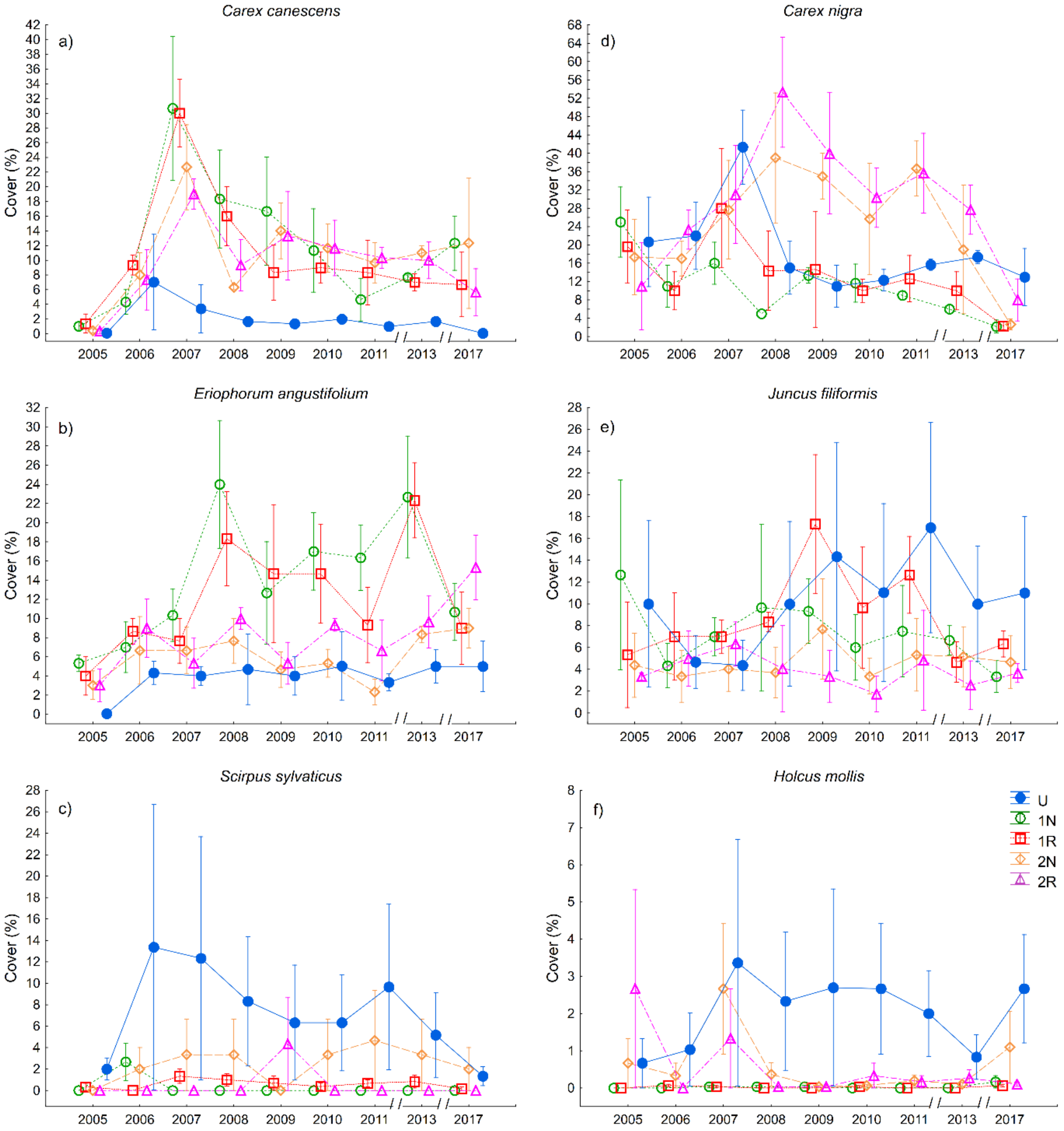
Appendix S4: Assignment of plant species to functional groups. Individual plant species within plant functional groups recorded in the experiment.

Tall graminoids	Short graminoids	Tall forbs	Short forbs	Legumes
Typha latifolia	*Agrostis* sp.	*Angelica sylvestris*	*Alchemilla* sp.	*Vicia cracca*
Alopecurus pratensis	*Anthoxanthum odoratum*	*Bistorta officinalis*	*Anemone nemorosa*	
Avenella flexuosa	*Briza media*	*Chaerophyllum hirsutum*	*Caltha palustris*	
Carex rostrata	*Carex canescens*	*Cirsium palustre*	*Cardamine amara*	
Deschampsia cespitosa	*Carex echinata*	*Crepis paludosa*	*Cardamine pratensis*	
Festuca rubra agg.	*Carex nigra*	*Filipendula ulmaria*	*Cerastium holosteoides*	
Glyceria fluitans	*Eriophorum angustifolium*	*Geum rivale*	*Dactylorhiza* sp.	
Holcus mollis	*Juncus* sp.	*Ranunculus acetosa*	*Epilobium palustre*	
Juncus effusus	*Juncus filiformis*	*Rumex crispus*	*Galium palustre*	
Phleum pratense	*Luzula multiflora*	*Valeriana excelsa* subsp.	*Galium uliginosum*	
Poa palustris	*Nardus stricta*		*Lychnis flos-cuculi*	
Poa pratensis			*Mentha arvensis*	
Poa trivialis			*Montia fontana*	
Scirpus sylvaticus			*Myosotis nemorosa*	
Trisetum flavescens			*Potentilla erecta*	
			Ranunculus auricomus	
			Ranunculus repens	
			Silene dioica	
			Stellaria graminea	
			Stellaria uliginosa	
			Tephroseris crispa	
			Valeriana dioica	
			Viola palustris	

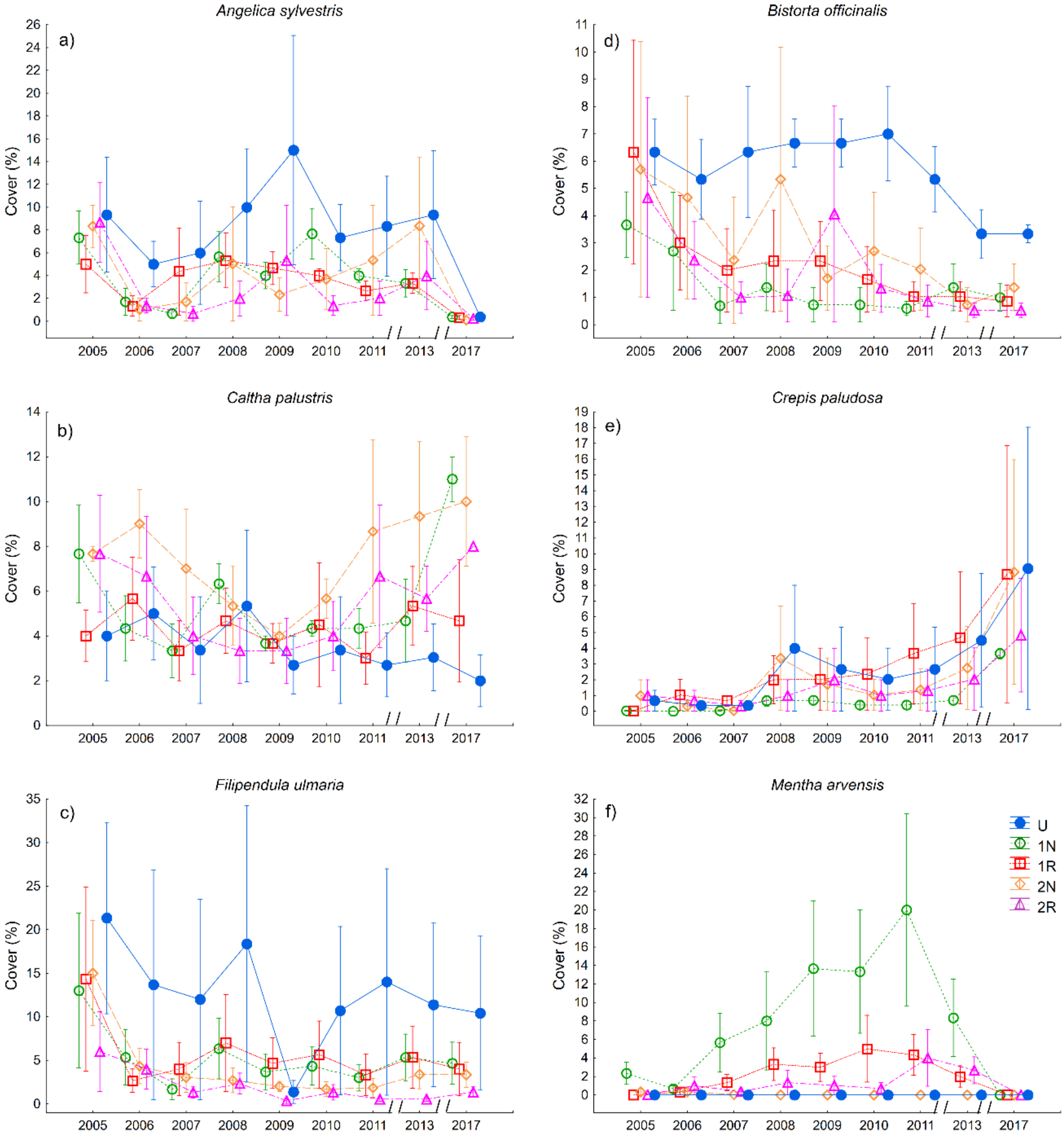
Appendix S5: The mean number of all plant species and species evenness index in the years 2005–2017. (a) number of all species and (b) evenness index. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1O** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2O** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).



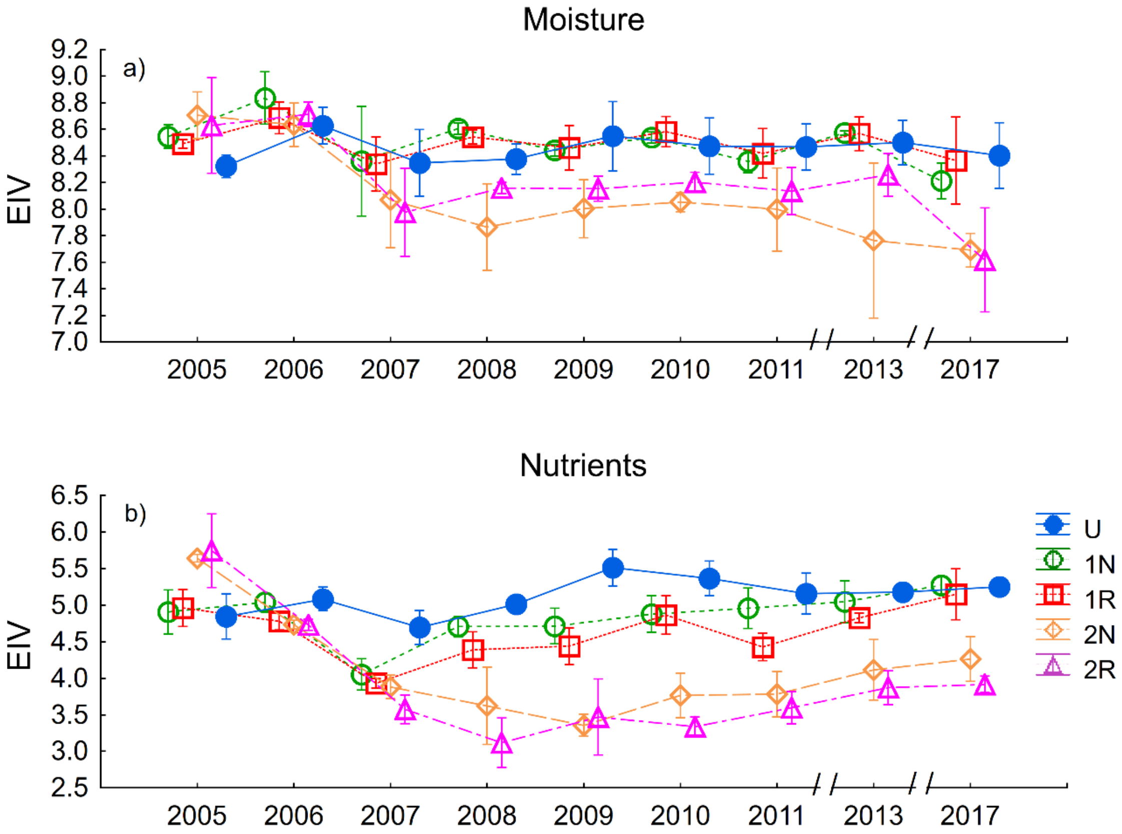
Appendix S6: The mean cover (%) of dominant graminoids species in the years 2005 – 2017. (a) *Carex canescens*, (b) *Eriophorum angustifolium*, (c) *Scirpus sylvaticus*, (d) *Carex nigra*, (e) *Juncus filiformis* and (f) *Holcus mollis* in investigated treatments. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1O** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2O** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).



Appendix S7. The mean cover (%) of dominant forb species in the years 2005 – 2017. (a) *Angelica sylvestris*, (b) *Caltha palustris*, (c) *Filipendula ulmaria*, (d) *Bistorta officinalis*, (e) *Crepis paludosa* and (f) *Mentha arvensis* in investigated treatments. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1O** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2O** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).



Appendix S8: The mean Ellenberg indication values in the years 2005 -2017. (a) EIV moisture and (b) EIV nutrients in investigated treatments. Error bars represent standard error of the mean. Applied treatments were: **U** (unmanaged control); **1O** (cut once a year without biomass removal); **1R** (cut once a year with biomass removal); **2O** (cut twice a year without biomass removal); **2R** (cut twice a year with biomass removal).



Appendix S9:

a) Photo of experimental site

b) Photo of unmanaged treatment

c) Photo of experimental site after cutting

Chapter VII

The main results and discussion

The main results

Central European semi-natural hay grasslands

Widely distributed plant species are indeed the **most important in terms of biomass production** in semi-natural grasslands, but the role of rare species should not be underestimated.

Worth noting is that **rare species** constituted about **three-fourths of all species recorded** in this study.

High biomass inequality among coexisting species.

Biomass inequality can be ascribed to the **five most widespread and most abundant grass species**, which constituted on average about **49.2 % of the t-AGB**.

Grasses were found to have **the highest relative proportion to the t-AGB.**

Nearly half of the t-AGB was dependent on 160 species. Among them, there were **145 species** with a **very low relative proportion to the t-AGB (< 1 %)**.

Non-dominant species can be of different sizes and have **various functional traits** and life strategies, and varying abilities to compete for resources.

Inequality in biomass distribution among species **decreases** with the **increasing functional diversity** of the community.

The result of this study supports the belief that **mass ratio hypothesis and niche complementarity** in semi-natural grasslands **are complementary to each other rather than mutually exclusive**.

The **highest portion of the variation** in the **s-AGB** data was explained by the soil **pH gradient**.

Species with a negative correlation to pH (positive correlation to low pH), including ***A. capillaris***, ***F. rubra, H. mollis,* and *H. maculatum***, are **diagnostic for nutrient-poor habitats** on acidic bedrock. These species are **the most important in terms of productivity at higher elevations**, possibly due to their **physiological adaptations related to acidification**.

Alternatively, **species** with a **positive correlation to pH** are mostly lowland species that also **thrive in low-altitude grasslands** in more **nutrient-rich and less acidic** habitats.

Land use management was found to be the **second most important explanatory variable**, and it accounted for about **one-third of the variation** explained in **s-AGB**.

This study also observed that **mowing facilitates** the **development of less competitive plant species**, especially low-productive short forbs, which are the **core of the diversity in semi-natural grasslands**

Abandonment of semi-natural grasslands is also often followed by the expansion of native **tall forbs** such as ***Aegopodium podagraria*, *Chaerophyllum aromaticum*, *Galium mollugo*, *Senecio nemorensis* agg. and *Urtica dioica***. The **progressive expansion** of these species may lead to the **exclusion of species** with life-history strategies **developed under mowing and grazing management**.

The significant response of **s-AGB** is the result of a **wide gradient in mean annual temperature (6.3 ◦C-9.6 ◦C) and precipitation (721–1600 mm)**, but as individual variables both were **weaker predictors of s-AGB than pH and land use management**.

Less frequent and less abundant species (especially short forbs) combined to play an important role in the production of biomass. What is crucial is that they are the **core of diversity in semi-natural grasslands**.

Regular biomass removal suppresses competitor species, thus **preventing the competitive exclusion of multiple subordinate species** which are an important component of grasslands productivity and diversity.

The **maintenance of species diversity** in grasslands should be **prioritized in nature conservation policies** to ensure the sustainability of ecosystem functioning and services.

Rengen Grassland Experiment (RGE)

Relatively similar response of the plant community **to the different fertilizer applications** throughout the ten years in RGE.

In the **unfertilized control**, there was a certain **directed trend** in plant species composition in response to **ongoing nutrient impoverishment**.

It seems that after 74 years, **some stage of equilibrium** of the grassland community was achieved in all treatments receiving **any type of fertilizer application**, but **less so in the unfertilized control** treatment.

Species-rich grassland communities with higher numbers of species with covers >1% are more resistant to species fluctuation. Therefore, the treatments fertilized by **NP and NPK** which have **lower numbers of species with cover >1%** can show the **greater vulnerability** of their grassland community **to perturbations.**

Tall grasses such as ***Alopecurus pratensis*, *Arrhenatherum elatius*, and *Trisetum flavescens*** had **higher cover** in the treatments fertilized by **N in combination with Ca, P and K**.

The variability of ***Briza media* and *Festuca rubra* agg**. in treatments with **Ca and CaN** application are an example of **compensatory dynamic**. The decrease in cover of one species is compensated by an increase of another species.

The stability in the cover of ***Nardus stricta*** in the **unfertilized control** underlined its preference for, or **adaptation to, oligotrophic acid soils**.

Plantago lanceolata was one of the forb species **present in all treatments**. However, the **addition of P reduced its cover** significantly, because in nutrient-rich soil **cannot fully compete for light with grasses** due to its leaf morphology.

Treatments **without N application** promoted the annual facultative root **hemi-parasitic plant *Rhinanthus minor* and the forb *Leucanthemum vulgare***. It seems that increased biomass **productivity (more than 3 t/ha DM)** was **detrimental to the competitive ability of both** these **species**, as observed throughout the study period.

Leguminous species such as ***Trifolium pratense* and *Lotus corniculatus*** were supported by **lime application without N fertilization**. The **negative response** of both species on **N application** was observed for the majority of years.

The treatments with **Ca and CaN application** had **greater amounts of biomass DM yield**, which led to **higher P and K depletion from the soil** in comparison with the control treatment.

The **combined application** of growth **limiting macro-nutrients** always resulted in a **strong diversity decline**.

The Admont Grassland Experiment (AGE)

All combinations of nutrients that included P had dry matter biomass **yield > 5 t ha^{-1}** whereas all treatments supplied with only **one of three nutrients (N, P, K treatments)** had **similar yield as in the unfertilized** treatment.

Although the **different fertilizer applications affected plant species composition**, there was **no** significant **effect on characteristics of species diversity**.

The **high frequency of cutting** was probably the main factor that caused the **predominance of short species** in all treatments.

Tall grasses were supported by the treatments **with P fertilization** (NPK, P, PN, and PK).

Short grasses had the **highest cover in the N** treatment.

The cover of both **tall and short forbs** was **lowest in the N** treatment.

The **applied fertilizers contained additional nutrients**, which may also have **contributed to changes in soil nutrient status**.

P was applied as a **superphosphate**, which **simultaneously supplied** the soil with **Ca.** It increased soil Ca content and also **raised the pH-value. Any** potential **harmful effect of P** fertilization was **diminished by the simultaneous increase** in soil **pH**-value.

N was applied at a moderate level as NH_4NO_3+$CaCO_3$ and the **content of Ca in this fertilizer reduced soil acidification**.

Limitation of P in the **N and the KN** treatments could have **reduced the negative effect of N** on species richness.

K fertilizer in the **form of KCl** causes **soil acidification,** because **Cl** from KCl fertilizer **binds Ca** from soil and is leached as $CaCl_2$ to lower soil depths.

In treatments where **nutrients were combined**, the **effects of individual fertilizers were multiplied or** they may have **acted against each other**. For example, in the PK and NPK treatments the effects of P and K fertilizers on Ca soil concentration acted against each other.

Mineral-rich alluvial clay soils such as occur on this site are able to **release considerable amount of K** and therefore maintain a **sufficient K level** in the soil despite the high amount of K removed with harvested herbage.

Trisetum flavescens was the only **tall grass** species that was **tolerant of the higher frequency** of cutting.

The **negative relationship** between ***Agrostis capillaris*** **and soil P** concentration.

The **highest grasses/forbs ratio** in the N treatment showed the **positive influence of N** fertilization on the **cover of grasses**.

The **low grasses/forbs ratio** in the **PK, NPK and K** treatments and the relatively balanced grasses/forbs ratios in the CTL and P treatments is connected with **high proportion of legumes** (especially ***Trifolium repens*, *Trifolium pratense*, *Lathyrus pratensis***) in these treatments.

Legumes were **suppressed** by treatments with **N application (N, KN, PN)**.

Leontodon hispidus was the most frequent non-legume forb **in the majority of treatments**, probably more supported by cutting frequency, rather than availability of Ca and Mg nutrients in the soil.

P. lanceolata showed a tendency for **prevalence in the PN and P** treatments, its cover was probably influenced indirectly by P fertilizers through its Ca content (positively correlated with soil Ca concentration and pH-value).

Negative relationships between the occurrence of ***A. millefolium*** and soil **Ca concentration** (preference to acid soils).

The occurrence of prostrate ***T. officinale*** in the NPK treatment was **supported by nutrient supply** and **frequent defoliation**.

Long term experiment in the Nízké Tatry Mts (Slovakia)

Rumex obtusifolius is very **well established in mountain nutrient-rich unmanaged** grasslands, especially in abandoned former cattle resting places.

The **highest cover of *R. obtusifolius*** was observed in the **unmanaged** treatment during all experimental years. The **cover** was relatively **stable (about 75%)** during the experimental time, probably because of nutrients enrichment.

A gradual **decrease of *R. obtusifolius*** from **77%** at the beginning of the experiment to **3%** in the last year of the experiment was recorded under **cutting twice per year**.

The **herbicide application** in combination **with reseeding of grass/forb mixtures** can **completely suppress *R. obtusifolius*** and establish species rich-grasslands with desirable species composition.

Long-term **cutting twice per year** seems to be **more suitable** and environmentally friendly management **to control *R. obtusifolius*.** (possible environmental risks the using of glyphosate)

Experiment with *Typha latifolia* in Malá Strana nature reserve

***Typha latifolia* characteristics**

The results of our study confirmed the findings of previous short-term studies, that **cutting** is suitable management **for *T. latifolia* control**; however, in terms of **intensity of cutting** our **results** are **not so straightforward**.

Thus, although higher frequency of cutting significantly reduced *T. latifolia* cover and its DMB and litter yield, the number and height of its tillers remained unaffected. A possible explanation for this could be that there are **changes in *T. latifolia* morphology under the different cutting frequencies**, for example in the number of leaves or thickness of plant stems, as well differences in stem or leaves tissue density.

There was only a **slight tendency of removal of cut biomass to affect the characteristics of *T. latifolia*** (apart from an effect on *T. latifolia* plant height).

The **moisture and temperature conditions were favourable** for supporting **easy decomposition of aboveground biomass** (including biomass of *T. latifolia*). Therefore, **undecomposed residues** of aboveground cut biomass **did not accumulate on the surface** for sufficient time to adversely affect plant growth.

Sward characteristics

The results from our experiment confirm findings from previous research on mezophilous meadows that **cutting reduces the height and yield of the sward in the next yea**r.

The results of this experiment **did not show the often-observed negative relationship between the number of species and yield/sward height** of the first cut.

This was probably due to the **wet and oligotrophic conditions** of this site which **did not allow the formation of a dense sward** and thus, even in the unmanaged treatment, **light conditions in the sward** were **still sufficient to support species diversity**.

Because of the relatively low **density of plants of *T. latifolia***, the **light conditions in the sward were also sufficient** to support **short and mostly competitively weak species**.

Nevertheless, **according to the RDA ordination diagram**, **three groups of plant species** were **promoted by different cutting intensities** that differed from each other in their plant species composition.

This was not only a result of light availability but also as a **direct response of individual species to the cutting management**, as tall species are damaged by defoliation more than short ones.

Biomass removal after cutting, compared with leaving cut biomass on the sward surface, **had no effect on plant species composition** and this was probably because of rapid decomposition processes of the cut biomass on the sward surface under the environmental conditions of the site.

The dominance of *E. angustifolium* in the group of **one-cut treatments** (1R, 1N) confirmed that **management regimes that are either too intensive**, such as multiple defoliation or on the other hand **too extensive (i.e., abandonment) are unfavourable** for the growth of this species.

The **dominance of two *Carex* species in the group of two-cut treatments** (2R, 2N) could be explained by the **positive effect of cutting on the cover of *C. nigra* and *C. canescens***.

The cover of the highly productive ***F. ulmaria* decreased in** plots with **cutting management** in our experiment. This is a typical response of this species to defoliation.

Bistorta officinalis is also a **typical species of abandoned grassland**. It is able to suppress the surrounding vegetation by its above-ground cover of leaves, large amount of litter and depletes nitrogen from the soil which was confirmed by its **higher presence in unmanaged plots** in our experiment.

Juncus filiformis is a species which prefers **sufficient availability of light** and although it is a shade-intolerant species, **in our experiment it was also present at a high cover in the unmanaged treatment**. Its occurrence in abandoned plots was probably **positively influenced by the absence of cutting and at the same time by quite low density of *T. latifolia***, even in abandoned plots, which enabled light to penetrate into the lower layers of the sward.

Management implications

Practical management should **not be focused only on reduction of *T. latifolia***, but it should also **take into account the requirements for nature conservation** in terms of the **target state of the whole plant community and the rarity and endangered status of individual plant species** in the region.

The **response of the whole community to the cutting intensity** appears to be a **very important factor** in the **choice of appropriate management**.
Present experiment led us to suggest that **biomass removal does not necessarily have a significant effect on vegetation** under certain conditions. It seems that **wet edaphic conditions**, as in our experiment, can probably **accelerate the decomposition of unremoved cut biomass**, and thereby preserve conditions of sufficient light penetration required for vegetation growth. Moreover, the issue of biomass removal is also closely linked to the soil nutrient status, as **regular and long-term removal of biomas**s can result **in oligotrophication.**

In wet meadows where there is a dominancy of *T. latifolia* we consider that **extensive management (with cutting once per year without biomass removal)** can be **sufficient to maintain the plant species community in a desirable state**. Furthermore, as a management technique, this is a **relatively low-cost option compared with multiple cutting and biomass removal**. Moreover, **cutting once per year without biomass removal protects** the meadow vegetation from **overgrowth by shrubs and trees**.

Defoliation management cutting once or twice per year, regardless of whether or not the cut biomass was removed, led to **the successive changes in plant species composition** but had **no effect on the number of plant species and species evenness index.**

Comments of the results and discussion

Responses of individual plant species on long-term fertilized experiments

I choose individual plant species and compared their responses to different fertilizers applications from 6 oldest long-term fertilized grassland experiments across Europe. I took the results from Rengen Grassland Experiment, Admond Grassland Experiment, Steinach Grassland Experiment, Veľká Lúka Permanent Grassland Experiment, Palace Leas Hay Meadow Experiment and Černíkovice grassland experiment (terminated). Botanical data about individual plant species from the oldest grassland experiment (Park Grass Experiment) and from Ossekampen Grassland Experiment (Netherland) I have not found.

The dominant grass species such as *Festuca rubra*, *Agrostis capillaris, Arrhenatherum elatius*, *Dactylis glomerata* and *Alopecurus pratensis* (discussed below) were also the most common species in the landscape study in the Sudetes Mountains (Central Europe) under managed as well us under abandoned grasslands regardless of the amount of nutrients in the soil. Because of the different dominant plant species in Nízké Tatry Mts and Malá Strana experiments, the results from these two experiments are not discussed in this section.

Trisetum flavescens

Tall grass *Trisetum flavescens* was supported by P application on both long-term fertilizers experiments in Admont (AGE) and Rengen (RGE). The presence of this species was on all treatments fertilized by P either individually or in combination with other nutrients (N or K) on both experiments. This is probably because P was limiting nutrient in both long-term grasslands experiments and growth of this species increased after the P application. This tall highly competitive species over-competed some of the other short species under two cut regime in RGE, but not so much in AGE with cutting three times per year. *T. flavescens* was only tall grass tolerating three cutting regime in AGE, contrary cutting regime twice per year under different fertilizers applications in RGE supported large number of tall grass species. Similarly, moderate and higher rates of NPK fertilizers increased cover of *T. flavescens* under cutting three times per year in Vel’ká Lúka long-term grassland experiment (Vargová et al., 2020). *T. flavescens* also increased cover in treatments fertilized by higher rates of NPK fertilizers in Steinach Grassland experiment (SGE) (Hejcman et al., 2014). Based on the results from 4 long-term grassland experiments it appears, that tall grass *T. flavescens* is well adapted on higher rates of different nutrients applications and their combinations.

Arrhenatherum elatius, Alopecurus pratensis

Arrhenatherum elatius was supported by the highest rates of NPK fertilization (Titěra et al., 2020; Vargová et al., 2020; Honsová et al., 2007), because it is highly competitive tall grass well adapted to higher nutrient levels. Similarly, *Alopecurus pratensis* prosper under high NPK nutrient levels (Titěra et al., 2020; Hejcman et al., 2014; Vargová et al., 2020). Contrary *A. pratensis* can decrease cover within some plant communities under high levels of NPK. This was observed in Černíkovice long-term grassland experiment. It was because other high competitive tall species (*Anthriscus sylvestris*, *Cirsium arvense*, *Elytrigia repens*, *Holcus lanathus*, *Poa pratensis*) increased cover as well, even they had lower total cover than *A. pratensis*. But under lower level of NPK they did not increased cover so much (Honsová et al., 2007). *A. pratensis* was supported by treatments with P application, but less under NPK (higher rate) fertilization in Palace Leas Hay Meadow. *A. pratensis* totally disappeared under N, K and NK treatments. This could be probably because N was supplied as either ($(NH_4)_2SO_4$) or (NH_4NO_3) and K as (KCl). These forms of application cause acidity, because of ions SO_4^{2-} NO_3^{-1} and Cl^{-1}, which are also part of NPK fertilizers (Kidd et al., 2017). The fertilization by NP and NPK was applied together with Ca in RGE. Similarly, the highest cover of *A. pratensis* was under the different combinations of N, P, K nutrients applied together with Ca in SGE. Granulated nitrogen fertilizer LAD, which was applied in Vel'ká Lúka also contain Ca^{2+} ions. Therefore it seems that *A. pratensis* could be a little bit sensitive to lower pH. Particularly in competition with other tall species well adapted to lower pH.

Holcus lanatus

Holcus lanatus had the highest cover in CaNPK treatments and the lowest cover in control, CaN and Ca treatments in RGE. *H. lanatus* had only marginal cover <1% in all treatments and the highest cover had in NPK and P treatment in AGE. *H. lanatus* had the highest cover in NPK treatments fertilized either with the lowest or with the highest N rate and the lowest cover in not fertilized (control) and N_{100}PK with lenient N rate treatment in Černíkovice grassland experiment (Honsová et al., 2007). *H. lanatus* had the highest cover in NPK treatments, PK, and NP treatment and the lowest cover in NK and control treatment in Palace Leas Hay Meadow (Kidd et al., 2017). *H. lanatus* had the highest cover in NPK treatments with different rates of applied nutrients and without Ca application and the lowest cover in K and control treatment in SGE (Hejcman et al., 2014). *H. lanatus* rather prefer nutrient-rich conditions and can successfully compete with other plants under the combined application of N, P and K, as was observed in 5 long-term grassland experiments.

Dactylis glomerata

Dactylis glomerata had the highest cover in CaNP treatment and CaNPK treatments and the lowest cover in not fertilized (control) treatment in RGE. *D. glomerata* had the highest cover in PK, and NPK treatment and the lowest cover in not fertilized (control) treatment in AGE. *D. glomerata* had the highest cover in lenient and moderate NPK fertilization in Černíkovice grassland experiment (Honsová et al., 2007). *D. glomerata* was present only in not fertilized (control) treatment and under all treatments with NPK fertilization with different rates of applied nutrients totally disappeared in Vel'ká Lúka grassland experiment (Vargová et al., 2020). *D. glomerata* had the highest cover in NPK (higher rate) treatment and in P treatment and totally disappeared in N, K, NK, and PK treatment in Palace Leas Hay Meadow (Kidd et al., 2017). *D. glomerata* had the highest cover in NPK treatments, CaNK, and CaPK treatment and the lowest cover in not fertilized (control) treatment and in NK treatment without liming. This species totally disappeared in K treatment in SGE (Hejcman et al., 2014). Based on the results from 6 long-term grassland experiments was found, that *D. glomerata* rather prefer NPK fertilization to no fertilization. But in some grasslands plant communities can be over-competed by other tall species such as *Arrhenatherum elatius*, *Trisetum flavescens*, *Alopecurus pratensis* (Vargová et al., 2020) or *Poa pratensis* (Honsová et al., 2007) or *Holcus lanatus* (Hejcman et al., 2014).

Anthoxanthum odoratum

Anthoxanthum odoratum increased cover in N, KN and PN treatments and decreased cover in P, K, PK and NPK treatments compared with the control treatment in AGE. NPK treatment had the lowest cover of *A. odoratum* under three cutting regime in AGE. *A. odoratum* had the lowest cover in treatments fertilized by N in combination with P and Ca and the highest cover in control treatment in RGE. *A. odoratum* had similar cover under control, lenient NPK and moderate NPK fertilized treatments. This species decreased cover in high NPK fertilized treatment under cutting three times per year in Vel'ká Lúka grassland experiment (Vargová et al., 2020). *A. odoratum* had the highest cover in NK and K treatments and the lowest cover in treatments with higher rates of NPK and PK fertilization in SGE (Hejcman et al., 2014). Marginal cover of *A. odoratum* was only in treatments not fertilized by higher rates of NPK in Černíkovice grassland experiment (Honsová et al., 2007). The lowest cover had *A. odoratum* in control, K, P, NPK (lower rate), NKP (higher rate) and the highest cover in N and NK treatments in Palace Leas Hay Meadow (Kidd et al., 2017). Based on the results from 6 long-term grassland experiments was revealed, that *A. odoratum* prefer rather N fertilization alone and decrease cover under high rates of NPK fertilization.

Agrostis capillaris

Agrostis capillaris was supported by N fertilization in AGE. Combined application of N and either K or P increased cover of this species pronouncedly. But NPK fertilization decreased cover of *A. capillaris* to the lowest level from all treatments. The highest cover had *A. capillaris* in NK, followed by NP, NPK (lower rate), NKP (higher rate) and N treatments. The lowest cover had in P treatment (Kidd et al., 2017). Similarly *A. capillaris* was supported by N and K fertilizers application and also by no fertilization in SGE (Hejcman et al., 2014). The cover of *A. capillaris* was very low in all treatments, but the highest cover (cca 1.5 %) was recorded in N-fertilized treatment in RGE. The results from 4 long-term grassland experiments showed, that *A. capillaris* is well adapted to N fertilization. *A. capillaris* can sometimes thrive in treatments with combined application of N with other nutrients such as K and/or P, but not so much in treatments fertilized only P.

Luzula campestris

Luzula campestris was present only in not fertilized, limed and CaN treatment in RGE. *L. campestris* had the lowest cover in NPK treatment and the highest cover in N, KN and not fertilized (control) treatment in AGE. *L. campestris* had the highest cover in not fertilized (control) treatment and in NPK treatments had only marginal cover or totally disappeared in Černíkovice grassland experiment (Honsová et al., 2007). *L. campestris* had the highest cover in N and NK treatment, in PK treatment had only marginal cover and in NPK (higher rate) treatment totally disappeared in Palace Leas Hay Meadow (Kidd et al., 2017). Species with similar ecological requirements *Luzula multiflora* had the highest cover in control and K fertilized treatment in SGE (Hejcman et al., 2014). Based on the results from 5 long-term grassland experiments, *L. campestris* prefer rather nutrient poorer conditions without higher nutrients inputs. *L. campestris* can be tolerant to K fertilization and sometimes even supported by N and NK fertilization such as similar species *L. multiflora*.

Festuca rubra agg.

Festuca rubra agg. had higher cover in control, limed and N fertilized treatments. Combined application of N and P led in decrease in the cover of this species throughout 10 years of study period in RGE. N fertilization also supported cover of *F. rubra* agg. in AGE, but in contrast with RGE under the combined application of N and P this species thrived as well. Cover of *F. rubra* agg. decreased on all treatments fertilized by K on both experiments (AGE and RGE). NPK fertilization even in higher rates did not change the cover of *F. rubra* agg. so much, as its cover was similar as under no fertilization. Moderate rate of NPK increased the most the cover of *F. rubra* agg. under cutting three times per year in Veľká Lúka (Vargová et al., 2020). *F. rubra* agg. had the highest cover in control treatment and exhibited a negative response to N application under three cutting regime in SGE (Hejcman et al., 2014). The highest cover of *F. rubra* agg. was in control and K fertilized treatments compared with the N, P treatments and treatments with combined application of nutrients (NP, NK, PK, NPK) under cutting once per year in July and aftermath cattle or sheep grazing in Palace Leas Hay Meadow. The second highest cover of *F. rubra* agg. in K treatment from all treatments is in contrast with findings from AGE and RGE (Kidd et al., 2017). *F. rubra* had the highest cover in control treatment and in N_{100}PK and N_{50}PK treatments. The lowest cover in PK, N_{150}PK and N_{200}PK treatments (Honsová et al., 2007). Based on the results from 6 long-term grassland experiments, the preference of *F. rubra* agg. to the soils with lower rather than higher nutrient status was found. However, *F. rubra* agg. can be also tolerant to higher nutrient rates of different fertilizers, but in that communities not prevail as a dominant plant species, but rather as subdominant or as a species with the lower cover.

Poa pratensis

Poa pratensis had the highest cover in PN and P treatments and the lowest cover in K, KN, PK and not fertilized (control) treatments under cutting three times per year in AGE. *P. pratensis* had the highest cover in NP treatment with Ca application and totally disappeared in not fertilized (control) treatment in RGE. *P. pratensis* had the highest cover in NPK treatment with the highest rate of applied nutrients, the lowest cover in not fertilized (control) treatment in Černíkovice grassland experiment (Honsová et al., 2007). *P. pratensis* was supported by PK fertilization and had only marginal cover (0.1 %) in not fertilized (control) treatment in SGE (Hejcman et al., 2014). *P. pratensis* had similar cover under not fertilized (control) treatment as in the treatments fertilized by different rates of NPK nutrients in Veľká Lúka

(Vargová et al., 2020). Based on the results from 5 long-term grassland experiments, there is a tendency of *P. pratensis* to prefer rather nutrient rich conditions. The presence of *P. pratensis* in Vel'ká Lúka grassland experiment in control treatment could be because of higher nutrient status of soil also under no fertilization. The different responses of this species to applications of either individual nutrients or their combinations under different long-term experiments probably also depend on plant community, where this species grows (Reader et al., 1994).

Plantago lanceolata

P application and PN application in AGE increased cover of *Plantago lanceolata*, in contrast P application together with N and Ca decreased cover of *P. lanceolata* in RGE. Application of K together with PN decreased cover of *P. lanceolata* on both experimental sites, but in AGE the cover of *P. lanceolata* on NPK treatment was still higher than in control treatment. In contrast *P. lanceolata* had the highest cover in control treatment throughout all 10 years of study period in RGE. Despite, that *P. lanceolata* is well adapted to the soils with reduced fertility (Honsová et al., 2007; Pavlů et al., 2012; Kidd et al., 2017), long-term cutting regime three times per year probably decreased level of P to minimum, which is then not sufficient even for this species. Control and K treatments had therefore the lowest cover of *P. lanceolata* in AGE. In contrast cutting regime twice per year in control treatment in RGE, even though the level of available P in the soil was very low, still did not limit the growth of *P. lanceolata* as in AGE. *P. lanceolata* was presented only in control treatment and under the all treatments fertilized by any rate of NPK nutrients disappeared in Vel'ká Lúka. But the cover of *P. lanceolata* was also very low (1%) in control treatment (Vargová et al., 2020). In contrast *P. lanceolata* was the most common species in all plots, irrespective of fertilizer input under three cutting regime in SGE (Hejcman et al., 2014). *P. lanceolata* had the highest cover in control treatment and in treatments with individual application either P or K. The lowest cover had in PK and NPK (lower rate) treatments and it was not present in N, NK and NKP (higher rate) treatments (Kidd et al., 2017). *P. lanceolata* was only present in control (1.3 %), PK (1.1 %) and N_{100}PK treatments. *P. lanceolata* disappeared totally from N_{50}PK, N_{150}PK, N_{200}PK treatments under cutting twice per year in the Černíkovice experiment (Honsová et al., 2007). Based on the results from 6 long-term grassland experiments it seems, that *P. lanceolata* prefer rather nutrients poorer grasslands, either unfertilized or with lower rates of nutrients applications. However, *P. lanceolata* can sometimes also over-stand higher rates of fertilizers applications, better under multiple cutting regimes, because of its lower competitive ability.

Leontodon hispidus

Leontodon hispidus was only present in control and limed treatment and totally disappeared in CaN, CaNP and CaNPK treatments in RGE. *L. hispidus* had the highest cover in NPK, PK, KN, P and K treatment and the lowest cover in N and PN treatment under three cutting regime in AGE. *L. hispidus* had the highest cover in not fertilized (control treatment) and the larger was rate of NPK fertilization, the lowest was cover of this species in Vel'ká Lúka permanent grassland experiment (Vargová et al., 2020). *L. hispidus* had the highest cover in K fertilized treatment and totally disappeared from NPK treatments with the highest rates of applied nutrients in SGE (Hejcman et al., 2014). The results from 4 long-term grassland experiments showed, that *L. hispidus* rather prefer no fertilization or individual fertilization of K or P nutrients. This species has probably lower competitive ability about the light compared with other grassland species, therefore disappeared from the most fertilized NPK treatments in long-term grasslands experiments. Cutting three times per year probably reduced competitiveness of other plant species and was a stronger factor than the soil nutrient status in AGE. Contrary, additional NPK fertilization even under three cutting regime in productive alluvial grassland in SGE or in permanent wet grassland with dominance of *Alopecurus pratensis* in Vel'ká Lúka experiment suppressed *L. hispidus*. This could be because the competition of other plant species was not to such an extend as in AGE reduced.

Achillea millefolium

Achillea millefolium had the highest cover in KN and K treatment and the lowest cover in PN, N and P treatment under three cutting regime in AGE. *A. millefolium* was only present in control, Ca and CaN treatment and had only marginal cover under two cutting regime in RGE. *A. millefolium* had the highest cover in the control and PK treatment and the lowest cover in NPK treatments with the highest rates of nutrient applications in the Černíkovice experiment (Honsová et al., 2007). *A. millefolium* had the same cover in all treatments independently of fertilizer application with exception of lenient NPK fertilization with the lowest cover in Vel'ká Lúka permanent grassland experiment (Vargová et al., 2020). *A. millefolium* had the highest cover in not limed NK treatment with higher rate of K application and in not limed NPK treatment with higher rate of K fertilization under three cutting regime in SGE. This species had the lower cover in K, KP and KN treatments and the lowest cover in not fertilized control and limed NPK treatment with lowest rate of NP fertilization. This species totally disappeared in limed NPK treatment with higher rates of NP fertilization (Hejcman et al., 2014). Based on the results from 5 long-term grassland experiments it seems, that *A. millefolium* prefer rather acidic soils with lower pH, but can survive in very marginal cover under liming as was found in RGE. Positive response of this species to K fertilization in the form KCl in many long-term grasslands experiments was due to Cl^- ions leading to soil acidity. Application of K nutrient and liming, eventually application of K with other nutrients together with Ca reduced this effect. NPK fertilization together with Ca application decreased cover or totally suppressed *A. millefolium.*

Taraxacum officinale agg.

Taraxacum officinale agg. had the highest cover in CaN and CaNP treatment and the lowest cover in not fertilized (control) treatment in RGE. *T. officinale* agg. had the highest cover in NPK and NP treatment and the lowest cover in K and not fertilized (control) treatment under cutting three times per year in AGE. *T. officinale* agg. had the highest cover in not fertilized (control) treatment and the lowest cover in NPK fertilization treatments with moderate and higher rate of nutrients applications in Vel’ká Lúka permanent grassland experiment (Vargová et al., 2020*). Taraxacum* spp. had only marginal cover in all treatments and in NPK treatment with lenient nutrients applications totally disappeared in the Černíkovice experiment (Honsová et al., 2007). *T. officinale* agg. was only present in P and PK treatment, from the other treatments totally disappeared in Palace Leas Hay Meadow (Kidd et al., 2017). *T. officinale* agg. had the highest cover in NK and CaNPK treatments with moderate and higher rates of nutrients applications in SGE (Hejcman et al., 2014). Based on the results from 6 long-term grassland experiments the responses of *T. officinale* agg. to fertilizer applications are not straightforward. Overall is *T. officinale* agg. supported by broader range of nutrient applications with respect to individual plant communities under different long-term grassland experiments with regular cutting regime and rather do not prefer no fertilization, especially under low cutting intensity or in abandonment (Supek et al., 2016).

Trifolium pratense

Leguminous species *Trifolium pratense* had higher cover in control and limed treatments throughout the 10 years of study period in RGE. Fertilization by N decreased cover of this species in all treatments in both experiments (AGE and RGE). PK application had positive effect on *T. pratense* in AGE, but in RGE, PK was applied with N, so the cover of *T.*

pratense was lower than in control and limed treatments during the study period. But in Veľká Lúka permanent grassland experiment low rate of NPK fertilization increased cover of *T. pratense* and higher rate of NPK decreased its cover under cutting three times per year (Vargová et al., 2020). It is highly probable that the application of N together with PK in very low rate is not detrimental, but even expedient for *T. pratense*. Similarly, *T. pratense* tolerated moderate rate of N fertilizers in SGE with cutting regime three times per year (Hejcman et al., 2014). *T. pratense* had the highest cover in control treatment and in treatments with individual application either P or K. The lower cover had in PK, NP and NPK (lower rate) treatments and it was not present in N, NK and NPK (higher rate) treatments (Kidd et al., 2017). *T. pratense* was only present in PK treatment in the Černíkovice experiment (Honsová et al., 2007). Based on the results from 6 long-term grassland experiments it appears, that *T. pratense* prefer rather no fertilization or application of individual nutrients either P or K or their combination (PK). But *T. pratense* can sometimes tolerate very low rates of N fertilization (cca 50 kg ha^{-1} y^{-1}) and combination this low rate of N with PK fertilization can even increase cover of this species.

Lathyrus pratensis and *Trifolium repens*

Lathyrus pratensis increased cover under PK fertilization in AGE and Palace Leas Hay Meadow (Kidd et al., 2017). *L. pratensis* supported PK and control treatments in Černíkovice experiment (Honsová et al., 2007) and moderate NPK fertilization in SGE (Hejcman et al., 2014) and in Veľká Lúka (Vargová et al., 2020) and NPK treatments in RGE. *Trifolium repens* had the highest cover under PK fertilization in AGE, Černíkovice experiment (Honsová et al., 2007) and in SGE (Hejcman et al., 2014). *T. repens* decreased cover under higher NPK fertilization in Veľká Lúka (Vargová et al., 2020). *T. repens* tolerate moderate rate of NPK fertilization in AGE, in Veľká Lúka (Vargová et al., 2020) and in SGE (Hejcman et al., 2014). Based on the results from long-term grassland fertilized experiments it seems, that *L. pratensis* and *T. repens* thrive under PK fertilization. Both species can also tolerate or even increase their covers under moderate NPK fertilization. *T. repens* also prosper under no fertilization in AGE, in Veľká Lúka (Vargová et al., 2020), in SGE (Hejcman et al., 2014), in Černíkovice experiment (Honsová et al., 2007) and in Palace Leas Hay Meadow (Kidd et al., 2017) rather than *L. pratensis*.

Based on these findings it seems that the results only from two long-term grasslands experiments are not so robust to formulate precise conclusions about the ecology of individual plant species, plant species communities and grassland ecosystems. Therefore it is necessary to combine results from many long-term grassland experiments to strengthen and clarify the general conclusions in grassland ecology.

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Chapter VIII

Summary of results

Future recommendations

Summary of results

One of the contributions of this dissertation from the landscape study in semi-natural grasslands is that widely distributed plant species are indeed as the most important in terms of biomass production. Hovewer rare species in terms of biomass production constituted about three fourths of all species recorded within the landscape study. There was high biomass inequality among coexisting species. The five most widespread and the most abundant grass species constituted on average almost half of the total aboveground biomass, whereas second half was dependent on 160 species. Then the highest portion of the variation in the species-specific aboveground biomass was explained by the soil pH gradient. Further, one of the main contributions of this dissertation is extension of knowledge about the long-term effects of different fertilizers on plant species composition, productivity and soil nutrients in the mountain grasslands. Long-term fertilizers applications caused large changes in the covers of individual plant species, plant species composition and even led to changes of grassland communities. It was found, that dynamic equilibrium of vegetation appeared after long-term fertilizers application and twice a year cutting regime, but still has not been achieved under no fertilization in Rengen grassland experiment. Not only long-term application of nutrients and their doses, but also the form of application is very important, as was showed in Admont grassland experiment. The time necessary for changes in vegetation and to reach equilibrium, may strongly depend on the initial nutrient status of the soil and the quantity of nutrients added per year by mineral fertilizers. Despite differences in plant species composition between long-term grassland experiments, similar responses of some individual plant species or functional groups to cutting and/or fertilization in variable soil and clime conditions were observed. Grasslands plant species composition is not only endangered by excessive fertilizers application, but also by weed and expansive species. This dissertation therefore also aimed to answer the effects of cutting and/or biomass removal on two problematic plant species on the grasslands. First is weed species *Rumex obtusifolius*. This species can reduce forage quality and species diversity of the grasslands. Cutting at least twice per year seems to be suitable management to reduce or even in long-term probably totally suppress *R. obtusifolius*. Herbicide application for its eradication is not necessary to use, considering the undesirable side effects on other species, soil and water quality. Second is expansive species *T. latifolia*. Cutting once per year without biomass removal seems to be suitable management for long-term control of *T. latifolia* to decrease its cover and maintain targeted plant species composition without decreasing plant species diversity. Long-term abandonment seems to be not suitable, so that ongoing spontaneous succession lead into the changes in plant species composition and to overgrowing of original vegetation by woody plants. However, spontaneous succession on mountain wet meadow can take a long time as was observed by almost no changes in species richness between abandonment and cutting management regimes throughout 14 years in our experiment. Plant species composition of fertilized grasslands with two or three cuts per year can by changed in shorter time, due to delivery of growth limiting nutrients, especially P by mineral fertilizers into the vegetation. Combined application of two main growth limiting nutrients, N and P supports high and competitive species, which can out-compete short species under two cutting regimes, but not or very little under three cutting regimes, despite higher productivity of the grasslands. This is probably because the competition between the species is much more about the light rather than about the nutrients. Thus short species still have an enough light supply and they can grow together with tall species under three or multiple cutting regimes. Fertilizer application and the management to control weed and expansive species need to be considered with respect of local soil and climate conditions, plant species composition and the history of management. All the results from this dissertation can be used by nature protection, farmers or by grasslands experts for the suggesting sustainable grassland managements.

Future recommendations

For the future research could be interesting to compare the same individual plant species from many long-term grasslands experiments as well as from landscape studies under same or similar treatments. This could help to expand our knowledge about the ecology requirements of the plants in variable communities, soil and clime conditions. Then it could help to suggest suitable grassland management for each individual plant species based on the large datasets from many countries over the Europe. Changes in the abundance of individual plant species as well as in plant species composition will be easier to predict with respect of all the most important factors observed on broader range of grasslands. Special attention could be devoted to weeds and expansive species on the semi-natural grasslands in central Europe.

The future research can be also focused on genetic differences of individual plants, especially weed and expansive species, under different long-term grassland experiments with respect of management and availability of nutrients in the soil. This could help to answer many questions, such as: why is some management type in one long-term grassland experiment suitable and in other not for the selected individual plant species?

The future research can also elucidate the effects of nutrients on expansive species *T. latifolia* under different cutting regimes with or without biomass removal in the wet meadows. Nutrient enrichment can increase growth and density of tall grass *T. latifolia* and change plant species composition and cover of individual plant species. Effects of different nutrients need to be considered under variable water levels and climate conditions and in the wide altitude range. Thus understanding the effects of management regimes or available soil nutrients on *T. latifolia* growth can help to suggest sustainable managements to protect diverse swards of the different wet meadows.

The possibilities of future research are very large and long-term grasslands experiments could serve as unique places, where the long-term effects of different managements on vegetation can be observed as well as landscape studies on various types of grasslands and the data from them can be used for the prediction of future development of grassland communities.

Curriculum vitae

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List of publications

PERSONAL INFORMATION

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WORK EXPERIENCE

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Czech University of Life Sciences, Prague (Czechia)
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TRAININGS AND COURSES

15/04/2022– 15/09/2022; Student internship (Pwllpeiran Upland Research Centre)
Aberystwyth (Wales, Great Britain)
Studying of the scientific articles published in the context of the grassland research in Wales
Joining a plant breeding team (breeding process of the plants, planting seeds, cutting plants in the greenhouse, plant selection, fertilization of permanent plots)
Collecting of botanical data in the long-term sheep grazing experiment and in the CSP experiment
Processing and analyses of the botanical data

05/08/2021– 25/02/2022; Student internship (Raumberg-Gumpenstein institute)
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Assistance with the data preparation in the SatGrass project (modelling yield dynamics and quality parameters of the forage from the grasslands in Austria by using satellite data)
Data analyses from the long-term grasslands experiments

01/08/2020 – 04/05/2021; DBU (Deutsche Bundesstiftung Umwelt); Student internship (Technical University Dresden, International Institute Zittau)

Zittau (Germany)
Soil analysis in laboratory (using spectroscopy) – to find available nutrients for the plants from different grasslands experiments
Work on the international projects
Grasslands research work (preparation of scientific articles)

06/02/2020–26/02/2020; 22. International winter language school from German

Osnabrück (Germany)
Intensive language course on the level B1 - certificate of successful completion (final mark 1.7)
Speaking with small groups (max. 10 people); Grammar practicing; Listening exercises; Individual work on various topics (music, policy, geography); Presentation of selected topics to other students

15/04/2019–15/09/2019; Student internship (Georg-August-Universität Göttingen)
Göttingen (Germany)
Work in long-term cattle grazing grassland experiment (Relliehausen); Ingrowth cores methods (net root mass productivity, total root mass); Greenhouse testing of phenotypic adaptations of *Dactylis glomerata* and *Festuce rubra* species under different grazing intensities and different patches types

27/02/2019–28/02/2019; Scientific writing

Liberec (Czechia)
Structure of scientific article; How to write different parts of article; Which mistakes to avoid; Ethical codex of scientific writing; How are scientific articles evaluated.

27/01/2019–03/02/2019; CANOCO course

České Budějovice (Czechia)
Lectures about multivariate data analysis; Learning how to use program CANOCO; Scientific data evaluating in CANOCO; Presenting of achieved results.

01/10/2018–04/10/2018; Staff training - Set up and management of field trials to determine ecosystem services

BOKU (University of Natural Resources and Life Sciences), Vienna (Austria)
Trial set up and statistics; Cover crops and their hidden halves; Nutrients mobilisation and rhizosphere processes; Soil fauna; Plant pathogens.

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Complete life cycle of bio-based products: bioenergy, biomaterials, food technology as well as socioeconomic and ethical aspects of a future economy.

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PUBLICATIONS

Scientific publications with IF

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Projects participation

Principal investigator, Small grant project (No. 30021/963-43/0) by Deutsche Bundesstiftung Umwelt (DBU).

Principal investigator, University grant project (IGA): Dynamics of nutrients in plants and soil and their relationship with plant species composition under long-term management of grasslands.

Co-investigator, EU, Interreg CZ/PL r.n. CZ.114.120/0.0/0.0/16_026/0001092 Grassland biomass as a renewable source of energy - Biodiversity-Biomass-Biogas.

Co-investigator, EU, Interreg SN/CZ r.n. 100264999 Sustainable grassland management for supporting biodiversity.

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