DEPARTMENT OF ECOLOGY

EFFECT OF MANAGEMENT ON THE VEGETATION AND FUNGI IN UPLAND AND MOUNTAIN GRASSLANDS

(Vliv obhospodařování na vegetaci a houby podhorských a horských travinných porostů)

/PhD Thesis/

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CONTENTS

Chapter 1

General introduction

1.1 Management of permanent grassland

At the beginning of the 20th century most of grasslands were grazed by cattle or cut for the hay and fresh forage, which are traditional ways of the management in the upland and mountain grassland areas. After the World War II large part of grasslands were converted into arable land or temporary grasslands. Semi-natural hay-meadows and pastures with low yield of forage were ploughed and reseeded, wet meadows were drained, limed and fertilized because of a forage yield and quality improvement. Intensification of grasslands management continued until the end of 1980s'. In 1990s the significance of grasslands for fodder production decreased dramatically by reason of rapid reduction of cattle and sheep herds due to enhancement of grasslands area in the Czech Republic (CSO, 2012; Gaisler et al., 2004). Many areas of formerly intensively managed grasslands were abandoned and an alternative ways of management were searched. The mulching can be one possible but temporary alternative management important for the interruption of secondary succession and afforestation of grassland. However mulching is often refused due to possible negative effect on the insect populations.

Grazing

Grazing is probably most natural method of grassland management. It is an economically interesting alternative to cutting in order to maintain species-rich grasslands in the landscape because grazing management is cheaper compared to cutting and consequent forage conservation (Isselstein et al., 2005). On the pastures a mosaic of ungrazed, little grazed, and severely grazed patches forms, which are created by selective grazing, trampling and excrement return (WallisDeVries et al., 1998).

In the practice two basic grazing systems are used: continuous and rotational. Under rotational grazing the area is divided into a series of fields or paddocks that are grazed in sequence, each use being followed by a rest period (Frame, 1992), whereas under continuous stocking (set stocking) the animals are in the grazing area for the whole grazing season or year (Hodgson, 1979). Set stocking is prevailing grazing system at present due to lower cost of fencing and labour with herd (Isselstein et al., 2005).

Grazing management with respect to grazing pressure, stocking rate and target sward height is divided to intensive and extensive ones. Extensive, in contrast to intensive grazing, promotes selective patch defoliation, which results in an uneven distribution of grazing pressure, both within and between plant communities and plant species (Tainton et al., 1996; Rook et al., 2004). Mosaics of short grazed turf and lightly grazed patches, provided the animals create and maintain by their grazing constant patterns in the vegetation. If such patterns develop, grazing at low stocking rates creates extra structural diversity in the sward. It should be note that the vegetation patterns in grazing intensity often seem to reflect the preference for or avoidance of the dominant species of different plant communities (Bakker, 1998). Management can have major impacts on the competitive interactions between species and on the composition on pasture however it will vary depending upon season and the growth processes that are going on at that time. The evaluation of management impacts can be done from a short or long-term perspective. In perennial pasture ecosystems, the impact on the long-term persistence of species can be more important (Kemp and King, 2001).

Cutting

Cutting is also frequently used management of grasslands. We distinguish the three basic ways of forage using: harvesting for fresh forage harvesting of wilted biomass for grass silage and haymaking. Traditional cut by the scythe was replaced by the different mowing machines with higher

productivity of the work. But effect of defoliation and removal plant biomass is similar for all the ways. The differences are only in the time and frequencies of the defoliation.
Forage yield can be successively decreased under yearly mowing without any fertilisation and this effect is stronger with the increasing of cutting frequency for a year. Regular mowing with biomass removal takes away nutrients (Schnitzler and Muller, 1998) that might cause nutrient depletion from the soil. The nutrient depletion is rather low and for many elements (N and P in particular) the effect of hay-making during several years cannot be detected by chemical soil analysis (Bakker, 1989). The botanical composition is more markedly influenced, plant species with higher tolerance to partial oligotrophication and especially species which are better adapted to disturbance can spread on mowed grasslands. The multiply cutting management is favourable for short legumes such as *Trifolium repens* and also prostrate forbs, e.g. *Taraxacum* sp., *Plantago* sp., *Leontodon* sp., etc. (Huhta et al., 2001; Correll et al., 2003; Gaisler et al., 2004; Hejcman et al., 2005; Pavlů et al., 2006, etc.) Cutting is often preferred management in cases where high species richness is a primary concern (Hansson and Fogelfors, 2000).

Mulching

One possible alternative grassland management largely used in the Czech Republic since the 1990s is mulching (Fiala, 2007; Gaisler et al., 2010). Mulching is a low-cost method, defined as the crushing of aboveground biomass into pieces several cm long, which are then spread evenly onto the grassland surface (Doležal et al., 2011; Metsoja et al., 2012). The plant biomass subsequently decomposes and released nutrients return into the ecosystem. Although mulching has been used as alternative grassland management in mountain and upland areas in the Czech Republic, long-term detailed studies to evaluate several mulching and cutting regimes differing in frequency and timing of management during the vegetation season are still lacking. The only existing studies have compared mulching performed once or twice per year, with cut and unmanaged treatments (Kahmen et al., 2002; Bakker et al., 2002; Duffková, 2008; Kahmen and Poschlod, 2008; Tonn and Briemle, 2008; Mašková et al., 2009; Römermann et al., 2009). In view of the absence of information on different mulching regimes and their effects on the vegetation of common grasslands in the Jizera Mts., we established a large-scale grassland management experiment on *Festuca rubra* grassland in 1997. Preliminary results published after five years of the experiment indicated that plant species composition and richness was highly affected by 1) the timing of mulching i.e. whether performed in spring or in autumn and by 2) the frequency of mulching i.e. whether performed once, two or three times a year. Mulching performed only in September decreased species richness of vascular plants to the same degree as that with no management (Gaisler et al., 2004).

1.2 Plant species diversity of semi-natural grasslands

In the temperate regions of Europe, the distribution of natural grassland was limited to sites where natural forests fail to grow, such as sites above the timberline in mountainous regions, very wet or dry sites in the lowlands, or marshlands close to the sea (Ellenberg, 1996). New studies on the history of grasslands suggest that, apart from these sites, grassland could also develop on fertile sites as a result of megaherbivore grazing (Vera, 2000). High diversity of the present species-rich semi-natural grasslands has origin in in the Middle Ages and in the early Modern Era (Speier, 1996; Prins, 1998; Korneck et al., 1998), when the human population increased. Traditional grassland utilisation systems were characterised by a generally low level of nutrient return to the swards and, on grazed grassland, by little adaptation of the stocking rates to the actually available herbage. Diverse, unimproved semi-natural grasslands covered a large percentage of the agriculturally utilised area throughout Europe. At the beginning of the 20th century grasslands were agriculturally improved and the management intensified. The grasslands were improved by liming and regular fertilisation. On wet soils, artificial drainage was introduced to remove surplus water (Isselstein et al., 2005). The process continued and increased still after World War II and in period of intensification of agriculture. After the change of the political regime in the 1990s large grassland areas were abandoned and changes of plant communities was started. The remaining semi-natural grasslands are now highly valued by nature conservationists and, in many countries, their maintenance has received priority in nature conservation activities and in agri-environment schemes (Söderstrom et al., 2001; Kumm, 2003; Jeangros and Thomet, 2004). Semi-natural grasslands are very important components of landscape of Central Europe and they represent a wide spectrum of communities (Mašková et al., 2009). Suitable management is necessary for the maintenance of high diversity in semi-natural grasslands, and the abandonment is risk for species richness in most of cases (**Chapters 2** to **5**). Cessation of grassland management leads to successional change and to a loss of plant species diversity. At a local scale, vegetation succession facilitates the invasion of shrubs, the dominance of tall growing species from later successional stages and the competitive exclusion of species typical for managed grasslands (Krahulec et al., 2001; Moog et al., 2002; Tasser and Tappeiner, 2002; Pykala, 2003; Hejcman et al., 2004; Kahmen andPoschlod, 2004).

1.3 Weeds in grasslands

Distribution of grasslands weeds is strongly depended on the present and as well as former management practices (**Chapter 6**). For example dicotyledonous species, namely undesirable perennial weeds such us *Cirsium arvense, Urtica dioica* or *Rumex obtusifolius* and *Elytrigia repens* among weed grasses**,** often begin predominate in mesic unmanaged in the past reseeded or fertilised grassland (Mikulka and Kneifelová, 2004; Pavlů et al., 2005). Reintroduction of management on formerly abandoned grassland is necessary to prevent natural forestation. Suitable management and its intensity depend on present vegetation, local possibilities and our target goal. Specific conditions for grassland managements under organic farming do not allow using herbicides. So weeds must be controlled by a combination of management practices such as different intensities of cutting and grazing. Because many grassland weeds have relatively good quality and palatability, they have often been tolerated in grasslands (Frame, 1992). However they reduce yield and higher abundance can cause health problems of grazed animals. Only a little is still known about the effects of different grazing systems on weedy species in pasture ecosystems (Zaller, 2004). In agricultural practice the effect of mulching on weeds occurrence is often discussed. Mulching is a simple and low-cost procedure that can help maintain many grassland locations and fallow farm land (Prochnow et al., 2000). However, rapid decomposition of the mulch is important, because a long cover and shading of the sward will cause unwanted changes in grassland composition. Zelený et al. (2001) reported that mulching can suppress predomination of undesirable tall herbs. Gaisler et al. (2004) compared effect of different mulching and cutting regimes on vegetation of an upland meadow and concluded that mulching is not able to restrict highly competitive dominant herbs if performed once per year only in late summer or in autumn.

Bread-leaved docks (*Rumex* spp.) are the most important weed species on the grasslands in condition of Central Europe, mainly in intensively managed areas (Frame, 1992). *Rumex crispus* belongs to the worst weeds in the world. It is toxic for ruminants and grows mainly on ruderal nutrients-rich areas. *R. obtusifolius* has similar ecological demands, it is not consumed by ruminants and it has lower resistance to cutting. However it can hybridize with the *R. crispus* (Grime et al., 1987). There are important some factors for their success: flowering several times in year, large amounts of seeds with long-term germination ability, fast germination in suitable conditions (Carves and Harper, 1964). Docks in grasslands usually decrease yield and quality of forage (Marten et al., 1987; Hejduk and Doležal, 2004), rumicin in their above-ground biomass can cause indigestions or dermatitides of animals (Holm et al., 1977). Difficult regulation of docks follows from great regeneration ability and mainly from high long-term germination ability, which was confirmed by the range of experiments (e.g. Toole and Brown, 1946; Darlington and Steinbauer, 1961; Kivillaan and Bandurski, 1981). For totally reduction of the *Rumex* plants cuts after each two or three weeks and similarly grazing with five to six grazing cycles would be necessary (Zaller, 2004).

1.4 Indication fungi of semi-natural grasslands

Fungi are unique groups of organisms which are characterised by close adaptation to a specific substrate, mycorrhizal partner or host and they have high sensitivity to changes in the environment (Hlad and Skoberne 2001). A lot of fungi species can grow also on meadows and pastures (**Chapter 7**). Most of these species belongs to endangered and/or rare fungi. They are especially sensitive to intensive treatment management such as fertilizing, ploughing, drainage and consequent long-term changes in grassland plant species composition. Members of genus *Hygrocybe*, *Entoloma* and families *Geoglossaceae* and *Clavariaceae* are used for determination of high value for nature conservation (McHugh et al., 2001; Griffith et al., 2002; Newton et al., 2003; Evans, 2004). Grasslands with high proportion of *Hygrocybe* species are called “Wax-cap grasslands”. Many species of *Hygrocybe* genus are distinguished by the prominently colours, especially red, orange and yellow. Substantial part of former localities was devastated as a consequence of intensification of forage production in the second half of 20th century, when mainly moist and acid grasslands were drainaged, fertilised and often reseeded by the mixtures of productive cultivars of grasses and legumes (Gaisler et al., 2008). Wax-caps grasslands are intolerant to high content of plant available phosphorus in the soil. Common *Hygrocybe conica* and *H. virginea* belong to relatively tolerant species to management, however in some cases they can be even present during 10 to 15 years after last intensification treatment, but majority of wax-caps need longer time (McHugh et al., 2001). For example *Hygrocybe splendidissima*, *H. intermedia* and *H. spadicea* are species very sensitive to intensive management and period of their rediscovery after extensification can last more than half a century. Therefore a lot of these species occur in nature of Czech Republic very sporadically. In 90s of last century number of wax-cap grasslands more decreased after large abandonment of grassland management and following secondary succession as a consequence of rapid decrease of grassland utilisation for forage production (Holec and Beran, 2006). Many of *Hygrocybe* species are recorded in the Red list of fungi (macromycets) of the Czech Republic. A lot of monitoring studies on the waxcap meadows was made in western Europe (McHugh et al., 2001; Vesterholt et al., 1999, etc.) As well as Öster (2008) also studied a congruence between the diversity of Waxcap (*Hygrocybe* spp.) fungi and vascular plants in semi-natural grasslands. The majority of these studies were made in countries with more humid climat (e.g. Great Britain, Ireland, Netherlands, Belgium and Scandinavian countries). For the assessing sites of mycological importance the different classification systems in the literature exist. Therefore we cannot assign their suitability for natural conditions of the Czech Republic. It is because of ecological conditions (clima, soil, plant species composition) and history of agricultural management on grasslands in the Central Europe are different. For example in Slovakia Adamčík and Kautmanová (2005) reported adjusted one-visit values of classification system of Vesterholt et al. (1999). It seems that the Slovak system can be adapted also for for monitoring on Czech grasslands. Earth-tongues of genus *Geoglossum*, *Trichoglossum* and *Microglossum* are rare fungi that represent mostly also good indicators of areas without former insensitive agriculture management (Hagara et al., 1999, Kříž and Skála, 2007). There are only sporadic informations about their occurrence and ecology (Deckerová, 2007; Gaisler and Kříž, 2007; Kříž and Skála, 2007) and also most of these species were not published in Czech mycologist literature. Occurrence of rare members of *Geoglossaceae* in Slovakia reported Kučera et al. (2008) and Kučera et al. (2010). Geesteranus (1964) and later Roobeek (2009) described morphology, microscopic characters and distribution of *Geoglossaceae* species in Nederland. Some species can be relatively easy distinguishable according fruitbody features (e.g. *G. fallax*, *G. glutinosum* or *M. olivaceum*) but determination of the most other species is possible only microscopically. The keys for determination of earth tongues were published in several publications (e.g. Massee, 1897; Imai, 1945; Mains, 1954; Hansen and Knudsen, 2000) and Ridge (2006) described these fungi in beginners guide. In some case even microscopic measures can give only ambiguous results (Benkert, 1992; Deckerová, 2007). It is because of variability of size and shape of spores, ascus and paraphyses among fruitbodies of the same species and particular dissension in different works. Therefore in last five years several studies are focused on DNA analyses, which can help solve problems with determination (e.g. Wang et al., 2006; Schoch et al., 2009)

Some meadows or pastures in Jizerské hory Mountains are relatively rich in occurrence of members of *Hygrocybe*, *Entoloma*, *Geoglossaceae* and *Clavariaceae* and lots of them were found in the experimental sites of Grassland Research Station in Liberec (Crop Research Institute in Prague), therefore we decided for their regular monitoring.

1.5. Research questions

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

1) How do plant species composition and functional traits change under the long-term grassland management experiments (cutting, mulching, grazing, abandonment)?
2) How rapidly and when do these changes occur?
3) What is the effect of abandonment on nutrient availability in previously intensively managed grassland?
4) How long-term management affects weeds occurrence in grasslands?
5) What is the distribution of fungi in relation to different grassland type?

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Chapter 2

Long-term effects of different mulching and cutting regimes on plant species composition of *Festuca rubra* grassland

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Long-term effects of different mulching and cutting regimes on plant species composition of *Festuca rubra* grassland

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Abstract
In the Czech Republic, the number of cattle has decreased by about 60% between the years 1990 and 2010 and one major question is therefore, how to manage mountain grasslands with reduced livestock and demand for forage. One possible alternative is low-cost mulching, which is defined as the crushing of aboveground biomass, which is then evenly spread onto the ground. In view of a lack of data on the effects of mulching, we established an experiment with different cutting and mulching regimes on mountain *Festuca rubra* grassland in 1997 and monitored plant species composition over twelve years.
Treatments with a similar response of plant species composition were i) unmanaged control and grassland mulched once a year in September; ii) grassland mulched once a year in May and in July; iii), grassland mulched twice a year in June and August and grassland mulched three times a year in May, July and September; iv) grassland cut twice a year with biomass removal in June and August.
We concluded that mulching performed at least twice a year can substitute for cutting management in low productive grassland, without substantial loss of plant species richness and diversity. Mulching only in September affected plant species composition of the grassland in a similar way to no management, therefore, mulching performed only in September can be used only for prevention of grassland encroachment by shrubs and trees. The majority of changes in plant species composition developed during the first five years, thus, experiments to study the effects of management on grasslands must be performed for at least this length.

Key words: biomass, disturbance, meadow, species richness and diversity, vegetation traits

Introduction

In Europe, considerable changes in grassland management occurred in many post-communist countries after the change of the political regime in the 1990s (Krahulec et al., 2001; Vassilev et al., 2011). In the Czech Republic for example, the number of cattle decreased from 3,360,000 in 1990 to 1,344,000 in 2010 (CSO, 2012), and consequently a large area of grasslands in upland areas lost its agricultural justification. Some grasslands were artificially or naturally reforested and 20–30% of the remaining grasslands were left unmanaged (Pavlů et al., 2005). In addition, some arable fields were artificially or naturally grassed in less favourable areas (Lencová and Prach, 2011; Hejcman et al., 2012) and therefore, the total area of grasslands increased from 833,000 ha in 1990 to 986,000 in 2010 (CSO, 2012). In unmanaged grasslands, considerable changes in plant species composition have been recorded, namely the spread of tall weedy or expansive species such as *Urtica dioica* (Prach, 2008) or *Calamagrostis epigeos* (Stránská, 2004; Somodi et al., 2008; Holub et al., 2012) and particularly, the expansion of trees and shrubs such as *Prunus spinosa*, *Rosa* spp. and *Crataegus* spp. at lower altitudes (Stránská, 2004) and *Picea abies*, *Betula pendula* or *Populus tremula* in the mountains (Prach and Pyšek, 2001, Hrivnák and Ujházy, 2005). In unmanaged grasslands, a rapid decrease in species richness of vascular plants or extinction of endangered plant species has frequently been reported, especially in highly productive plant communities (Krahulec et al., 2001; Pavlů et al., 2005; Mašková et al., 2009). For all these reasons, large areas of grasslands cannot be left unmanaged in the cultural landscape of Central Europe and economically feasible alternatives to grassland management must be investigated. Many grassland studies have focused on the effects of various cutting and livestock grazing management regimes on plant species composition under different environmental conditions (e.g. Ryser et al., 1995; Losvik, 1999; Krahulec et al., 2001; Spiegelberger et al., 2006), but the central question concerning how to manage large areas of grasslands without any livestock and without any demand for forage has not been adequately answered yet.

One possible alternative largely used in the Czech Republic since the 1990s is mulching (Fiala, 2007, Gaisler et al., 2010). Mulching is a low-cost method, defined as the crushing of aboveground biomass into pieces several cm long, which are then spread evenly onto the grassland surface (Doležal et al., 2011; Metsoja et al., 2012). The plant biomass subsequently decomposes and released nutrients return into the ecosystem. Although mulching has been used as alternative grassland management in mountain and upland areas in the Czech Republic, long-term detailed studies to evaluate several mulching and cutting regimes differing in frequency and timing of management during the vegetation season are still lacking. The only existing studies have compared mulching performed once or twice per year, with cut and unmanaged treatments (Kahmen et al., 2002, Bakker et al., 2002, Duffková, 2008, Kahmen and Poschlod, 2008, Tonn and Briemle, 2008, Mašková et al., 2009, Römermann et al., 2009).

In view of the absence of information on different mulching regimes and their effects on the vegetation of common grasslands in the Jizera Mts., we established a large-scale grassland management experiment on *Festuca rubra* grassland in 1997. Preliminary results published after five years of the experiment indicated that plant species composition and richness was highly affected by 1) the timing of mulching i.e. whether performed in spring or in autumn and by 2) the frequency of mulching i.e. whether performed once, two or three times a year. Mulching performed only in September decreased species richness of vascular plants to the same degree as that with no management (Gaisler et al., 2004).

The aim of this paper was to evaluate the effect of different mulching and cutting management regimes on plant species composition over 12 years and to compare them with results published after five years of the experiment. We asked how the plant species composition and species richness was affected by different mulching and cutting regimes and whether some mulching regimes can substitute for traditionally used cutting and herbage removal management without any detrimental effects on the species richness of vascular plants.

Materials and methods

Study site and design of the experiment

The experiment was carried out in the Jizera Mts. (50°49’N, 15°02’E) 5 km north-west of Liberec, in the Czech Republic. The altitude of the site is 420 m, the mean annual temperature is 7.2°C and mean annual precipitation, 803 mm. The bedrock is a biotic granite underlying acid Cambisol. In the upper 10 cm soil layer, pH (KCl) was 4.8, the content of organic C was 3%, and plant-available (Mehlich III extraction; Mehlich, 1984) P, K, and Mg concentrations were 43, 70, and 68 mg kg^{-1}, respectively. The meadow in the study area was abandoned for about ten years before establishment of the experiment. According to Chytrý et al. (2007), the vegetation of the experimental grassland was classified as *Arrhenatherion*. The dominant vascular plant species before the start of the study in descending order according to their cover were: *Festuca rubra* agg., *Alopecurus pratensis*, *Vicia sepium*, *Anthriscus sylvestris*, *Galium album* and *Veronica chamaedrys*. The mean forage yield of the meadow ranged from 2 to 4 t ha^{-1} of dry matter per year. No fertilisers were applied for at least ten years prior to the start of the experiment or during the study.

Design of the experiment

The experiment was established in four completely randomised blocks in spring 1997. Each plot was a rectangle with the dimensions 10.0 m × 3.7 m. The treatments performed were: unmanaged control (U), two cuts per year with biomass removal in June and August (2C), mulching once per year in May (1MM), once per year in July (1MJ), once per year in September (1MS), twice per year in June and August (2M) or three times per year in May, July and September (3M). Mulching was performed using the tractor-driven Uni Maher UM 19 machine and cutting was performed using a tractor-driven rotary mower. The mulching machine crushed the plant biomass into pieces 5–10 cm long and inserted them into the homogenous layer on the sward surface. Cut biomass (in the 2C treatment) was removed immediately following cutting. The height of mulching and cutting was 5 cm above the soil surface and mulching and cutting were performed in the same way as by local farmers.

Plant species composition and cover of functional groups

The cover of all present plant species rooting in the monitoring plot was visually estimated directly in percentages. To remove an edge effect, we estimated cover in the central 8.0 × 2.5 m rectangle of each permanent plot. The collection of relevés was performed annually in May before the first management application. To obtain the baseline data, the initial plant cover estimation was performed before the first experimental manipulation in 1997. The nomenclature of vascular plant species follows Kubát et al. (2002).

Based on the description of vascular plants in the regional flora (Kubát et al., 2002), all plant species within the study area were classified *a priori* according to their main traits. We recognised four categories: tall graminoids (mean height ≥0.5 m), short graminoids (below 0.5 m), and similarly, tall and short forbs (Table 1).

To evaluate changes in plant species diversity, the Shannon diversity (SD) index was calculated using cover data of all species in a particular relevé.

The C-S-R signature (Grime et al., 1988) for each plot was calculated by means of C, S and R values, weighted using the cover of each species present in the individual plot (Hunt et al., 2004). The sum of calculated C-S-R strategies was one for each plot. Repeated analysis of variance (ANOVA) measures were used to evaluate C-S-R strategies.

Data analyses

Redundancy analysis (RDA) in the CANOCO 4.56 program (ter Braak and Šmilauer, 2002) was used to evaluate multivariate vegetation data in particular years. The blocks were used as covariables in all analyses to restrict permutations into blocks. Cover data in RDA were logarithmically transformed [y = log (y + 1)] to down-weight dominant species (Lepš and Šmilauer, 2003) and 999 permutations were used in all performed analyses. An ordination diagram, constructed by the CANODRAW program (ter Braak and Šmilauer 2002), was used to visualise the results of the RDA analysis of data collected in 2009.

Repeated measures ANOVA was used to evaluate the cover of the most abundant vascular plant species, species diversity and richness and cover of functional groups, and C-S-R strategy.

Results

Species diversity and richness

The Shannon index of diversity, species richness of all vascular plants, and richness of species with a cover greater than 1% were all significantly affected by time and treatment, but not by time × treatment interaction (Table 2). The Shannon index of diversity ranged from 1.7 to 2.3 at the start of the experiment (Fig. 1a). Diversification between all managed treatments and the unmanaged control developed immediately after the start of the experiment and lasted for the following twelve years. The highest values of the Shannon index of about 2.5 were recorded in the 2M, 2C and 3M treatments and the lowest values of approximately 1.7 were recorded in the unmanaged control.

During the experiment, a successive increase in species richness was recorded in all managed treatments (Fig. 1b). The highest species richness was recorded in frequently managed treatments (2C, 2M and 3M treatments) and varied around 30 species per 20 m^2, followed by once-mulched treatments (1MS, 1MJ, 1MM) where species richness ranged from 25 to 28. In all years, the lowest species richness ranged from 19 to 24 species per 20 m^2 and was recorded in the unmanaged control. The mean richness of species with a cover more than 1% slightly increased in all managed treatments during the experiment, whereas it decreased in the unmanaged control (Fig. 1c).

Plant species composition and cover of functional groups

No significant effect of treatment on plant species composition calculated by RDA was recorded in 1997 (Table 3). The variability of plant species composition explained by treatments subsequently increased from initially 25% in 1997, to more than 50% after five years of the experiment in 2002. Variability explained by treatments was constantly above 50% between years 2002 and 2009. Four groups of treatments with similar plant species composition were recognised on the ordination diagram based on RDA analysis of data collected in 2009 (Fig. 2): 1MM and 1MJ treatments as the first group, 1MS and U treatments as the second group, 2C and 3M as the third group, and 2M treatments as the fourth group. The association of individual species with particular management treatments is clearly visible from the ordination diagram (Fig. 2) and only the main species are described in detail in the following paragraph.

The cover of short and tall graminoids and short and tall forbs was significantly affected by time and treatment, but not by time × treatment interaction (Table 2, Fig. 3). The most remarkable effect of treatment was found for the cover of tall and short forbs. The cover of tall forbs rapidly decreased in all managed treatments during the first two years. However, this decrease was only temporary in less frequently managed treatments and a low cover of tall forbs was permanent only in 2C and 3M treatments. The highest cover of tall forbs was found in the unmanaged control during the experiment. The opposite was observed for short forbs and their cover subsequently increased, particularly in frequently managed treatments (2C, 3M, 2M). The cover of short grasses was lowest in the unmanaged control at the end of the experiment.

Two cut (2C) and multiple mulching treatments (2M, 3M) promoted short forbs with creeping growth or a leaf rosette such as *Trifolium repens* (Fig. 2 and 4a), *Hypochoeris radicata*, *Leontodon hispidus* (Fig. 4c) and *Plantago lanceolata* (Fig. 4d). However, tall forbs such as *Anthriscus sylvestris*, *Cirsium arvense* and *Hypericum maculatum*, together with tall grasses *Alopecurus pratensis* (Fig. 5b) and *Arrhenatherum elatius* (Fig. 5b), spread in once-mulched treatments (1MS, 1MJ, 1MM) and in the unmanaged control. Similarly, the cover of tall forbs such as *Galium album* (Fig. 4b), was highest in the unmanaged control, followed by in the once-mulched treatments. The cover of the dominant tall grass *Festuca rubra* (Fig. 5d) was also highest in the unmanaged control in comparison to all managed treatments in the last years of the experiment. The cover of *Agrostis capillaris* (Fig. 5a) was negligible in baseline data and this short grass gradually spread into all treatments but especially into frequently cut (2C) or mulched (2M, 3M) treatments. *Agrostis capillaris* and *Trifolium repens* were only two dominant species whose cover was significantly affected by time, treatment and their interaction.

The percentage proportion of C-S-R strategies was significantly affected by time and treatment, but not by time × treatment interaction (Table 2, Fig. 6). The C strategy was the most successful, with means of 0.87, 0.78, 0.78, 0.77, 0.75, 0.69 and 0.68 for U, 1MM, 1MJ, 1MS, 2M, 3M and 2C treatments, respectively). The unmanaged treatment was the most favourable for the C strategy, but was the most harmful for the S and R strategies. Treatments with frequent management (3M, 2C, 2M) affected the C-S-R strategies in the opposite way compared to the unmanaged treatment.

Discussion

Species diversity and richness

With the exception of the U treatment, the differences in the number of species with cover greater than 1% were small. This indicated that the persistence of the fundamental composition of species was at a relatively similar level in all managed treatments. Alternatively, lower values for unmanaged meadows showed a higher degree of dominance in swards.

The highest Shannon diversity index and species richness was recorded in twice-cut and multiply-mulched treatments and the lowest was recorded in the unmanaged control. Similarly, after 22 years of another grassland management experiment, the lowest species richness was recorded in treatments mulched once per year and in the unmanaged control (Laser, 2002).

A positive effect of cutting management performed twice per year on species richness and a decrease in species richness after cessation of management in *Festuca rubra* grassland was also recorded by Pavlů et al. (2012). Positive effects of cutting and mulching management on species richness of vascular plants were also recorded by Mašková et al. (2009) and Nadolna (2009) in mountain grasslands with *Festuca rubra*. Mulching performed twice per year thus appears to be a suitable alternative to traditional cutting management performed twice yearly in lowly productive *Festuca rubra* grasslands. Mulching performed twice a year was also recommended by Römermann et al. (2009) as the most suitable long-term alternative management of species-rich calcareous grassland formerly managed by sheep grazing.

Plant species composition and cover of functional groups

In this study, multiple mulching affected most plant species in the same way as cutting management performed twice yearly and therefore, mulching performed twice a year over twelve years can substitute for traditional cutting management performed twice a year, without any detrimental effects on plant species composition. Similar results were also found by other authors from different regions with different lowly productive grasslands (Laser, 2002; Moog et al., 2002; Römermann et al., 2009). This conclusion is probably valid only for grasslands with an annual dry matter herbage production below 4 t ha^{-1}, but not for highly productive grasslands, where mulching without biomass removal can substantially increase nutrient availability and support the spread of nitrophylous species, as we observed in different regions of the Czech Republic (unpublished results). Mulching performed once a year affected plant species composition depending on its timing within the vegetation season. We concluded that mulching performed once a year should be applied early in the vegetation season up to July, but not in September, as was frequently performed by farmers. This is because mulching in September affected plant species composition in a similar way to that of the unmanaged control and thus enabled the spread of tall species. Mulching performed in September can therefore only prevent the expansion of shrubs and trees in contrast to the unmanaged control.

The C strategy (*sensu* Grime et al., 1988) predominated in all treatments and its proportion was suppressed with increasing defoliation intensity. Similarly, the positive effect of extensive management on the C strategy was found in other experiments in upland grasslands (see Ludvíková et al., 2010; Pavlů et al., 2010). However R and S strategies had similar proportions in all treatments because the C-S-R strategy was the most widespread strategy in the sward.

The majority of changes in plant species composition developed during the first five years of the experiment (Gaisler et al., 2004). Changes in subsequent years were not as obvious as those during the first five years. Therefore, the minimum recommended duration for experiments testing different defoliation managements of grasslands is five years.

Agrostis capillaris was a common grass in all treatments in the study, although *A. capillaris* is a competitively weak species, especially in unmanaged grasslands (Pavlů et al., 2007, 2012). The high presence of *A. capillaris* also in the unmanaged control in the experiment was probably due to low productivity of the experimental grassland on shallow soil with granite in the background. This explanation is consistent with results from Pavlů et al. (2011a) and Mašková et al. (2009), where *A. capillaris* was a frequent grass in plots left for a decade without any management. This short grass is evidently able to grow under various types of grassland management including unmanaged plots if the nutrient status is low (see also Venterink and Güsewell, 2010).

The positive effect of frequent defoliation management on cover of the short forb *T. repens* agrees with that found in other studies from various grasslands throughout Europe (Belsky, 1992; Correll et al., 2003; Hejcman et al., 2010; Pavlů et al., 2011b; Pavlů et al., 2012). The higher cover of *T. repens* in the frequently defoliated treatment was connected with its high light requirements and intolerance to shading from tall vegetation (Grime et al., 1988).

Although *L. hispidus* responded to applied management treatments similarly to *T. repens*, most notable was its positive response to mulching performed in May. This short forb with rosettes of leaves was only minimally damaged by mulching in this early period because the majority of its leaves were below the height of mulching and thus escaped defoliation. Its aboveground vegetative and generative organs were then able to quickly grow through the relatively thin layer of mulched material and developed better than those of other species. The layer of mulched litter was thicker in treatments with later mulching during the vegetation season and this was not favourable for the growth of *L. hispidus* and other short herbs.

Traditional two-cut management was the most favourable of all investigated treatments for growth of *P. lanceolata*, which agrees with results by Wahlman and Milberg (2002) that this medicinal herb is associated with cutting management. Alternatively, a two-cut management suppressed both tall dominant grasses *A. pratensis* and *A. elatius.* This was due to the high sensitivity of both grasses to a shortage of nutrients, as both grasses are characteristic dominants of two-cut or unmanaged grasslands under relatively high nutrient availability in the soil (Hrevušová et al., 2009; Pavlů et al., 2012).

The dominant tall forb *G. album* preferred unmanaged and infrequently managed treatments, whereas it was less abundant in treatments with frequent defoliation. Similar results were also obtained by other authors (Stránská, 2004; Pavlů et al., 2007). Generally, tall forbs were encouraged by regular management, especially if performed two or three times a year (see also Laser, 2002; Pavlů et al., 2012) In contrast to our results, *G. album* benefited from hay-making in the Russian Karelia (Jantunen and Saarinen, 2002)., but this might be due to varying ecological conditions at different sites.

The dominant tall grass *F. rubra* was indifferent to all managed treatments and was slightly more frequent under no management, similar to in the study by Pavlů et al. (2012) performed in a similar grassland type in the Jizerske Mts. *Festuca rubra* is thus a highly plastic species that tolerates not only no management in low productive mountain grasslands, but also frequent defoliation (Gaisler et al., 2004; Pavlů et al., 2007; Pavlů et al., 2011a; Krahulec et al., 2001; Pavlů et al., 2011b). This can be explained by its high genetic variability and its high ecological plasticity is connected with the ability to grow in different habitats and under different management (Grime et al., 1988).

Conclusions

After 12 years of the experiment, four groups of treatments were found that had a similar response of plant species composition to long-term defoliation management: i) unmanaged control and grassland mulched once a year in September; ii) grassland mulched once a year in May and in July; iii), grassland mulched twice a year in June and August and grassland mulched three times a year in May, July and September; iv) grassland cut twice a year with biomass removal in June and August. The main finding of this study is that the mulching at least twice a year can substitute for cutting management in low productive *Festuca rubra* grassland, without substantial losses of plant species richness and diversity. Mulching applied once a year in September affected plant species composition of the grassland in a similar way to no management. Mulching performed in September is only useful to prevent grassland encroachment by shrubs and trees. The majority of changes in plant species composition developed during the first five years of the experiment, therefore, experiments designed to study the effects of defoliation management on plant species composition of grasslands must be performed for at least this length.

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Table 1
Functional groups of plant species recorded in the experiment

Tall graminoids	Short graminoids	Tall forbs	Short forbs
Alopecurus pratensis	*Agrostis capillaris*	*Lathyrus pratensis*	*Lotus corniculatus*
Arrhenatherum elatius	*Anthoxanthum*	*Vicia sepium*	*Trifolium dubium*
Dactylis glomerata	*Carex ovalis*	*Anthriscus sylvestris*	*Trifolium medium*
Elytrigia repens	*Luzula campestris*	*Campanula*	*Trifolium pratense*
Festuca pratensis		*Chaerophyllum*	*Trifolium repens*
Festuca rubra agg.		*Cirsium arvense*	*Alchemilla* spp.
Holcus lanatus		*Cirsium palustre*	*Arabidopsis thaliana*
Holcus mollis		*Galium album*	*Campanula patula*
Poa pratensis		*Heracleum*	*Cerastium holosteoides*
Trisetum flavescens		*Leucanthemum*	*Glechoma hederacea*
		Pimpinella saxifraga	*Hypericum maculatum*
		Ranunculus acris	*Hypochoeris radicata*
		Rumex acetosa	*Leontodon autumnalis*
		Rumex obtusifolius	*Leontodon hispidus*
		Tanacetum vulgare	*Linaria vulgaris*
		Urtica dioica	*Platanthera chlorantha*
			Plantago lanceolata
			Ranunculus repens
			Rhinanthus minor
			Rumex acetosella
			Stellaria graminea
			Taraxacum spp.
			Veronica arvensis
			Veronica chamaedrys
			Veronica serphyllifolia
			Vicia cracca
			Viola arvensis

Table 2
Results of repeated measurements ANOVA (time, treatment, time × treatment) of dominant plant species, functional groups and number of plant species. *Df* = degrees of freedom, *F* = value derived from *F* statistics in repeated measurements ANOVA, *P* = probability value.

	Effect					
	Time; *Df*=12		Treatment; *Df*=6		Time x treatment;	
	F-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Shannon index	10.23	<0.001	18.54	<0.001	0.44	1.000
Number of all plant species	16.87	<0.001	44.73	<0.001	0.97	0.540
Number of plant species ≥1%	5.61	<0.001	11.56	<0.001	0.55	0.999
Short graminoids	6.97	<0.001	6.47	<0.001	0.55	0.999
Tall graminoids	11.07	<0.001	11.20	<0.001	0.78	0.894
Short forbs	4.86	<0.001	42.72	<0.001	1.27	0.093
Tall forbs	2.27	0.009	5.86	<0.001	0.28	1.000
C-strategy	30.91	<0.001	7.41	<0.001	0.61	0.995
S-strategy	31.77	<0.001	8.29	<0.001	0.66	0.986
R-strategy	29.86	<0.001	6.58	<0.001	0.56	0.999
Agrostis capillaris	38.60	<0.001	9.23	<0.001	1.56	0.006
Alopecurus pratensis	0.64	0.809	2.03	0.062	0.25	1.000
Arrhenatherum elatius	6.78	<0.001	5.98	<0.001	0.87	0.705
Festuca rubra	8.19	<0.001	7.72	<0.001	0.44	1.000
Trifolium repens	15.34	<0.001	66.84	<0.001	3.85	<0.001
Galium album	0.96	0.487	20.00	<0.001	0.36	1.000
Leontodon hispidus	2.99	<0.001	7.91	<0.001	0.63	0.990
Plantago lanceolata	1.58	0.096	15.17	<0.001	0.61	0.994

Table 3

Results of RDA analyses of cover estimates performed separately for each year. Applied treatments were unmanaged control (U), two cuts a year with biomass removal in June and August (2C), mulching once a year in May (1MM), mulching once a year in July (1MJ), mulching once a year in September (1MS), mulching twice a year in June and August (2M) and mulching three times a year in May, July and September (3M); % expl. var. = species, variability explained by one (all) ordination axis (measure of explanatory power of the explanatory variables); *F*-ratio = F statistics for the test of particular analysis; *P*-value = probability value obtained by the Monte Carlo permutation test. Tested hypothesis: is there any effect of treatment on plant species composition in any particular year?

Year	Explanatory variables	Covariables	% expl. var. 1st axis (all axes)	*F*-ratio 1st axis (all axes)	*P*-value 1st axis (all axes)
1997	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	10.2 (25.6)	2.0 (1.0)	0.493 (0.379)
1998	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	14.6 (35.1)	3.1 (1.6)	0.003 (0.001)
1999	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	20.8 (41.9)	4.7 (2.2)	0.001 (0.001)
2000	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	20.2 (41.0)	4.5 (2.1)	0.001 (0.001)
2001	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	31.1 (47.0)	8.1 (2.7)	0.001 (0.001)
2002	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	35.0 (53.8)	9.7 (3.5)	0.001 (0.001)
2003	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	37.2 (55.1)	10.7 (3.7)	0.001 (0.001)
2004	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	39.3 (55.9)	11.7 (3.8)	0.001 (0.001)
2005	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	39.3 (56.1)	11.6 (3.8)	0.001 (0.001)
2006	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	36.6 (53.9)	10.4 (3.5)	0.001 (0.001)
2007	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	33.1 (55.7)	8.9 (3.8)	0.001 (0.001)
2008	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	35.5 (55.2)	9.9 (3.7)	0.001 (0.001)
2009	2C, U, 1MM, 1MJ, 1MS, 2M, 3M	blocks	35.6 (53.8)	10.0 (3.5)	0.001 (0.001)

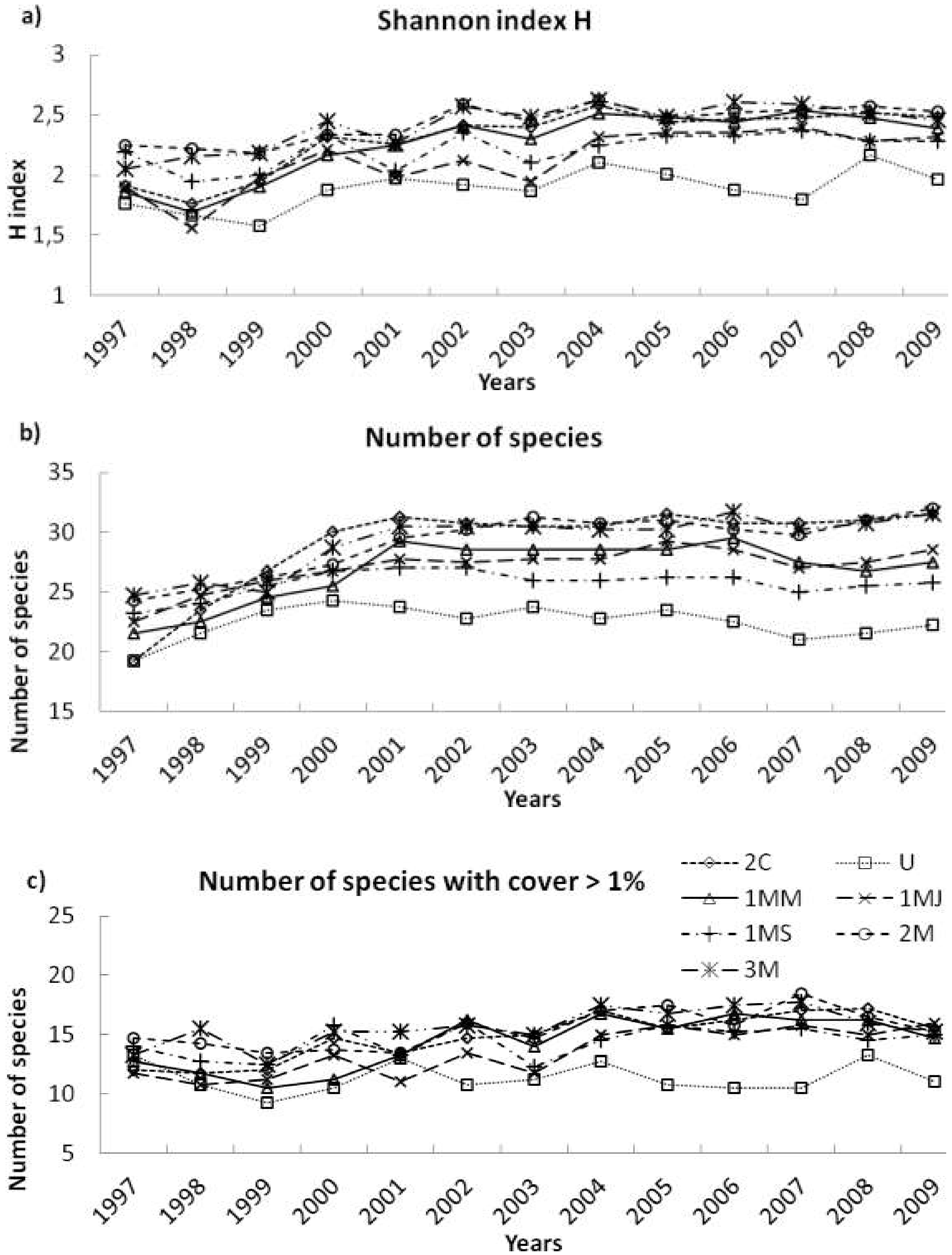

Fig. 1. (a) Changes in mean Shannon species diversity index, (b) total number of vascular plant species per 20 m^2 and (c) number of vascular plant species with more than 1% cover for treatments in the years 1997–2009. Applied treatments were: unmanaged control (U), two cuts a year with biomass removal in June and August (2C), mulching once a year in May (1MM), mulching once a year in July (1MJ), mulching once a year in September (1MS), mulching twice a year in June and August (2M) and mulching three times a year in May, July and September (3M).

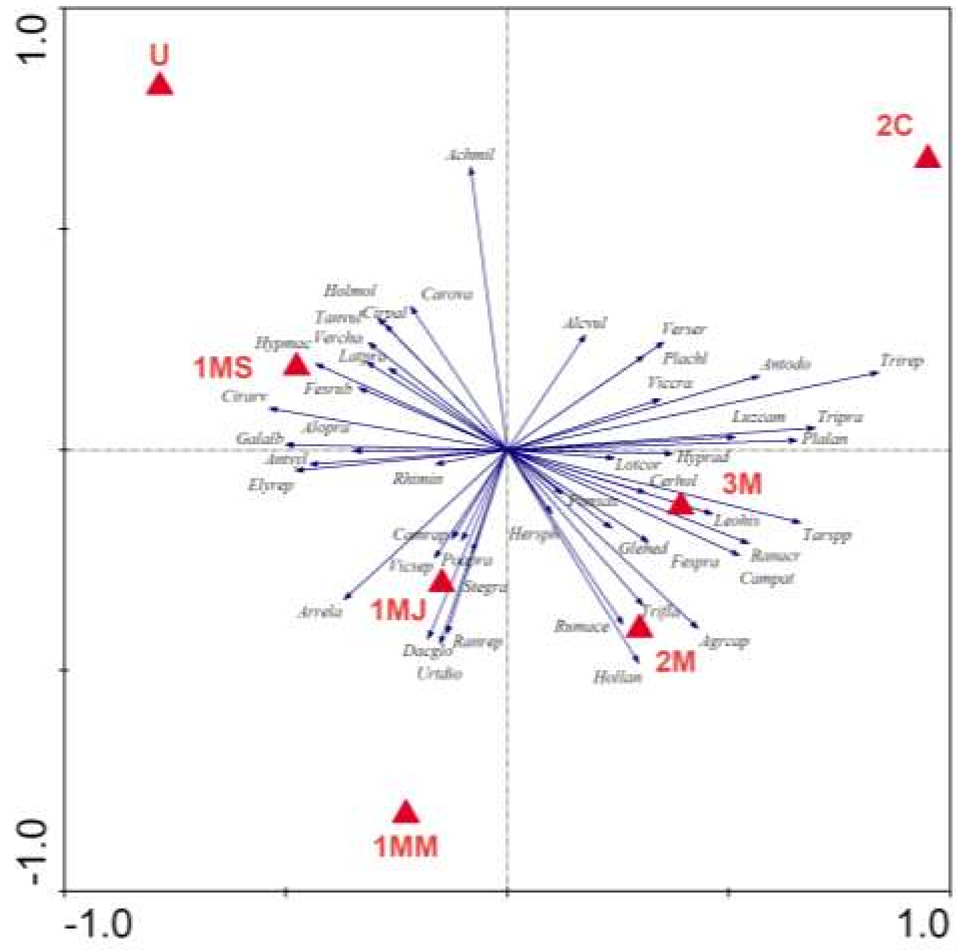

Fig. 2. Ordination diagram showing the results of RDA analysis of plant species composition data collected after twelve years of different management in 2009 (see Table 3 for details). Treatment abbreviations (U, 2C, 1MM, 1MJ, 1MS, 2M and 3M) are explained in Fig. 1. Species abbreviations: *Achmil* = *Achillea millefolium*, *Agrcap* = *Agrostis capillaris*, *Alcvu l*= *Alchemilla vulgaris* agg., *Alopra* = *Alopecurus pratensis*, *Antodo* = *Anthoxantum odoratum*, *Antsyl* = *Anthriscus sylvestris*, *Arrela* = *Arrhenatherum elatius*, *Campat* = *Campanula patula*, *Camrap* = *Campanula rapunculoides*, *Carova* = *Carex ovalis*, *Cerhol* = *Cerastium holosteoides*, *Cirarv* = *Cirsium arvense*, *Cirpal* = *Cirsium palustre*, *Dacglo* = *Dactylis glomerata*, *Elyrep* = *Elytrigia repens*, *Fespra* = *Festuca pratensis*, *Fesrub* = *Festuca rubra*, *Galalb* = *Galium album*, *Glehed* = *Glechoma hederacea*, *Hersph* = *Heracleum sphondylium*, *Hollan* = *Holcus lanatus*, *Holmol* = *Holcus mollis*, *Hypmac* = *Hypericum maculatum*, *Hyprad* = *Hypochaeris radicata*, *Latpra* = *Lathyrus pratensis*, *Leohis* = *Leontodon hispidus*, *Lotcor* = *Lotus corniculatus*, *Luzcam* = *Luzula campestris*, *Plachl* = *Platanthera chlorantha*, *Plalan* = *Plantago lanceolata*, *Pimsax* = *Pimpinella saxifraga*, *Poapra* = *Poa pratensis*, *Ranacr* = *Ranunculus acris*, *Ranrep* = *Ranunculus repens*, *Rhimin* = *Rhinanthus minor*, *Rumace* = *Rumex acetosa*, *Stegra* = *Stellaria graminea*, *Tanvul* = *Tanacetum vulgare*, *Tarspp* = *Taraxacum* spp., *Trifla* = *Trisetum flavescens*, *Tripra* = *Trifolium pratense*, *Trirep* = *Trifolium repens*, *Urtdio* = *Urtica dioica*, *Vercha* = *Veronica chamaedrys*, *Verser* = *Veronica serpyllifolia*, *Viccra* = *Vicia cracca*, *Vicsep* = *Vicia sepium*.

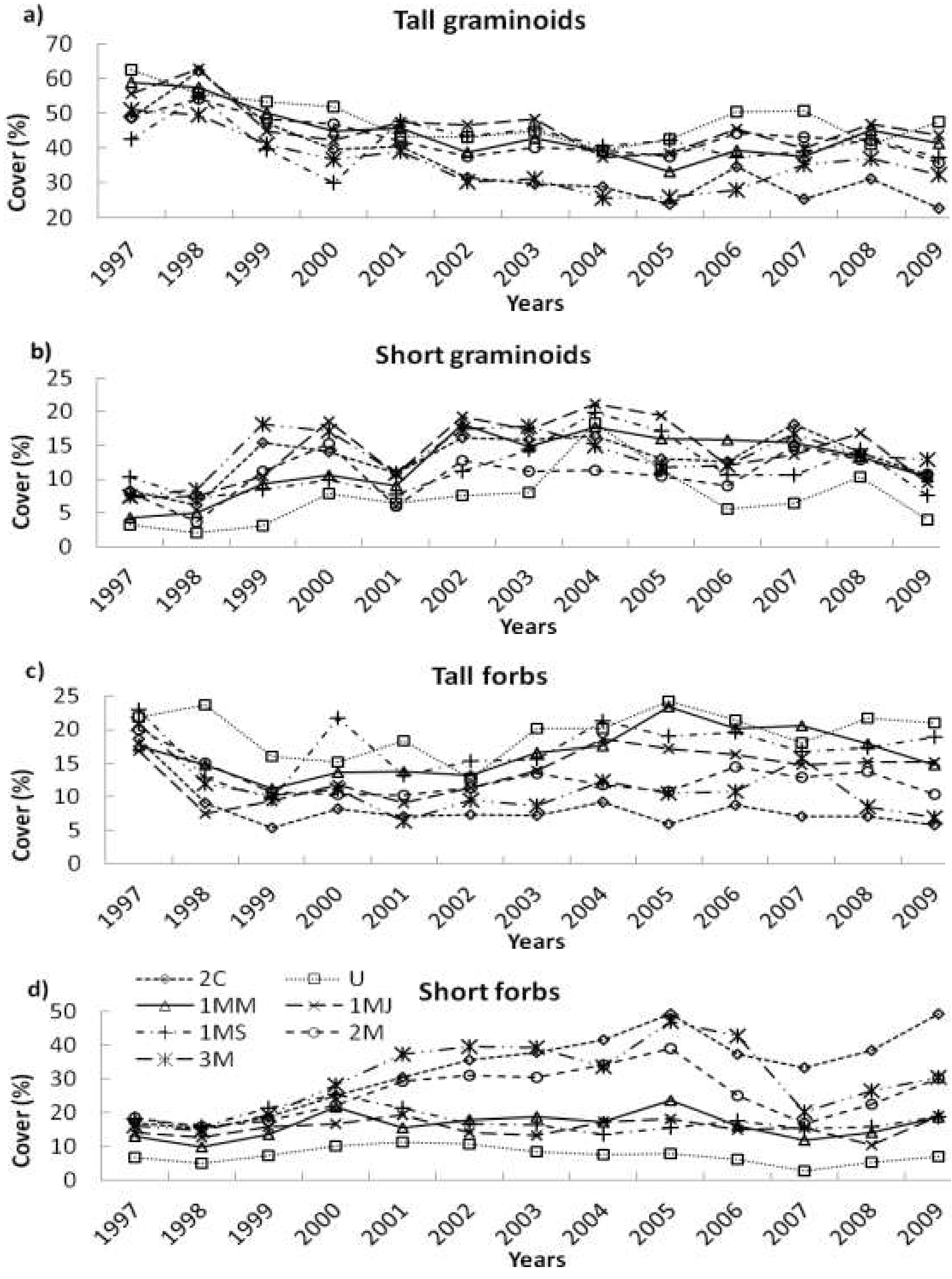

Fig. 3. Changes in mean cover (%) of main functional groups – a) tall graminoids, b) short graminoids, c) tall forbs and d) short forbs in investigated treatments in the years 1997–2009. Treatment abbreviations (U, 2C, 1MM, 1MJ, 1MS, 2M and 3M) are explained in Fig. 1.

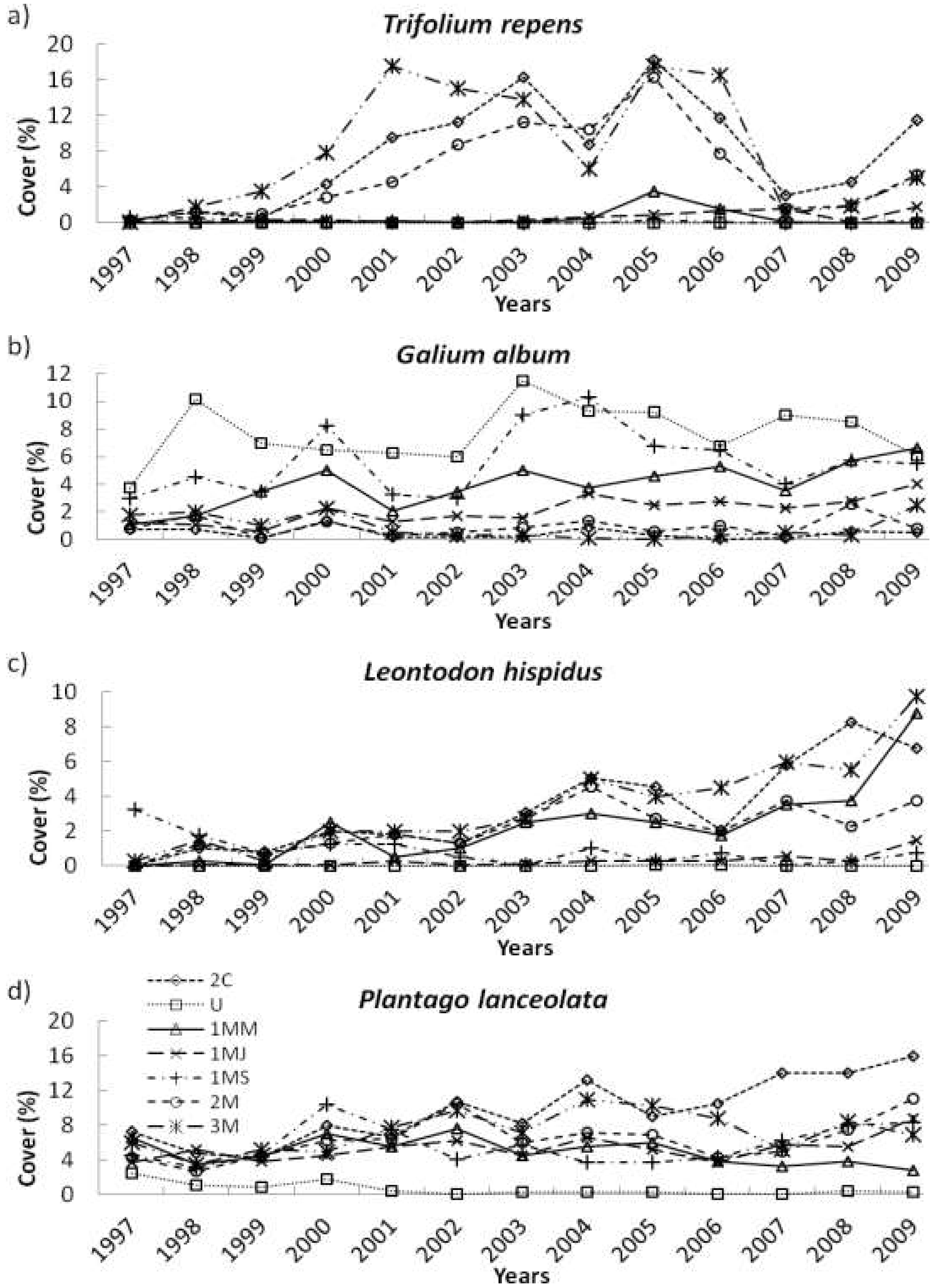

Fig. 4. Changes in mean cover (%) of dominant forb species – a) *Trifolium repens*, b) *Gallium album*, c) *Leontodon hispidus* and d) *Plantago lanceolata* in investigated treatments in the years 1997–2009. Treatment abbreviations (U, 2C, 1MM, 1MJ, 1MS, 2M and 3M) are explained in Fig. 1.

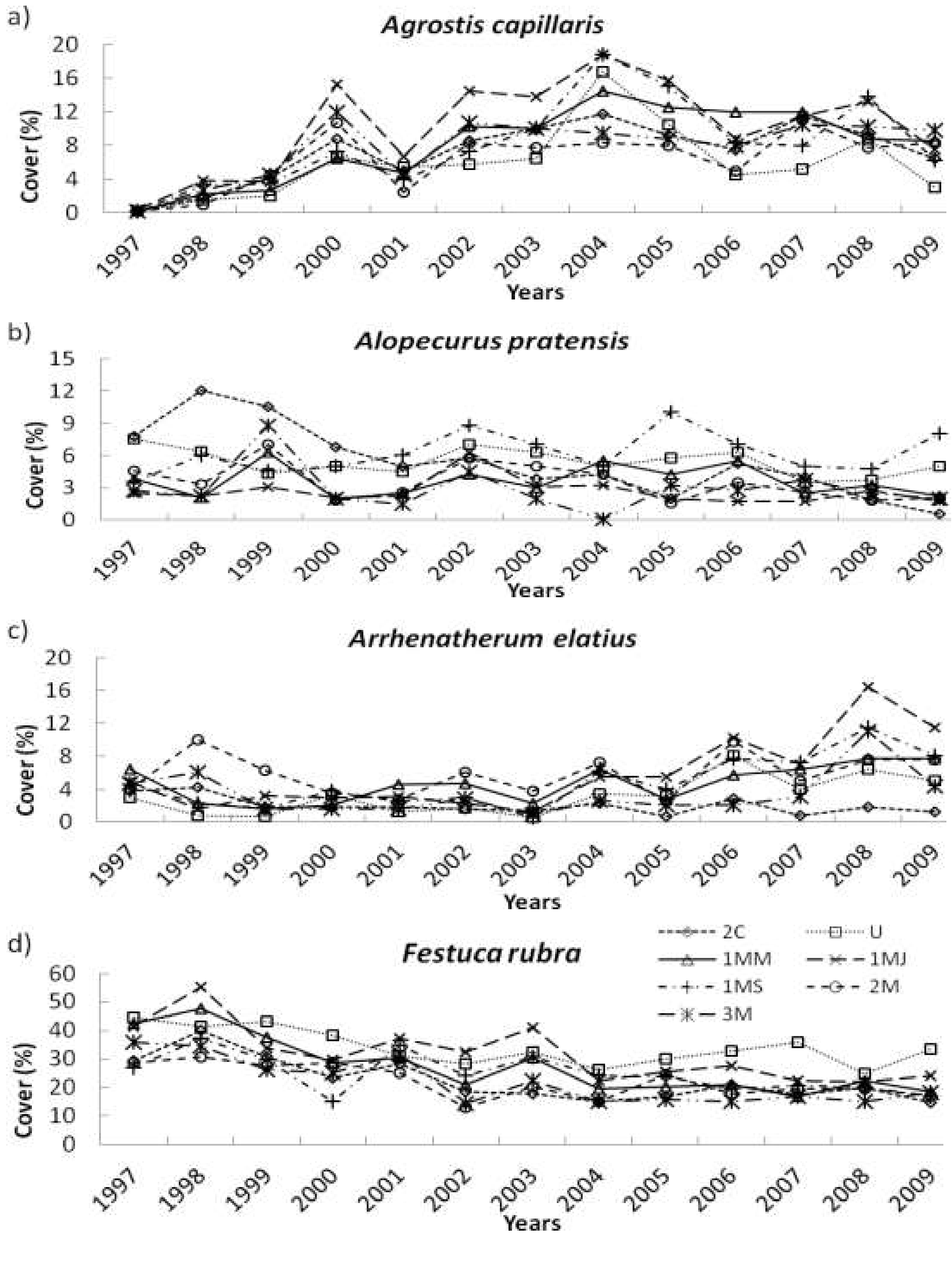

Fig. 5. Changes in mean cover (%) of dominant grass species – a) *Agrostis capillaris*, b) *Alopecurus pratensis*, c) *Arrhenatherum elatius* and d) *Festuca rubra* in investigated treatments in the years 1997–2009. Treatment abbreviations (U, 2C, 1MM, 1MJ, 1MS, 2M and 3M) are explained in Fig. 1.

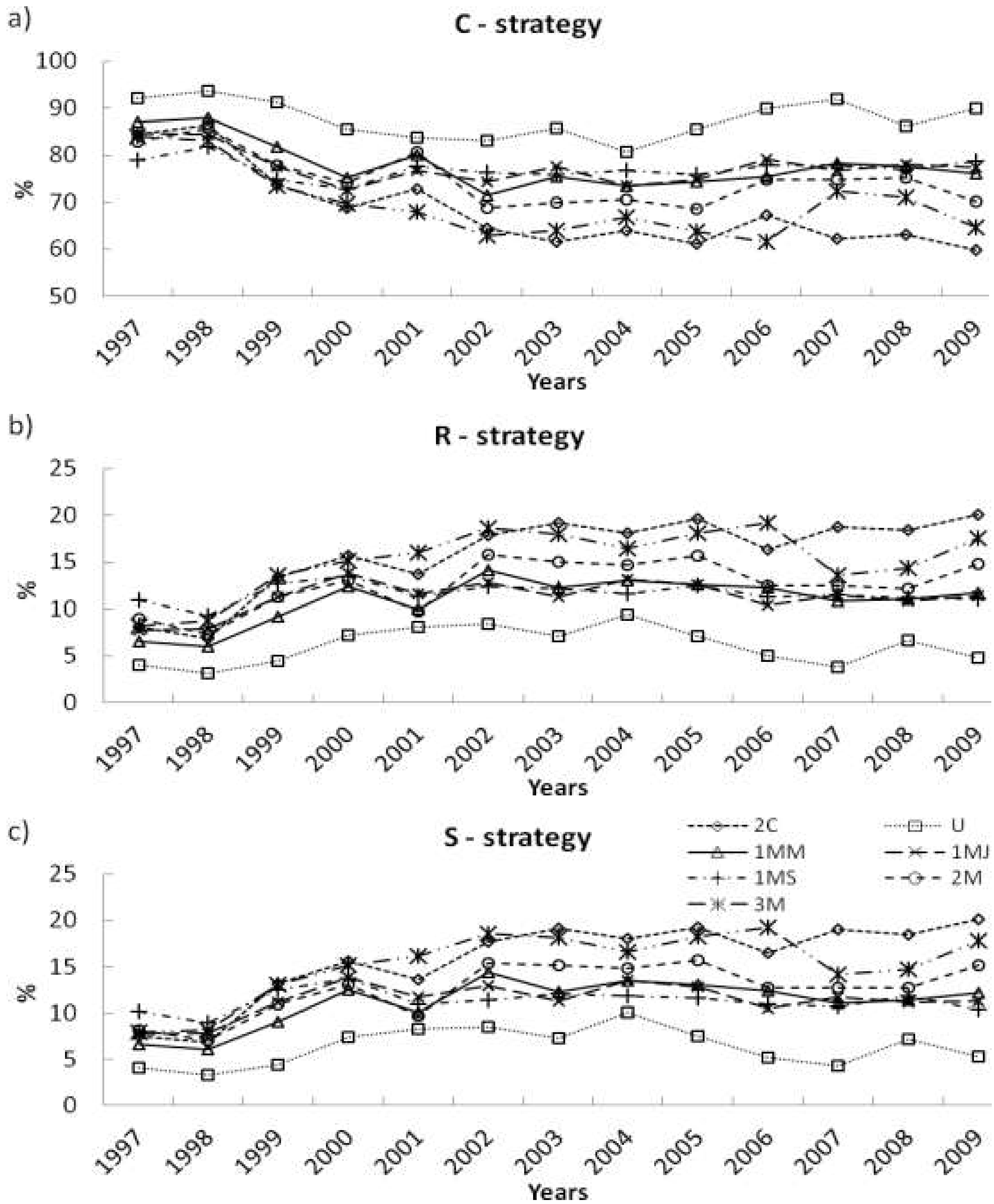

Fig. 6. Changes in C-S-R signature for a) C, b) S, c) R strategy in investigated treatments in the years 1997–2009. Treatment abbreviations (U, 2C, 1MM, 1MJ, 1MS, 2M and 3M) are explained in Fig. 1.

Chapter 3

Effect of long-term cutting versus abandonment on the vegetation of a mountain hay meadow (*Polygono-Trisetion*) in Central Europe

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Effect of long-term cutting versus abandonment on the vegetation of a mountain hay meadow (*Polygono-Trisetion*) in Central Europe

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Abstract

The aim of this study was to identify changes in the plant species richness, diversity and composition of a mountain hay meadow (alliance *Polygono bistortae-Trisetion flavescentis*) after abandonment in comparison with a control cut once per year. The experiment was carried out from 1999 to 2008 in a mountain hay meadow in the Bukovec nature reserve in the north-eastern part of the Jizera Mountains (Jizerské hory, Góry Izerskie, Isergebirge), Czech Republic.

The number of vascular plants species with cover greater than or equal to 1% remained almost the same throughout the study period; however, the total number of vascular plants was higher in the cut treatment after the first four years of the study. The cover of *Festuca rubra*, *Agrostis capillaris*, *Anthoxanthum odoratum*, *Briza media* and *Trifolium repens* was positively affected by cutting. On the other hand, the cover of *Cirsium heterophyllum*, *Geranium sylvaticum*, *Hypericum maculatum*, *Trisetum flavescens* and *Luzula luzuloides* was higher on the abandoned treatment.

The main effect of the abandonment on plant species composition was the shift in cover of the dominant species. Despite ten years of contrasting management, changes in the vegetation were relatively small with no shift to a different plant community. Therefore the cutting regime combined with several years of no management may be a suitable management strategy for maintenance of *Polygono-Trisetion* grasslands and will not be detrimental to the preservation of the target vegetation.

Keywords: Grassland management, Functional groups, Plant species composition, Diversity and richness, Jizera Mountains.

Introduction

In agricultural regions throughout Europe considerable changes in the utilization of grassland have occurred within the past 100 years. The decline in grassland diversity over the last few decades is threatening biological diversity and is a major conservation problem. Such decline is due to changes in agricultural management (intensive milk husbandry in cowsheds), as only a small proportion of grasslands are now being used for forage production and large areas of marginal grasslands have been abandoned (Isselstein et al., 2005; Hejcman et al., 2008 and 2010a). The situation is the worst in less accessible mountain areas with low productivity, where seminatural grasslands predominate.

Regular cutting or grazing management is necessary to maintain a desirable community structure and cutting is often preferred in cases where high species richness is a primary concern (Hansson and Fogelfors, 2000). Another possible strategy favouring nature conservation is periodic cutting (syn. intermittent), which may maximize faunal and floristic diversity (Billeter et al., 2003), as many species can finish reproducing in those years in which cutting does not take place. On the other hand, the absence of grassland defoliation frequently leads to a decrease in plant species diversity (Pecháčková and Krahulec, 1995; Pavlů et al., 2005), and the abundance of tall species tends to increase as litter accumulation elevates nutrient availability and hinders seedling recruitment (Huhta et al., 2001; Hejcman et al., 2009). The responses of individual plant species to grassland abandonment are not straightforward and vary widely. For example Eler et al. (2005) reported an increase in cover of grasses and a decrease in cover of forbs and legumes after abandonment while other authors recorded completely different results (see Huhta et al., 2001; Gaisler et al., 2004; Hejcman et al., 2005; Pavlů et al., 2006). Such differences may be due not only to environmental conditions and the original species composition but also to the management history (Moog et al., 2002; Chýlová and Münzbergová, 2008; Semelová et al., 2008). However, the majority of studies have confirmed that changes in the structure of vegetation can be fatal when management ceases for long periods because of the disparate behaviour of many taxa upon abandonment.

Long-term management studies are indispensable because changes in plant species composition after initial management intervention can take many years and periods of early resistance can occur (Bakker et al., 2002; Pavlů et al., 2007). There is a need to determine the underlying factors that control vascular plant species richness and composition in managed grasslands (Klimek et al., 2007).

Mountain hay meadows (alliance *Polygono-Trisetion)* remain common in the Alps, but in the past, such meadows were also common in the western part of the middle mountains of Central Europe (Chytrý, 2007). However, they disappeared due to changes in land use involving extensive ploughing and grassland renovation, especially in the second half of the twentieth century. Nowadays they are threatened by no or inappropriate management and large areas of meadows have been degraded (Krahulec et al., 1996; Chytrý, 2007; Dierschke and Peppler-Lisbach, 2009).

Given the low demand for forage from mountain hay meadows and the high cost of management, it is essential to determine how long these grasslands can be left unmanaged without losing their species richness. To address this question, we established a long-term grassland management experiment in the Jizera Mountains on the border between the Czech Republic and Poland.

The following research questions were addressed: 1) How do plant species composition and functional traits change in the 10 years after the cessation of cutting of mountain hay meadows? 2) How rapidly and when do these changes occur?

Material and methods

The experiment was carried out from 1999 to 2008 in a mountain hay meadow in the Bukovec nature reserve in the north-eastern part of the Jizera Mts (Jizerské hory, Góry Izerskie, Isergebirge), Czech Republic (50° 48´42´´ N, 15° 21´21´´ E) (Fig. 1). The altitude of the study site is 910 m a.s.l., the average annual precipitation is 1500 mm and the mean annual temperature is 4.5 °C. The bedrock of the Jizera Mts is mainly granite, but patches of basalt occur at the study site. The following soil chemical properties were recorded in the upper 0–10 cm at the beginning of the experiment: pH/KCl 4.4, plant available P content 5 mg kg^{-1} (very low), plant available K content 198 mg kg^{-1} (good) and plant available Mg content 300 mg kg^{-1} (good) (extracted by Mehlich III reagent; Mehlich, 1984). Prior to the start of the experiment the study site was generally cut once per year and biomass was removed (relatively regular management, at least from 1945). According to the phytosociological nomenclature (Chytrý, 2007), the vegetation of the experimental sites belonged to the alliance *Polygono bistortae-Trisetion flavescentis*. The dominant species at the beginning of the experiment were *Festuca rubra*, *Agrostis capillaris*, *Trisetum flavescens*, *Cirsium heterophyllum* and *Geranium sylvaticum*. The nomenclature of the plant species follows Kubát et al. (2002).

The experimental treatments were one cut per year with the removal of cut biomass in mid-July (control) and no cutting (unmanaged grassland). Vegetation monitoring was carried out annually in five permanent completely randomised blocks with an individual plot size of 5 m × 5 m before being cut in mid-July. The cover of all vascular plant species was recorded using the percentage scale. Based on the mean height of vascular plants in the regional flora (Kubát et al., 2002), all plant species within the study area were *a priori* categorized according to their main traits: short graminoids, tall graminoids, short forbs and tall forbs. The species with a mean height ≥ 0.5 m were considered tall and those below this were considered to be short (Appendix 2). The plant species diversity was assessed by plant species richness, Simpson´s (D) species diversity index and Simpson´s (E) species evenness index (Begon et al., 2005).

One-way ANOVA was performed to identify significant differences between treatments within each year for the most abundant plant species, main plant traits, species richness, and D and E indices. Repeated-measures ANOVA was used to evaluate plant species richness, cover of individual species and cover of main plant traits for the whole study period. Redundancy analysis (RDA, Lepš and Šmilauer, 2003) in the Canoco package (ter Braak and Šmilauer, 2002) followed by a Monte Carlo permutation test was used to evaluate trends in plant species composition. A split plot design was used in the permutation to cope with repeated measures. We used 999 permutations in all analyses. Our data form repeated observations along with the baseline data (measurements performed before the introduction of treatments), and thus the interactions between treatments and year were the most important variables. A standard biplot ordination diagram as well as the principal response curves (PRC) constructed by the CanoDraw program (ter Braak and Šmilauer, 2002) were used to visualise the results of the analyses. The resulting PRC showed the extent and directions of development of grassland vegetation under experimental treatment compared with the control treatment (Lepš and Šmilauer, 2003). The vertical scores of the PRC curves were based on the scores of environmental variables from RDA; the sampling time indicators were used as covariables and the interaction between the treatment levels and sampling times were used as environmental variables.

Results

Species richness

At the beginning of the experiment a total of 59 vascular plant species (18 graminoids and 41 forbs) were recorded across the study site. The mean cover of all recorded species is given in Appendix 1. During the 10 years of the study 79 vascular plant species were recorded (22 graminoids, 55 forbs and the seedlings of two woody species). In 1999 the mean number of vascular plants with cover ≥ 1% ± SE (standard error) was 16.8 ± 0.7 and 17.6 ± 0.7 in the unmanaged and cut treatments, respectively. The number of plants in this category was similar in both treatments throughout the study period (Table 2, Fig. 2). At the beginning of the experiment in 1999 the mean number and ± SE of all species per plot was 34.0 ± 0.9 and 34.0 ± 0.5 in the unmanaged and cut treatments, respectively. The number of all vascular plants, without reference to cover, increased significantly during the study in the cut treatment (Table 2, Fig. 2).

However, the occurrence of some of the newly recorded species was frequently random and only temporary (*Hieracium pilosella*, *Filipendula ulmaria*, *Veronica officinalis*, *Carex pallescens*, *Nardus stricta)*. The cover of the species *Trifolium spadiceum* and *Hieracium aurantiacum* exceeded 1% for only a short time and subsequently decreased below 1%. *Botrychium lunaria* occurred sporadically in some study plots, whereas *Arnica montana* and *Gymnadenia conopsea* occurred only once in the cut plots.

The D species diversity index and E species evenness index were similar at the beginning of the experiment, whereas the D species diversity index was significantly lower in unmanaged treatment plots after seven years compared to the control (P < 0.05, one way ANOVA) and the E species evenness index was significantly lower after eight years (P < 0.05, one way ANOVA).

Plant species composition

Significant differences between the study treatments and remarkable changes over time independent of treatment were detected by redundancy analysis (Table 1). The species that thrived under the cut treatment were *Festuca rubra*, *Briza media*, *Trifolium repens* and *Ranunculus acris*. The species associated with the unmanaged treatment were *Alopecurus pratensis*, *Lathyrus pratensis*, *Hypericum maculatum* and *Cardaminopsis halleri*. Those species that were not strongly affected by the treatments were *Potentilla erecta*, *Silene dioica*, *Crepis mollis* subsp. *hieracioides* and *Bistorta major* (Fig. 3).

PRC showed that the diversification in plant species composition created by the different treatments had already begun by the second year of the study (Fig. 4). Species with negative PRC scores had a higher abundance in the cut treatment whereas species with positive PRC scores had a higher abundance in the unmanaged treatment.

Graminoids

The cover of graminoids (both tall and short) was significantly higher in the cut than in the unmanaged treatment during the experiment (Figs. 5a, b). Short graminoids responded significantly to the cut treatment after two years whereas tall graminoids responded significantly after nine years. *Festuca rubra*, *Agrostis capillaris*, *B. media* and *Anthoxanthum odoratum* in particular showed a significant increase in cover in the cut treatment (Fig. 6). On the other hand the cover of *Luzula luzuloides* and *Alopecurus pratensis* in the cut treatment significantly decreased throughout the study, with an initially rapid decrease after which it was maintained at a lower level over the course of the experiment. The cover of *T. flavescens* decreased in both the cut and unmanaged treatment but the cover in the latter remained significantly higher.

The proportions of the majority of graminoids differed significantly over the years, with the exception of *Luzula luzuloides* (Table 2).

There was a significant effect of the time and treatment interaction on the proportions of total and short graminoids, but not in the case of tall graminoids (Table 2). Only two graminoids, *Festuca rubra* and *Agrostis capillari*s, were significantly influenced by the interaction between time and treatment.

Forbs

The absence of management significantly increased the cover of tall forbs over the course of the experiment but the cover of short forbs was significantly higher in the cut treatment. The proportion of tall forbs started to increase significantly in the unmanaged treatment after one year without management; however, the response of short forbs was not as straightforward, as their cover in the cut treatment increased significantly after seven years (Figs. 5c and 5d).

The cover of *Cirsium heterophyllum*, *Geranium sylvaticum, Hypericum maculatum* and *Rumex acetosa* (tall forbs) was significantly higher in the unmanaged treatment (Table 2, Fig. 7). On the contrary the cover of *Meum athamanticum*, *Trifolium repens* and *Rhinanthus minor* (short forbs) significantly increased in the cut treatment over the course of the experiment. The proportions of other less abundant forb species, such as *Bistorta major*, *Veronica chamaedrys*, *Vicia cracca*, *Potentila erecta*, *Crepis mollis* subsp*. hieracioides* and *Achillea millefolium*, did not change significantly under either treatment during the experiment.

Only four of the forbs (*Cirsium heterophyllum*, *Geranium sylvaticum*, *Bistorta major* and *Potentila erecta*) remained relatively stable with no significant changes over the course of the study.

There was a significant effect of the time and treatment interaction on the total cover of forbs (both tall and short) (Table 2). Species that were affected by this interaction were *Hypericum maculatum*, *Ranunculus acris*, *Trifolium repens* and *Rhinanthus minor*.

Discussion

Species richness

The 79 plant species recorded during the ten years of this experiment comprised 75.5% of the total species richness of the surrounding grasslands detected by Pavlů and Burda (1999). The floristic composition was affected by the local micro-relief, which allows small-scale floristic differentiation, reflecting spatially variable moisture conditions (*Nardion*< *Trisetion*) and the presence of basalt in the dominant granite bedrock. Therefore, although the study area belongs to the alliance *Polygono-Trisetion* some species from the alliance *Nardion* (e.g. *Botrychium lunaria*, *Arnica montana*, *Nardus stricta*) also occurred, as they are present in the vicinity of the experiment.

Despite abandonment, none of the species with a cover ≥1% disappeared from the study plots during the experiment, although their proportions changed considerably. This shows the high plasticity of the existing species to a change in management. Our study revealed that the number of vascular plants with a cover ≥ 1% was not affected by the different treatments whereas the total number of vascular plants, regardless of cover, was higher in the cut treatment after the first four years of the study. It should be noted that three new species that appeared during the study with a low frequency of occurrence (*Botrychium lunaria*, *Gymnadenia conopsea* and *Arnica montana*) are considered threatened in the Czech Republic (Holub and Procházka, 2000). *Botrychium lunaria* and *Arnica montana* predominantly occur in the alliance *Nardion*. *Botrychium lunaria* is a rhizome-geophyte and may develop from belowground organs; therefore it can remain invisible for several years. Nevertheless, the presence of sparse species was subject to variation. Therefore interpretation of results based on species with low cover should be carried out with care because of the potential to confuse the conclusions. Moreover it seems that a ten-year study period is too short to reveal substantial changes. However, the higher total species richness in the cut than in the unmanaged treatment recorded in our study is consistent with the results of other authors (Smith and Rushton, 1994; Ryser et al., 1995; Hansson and Fogelfors, 2000; Wahlman and Milberg, 2002; Pavlů et al., 2005).

In contrast to our results, Hellström et al. (2006), Bakker et al. (2002) and Hejcman et al. (2010b) found no effect of abandonment on the number of vascular plant species and Huhta and Rautio (1998) even reported higher species diversity in unmanaged meadows. A possible explanation for their results could be that their cutting regime eliminated tall growing species, which did not happen in our experiment. Therefore a temporary increase in species richness due to management cessation in some short-term studies may create the illusion that abandonment is more desirable from the nature conservancy point of view (Bakker et al., 2002).

Plant traits and plant species composition

Only tall forbs increased their cover due to abandonment because, as strong competitors, they do not tolerate disturbance. However, short graminoids and short forbs preferred managed grassland due to the better light conditions with more opportunity to colonise the open space. It seems that this mechanism is the key factor for promoting plant coexistence, as reported by Klimeš and Klimešová (2002). However, different species responses and functional traits were observed by other authors (e.g. Huhta et al., 2001; Eler et al., 2005; Hellström et al., 2006). These different responses may be due not only to environmental conditions but also the time of cutting. It should be noted that the effect of past land use may be even more important than the effect of some abiotic conditions (Chýlová and Münzbergová, 2008).

It seems that this mechanism is the key factor for promoting plant coexistence, as was described by Klimeš and Klimešová (2002). However, different responses of species and functional traits were revealed by some other authors (e.g. Huhta et al., 2001; Eler et al., 2005; Hellström et al., 2006). These different responses may be due not only to site-specific situations, the time of cutting but also the oceanity-continentality gradient and as well as South-North gradient of Europe. It should be noted that also the effect of past land use may be even more important than the effect of some abiotic conditions (Chýlová and Münzbergová, 2008).

The positive effect of cutting on the cover of *Festuca rubra* and *Agrostis capillaris* is well documented by other studies of temperate grasslands in mountain areas (e.g. Pecháčková and Krahulec, 1995 (*Nardo-Agrostion*); Krahulec et al., 2001 (*Nardion* and *Polygono-Trisetion*); Huhta and Rautio, 1998; Hellström et al., 2006). On the other hand, Mašková et al. (2009) observed a positive effect of abandonment on the proportion of *Agrostis capillaris* in an acidophilous meadow (*Polygono–Trisetion*) in the Šumava Mts, and Stampfli (1992) on poor soil in Switzerland (*Mesobromion/Trisetion*). This was probably due to the timing of cutting as *Agrostis capillaris* is a late-growing species that is favoured by late cutting. Moreover it seems that the cover of *Agrostis capillaris* depends not only on management, but also on the nutrient status of the soil, as mentioned by Olde Venterink and Güsewell (2010). And finally, it should be noted that although *Agrostis capillaris* was more abundant on managed plots in the study by Huhta and Rautio (1998), it also occurred frequently on all of their experimental grasslands. This is consistent with our experiment in which *Agrostis capillaris* was also remarkably abundant in all unmanaged plots, where its cover remained relatively constant during the experiment.

The positive effect of cutting on *Anthoxanthum odoratum* is consistent with the results of Hansson and Fogelfors (2000), who reported an increase in *Anthoxanthum. odoratum* in cut meadows in southern Sweden on soils with a medium nutrient status and pH of 5.5. However it contradicts the results of Huhta and Rautio (1998) and Stampfli (1992). These differences could be due to the different types of plant community according to local nutrient and moisture conditions. The high sensitivity of *Anthoxanthum odoratum* to shading in tall grassland might explain the positive effect of cutting on its abundance. On the other hand abandonment may create better conditions for *Anthoxanthum odoratum* on dry and nutrient poor soils.

Broad-leaved forbs usually suppress other plants in abandoned meadows that lack expansive tall grasses (Huhta et al., 2001). In our experiment the diagnostic species of the *Polygono-Trisetion* alliance (Chytrý, 2007), *Cirsium heterophyllum*, was the dominant tall forb, and thus suppressed other plants.

The rapid increase in the proportion of *Hypericum maculatum* in the unmanaged treatment is consistent with the results of other authors from mountain hay meadows in Central Europe (Krahulec et al., 2001; Hejcman et al., 2005; Pavlů et al., 2007). However Mašková et al. (2009) found no significant difference in the cover of *Hypericum maculatum* between cut and abandoned mountain grasslands (*Polygono-Trisetion*) in the Šumava Mts after ten years. The higher altitude (1150 m.a.s.l.), nutrient-poor acid soil and dominance of *Deschampsia cespitosa* in the Šumava stand are probably responsible for these differences.

The higher cover of *Trifolium repens* in the cut treatment probably reflected its intolerance of shade (Grime et al., 1988). The positive effect of defoliation management on the cover of *T. repens* is consistent with other studies from various grasslands throughout Europe (Belsky, 1992; Correll et al., 2003; Hejcman et al., 2010b). However, Stampfli (1992) included *T. repens* as one of the species that increased their cover on abandoned plots because of their ability to use the improved nutrient and water resources. This difference in the behaviour of *T. repens* may be due to differences in the ecological conditions of the study stands. Moreover, Stampfli (1992) only studied the grasslands for four years and thus it is not known whether this result can be extrapolated into later years. In our experiment, *Trifolium repens* started to increase progressively in the cut treatment after only five years.

Our results confirmed that *Geranium sylvaticum* is a characteristic species of abandoned meadows, as reported for meadows in the middle boreal zone (Huhta and Rautio, 1998), the central European mountains (Krahulec et al., 2001) and the European Alps (Spiegelberger et al., 2006). It is generally regarded as a problematic species in recently abandoned meadows because it often spreads at the expense of uncommon or rare plant species characteristic of traditionally managed meadows. However, it is notable that, in England, *Geranium sylvaticum* is a rare species and is accepted as an indicator of good ecological conditions in British hay meadows (Pacha and Petit, 2008).

Conclusions

Our ten-year study assessed the impact of the cessation of cutting on the plant species diversity and the composition of vegetation in a species-rich mountain hay meadow (*Polygono-Trisetion*). The total number of plant species regardless of cover was higher in the cut treatment after the first four years of the study. However the number of species with a cover ≥ 1% was the same in both treatments throughout the experiment. The main effect of the abandonment on plant species composition in our experiment was the change in abundance of some of the dominant species (e.g. *Festuca rubra*, *Cirsium heterophylum, Geranium sylvaticum, Hypericum maculatum*), but there was no change in the overall plant community. Ten years of contrasting management resulted in relatively small changes in plant species composition, but these rather slow changes are still in progress and therefore monitoring of grassland management requires really long-term studies. The main practical message of the study is that some types of mountain grasslands can be left unmanaged for several years without substantial changes in plant species composition and that such lack of management is not detrimental to the preservation of the target vegetation. Given the low demand for forage from *Polygono-Trisetion* grasslands and the high price of grassland management, cutting management combined with several years of no management may be a suitable strategy for the maintenance of *Polygono-Trisetion* grasslands.

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Table 1 Results of the RDA of cover estimates. U (unmanaged), C (cutting) = treatment abbreviations; Plot ID = plot identifier; % explained variability = species variability explained by the 1st (all) ordination axes (which measure the explanatory power of the explanatory variables); *F*-ratio = F statistics for each particular analysis; *P*-value = corresponding probability value obtained by the Monte Carlo permutation test.
Tested null hypotheses: A1 The temporal trend in the species composition is independent of the treatment; A2 There are no directional changes in time in the species composition that are common to both treatments.

Tested hypotheses	Explanatory variables	Covariables	% explained variability 1st (all) axes	*F*-ratio 1st (all) axes	*P*-value 1st (all) axes
A1	Year*U, Year*C	Year, Plot ID, Block	15.5	16.1	0.001
A2	Year, Year*U, Year*C	Plot ID, Block	16.1 (27.3)	16.9 (16.5)	0.001 (0.001)

Table 2 Results of repeated measurements ANOVA (time, treatment, time x treatment) of dominant plant species, functional groups and number of plant species. *F* represents value derived from *F* statistics in repeated measurements ANOVA and *P* represents related probability value.

	Effects					
	time		treatment		time x treatment	
	F-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Agrostis capillaris	2.62	0.010	50.63	<0.001	3.52	0.001
Alopecurus pratensis	3.37	0.002	4.74	0.032	1.35	0,220
Anthoxantum odoratum	3.80	<0.001	10.40	0.002	0.93	0.500
Bistorta major	1.75	0.091	2.67	0.106	0.45	0.903
Briza media	4.29	<0.001	46.40	<0.001	1.87	0.070
Cardaminopsis halleri	6.88	<0.001	25.96	<0.001	4.58	<0.001
Cirsium heterophyllum	0.43	0.91	38.71	<0.001	1.38	0.210
Festuca rubra	5.33	<0.001	222.23	<0.001	10.23	<0.001
Geranium sylvaticum	1.57	0.14	30.56	<0.001	0.82	0.600
Hypericum maculatum	10.18	<0.001	175.93	<0.001	10.18	<0.001
Luzula luzuloides	1.18	0.316	5.03	0.028	1.04	0.417
Meum athamanticum	2.54	0.01	9.53	0.003	0.40	0.931
Ranunculus acris	3.61	<0.001	5.74	0.019	4.83	<0.001
Rhinanthus minor	5.21	<0.001	40.78	<0.001	4.80	<0.001
Rumex acetosa	3.59	0.001	6.96	0.010	1.54	0.149
Trifolium repens	8.99	<0.001	77.56	<0.001	6.74	<0.001
Trisetum flavescens	12.00	<0.001	7.34	0.008	1.40	0.200
total graminoids	1.87	0.069	81.41	<0.001	2.64	0.010
tall graminoids	3.41	0.001	21.96	<0.001	1.34	0.231
short graminoids	3.60	<0.001	75.54	<0.001	4.13	<0.001
total forbs	8.40	<0.001	83.87	<0.001	4.81	<0.001
tall forbs	3.14	0.003	2003.18	<0.001	6.74	<0.001
short forbs	5.23	<0.001	31.33	<0.001	2.52	0.013
number of all plant species	2.06	0.043	16.08	<0.001	1.92	0.06
number of plant species ≥ 1%	9.00	<0.001	0.16	0.687	1.36	0.22

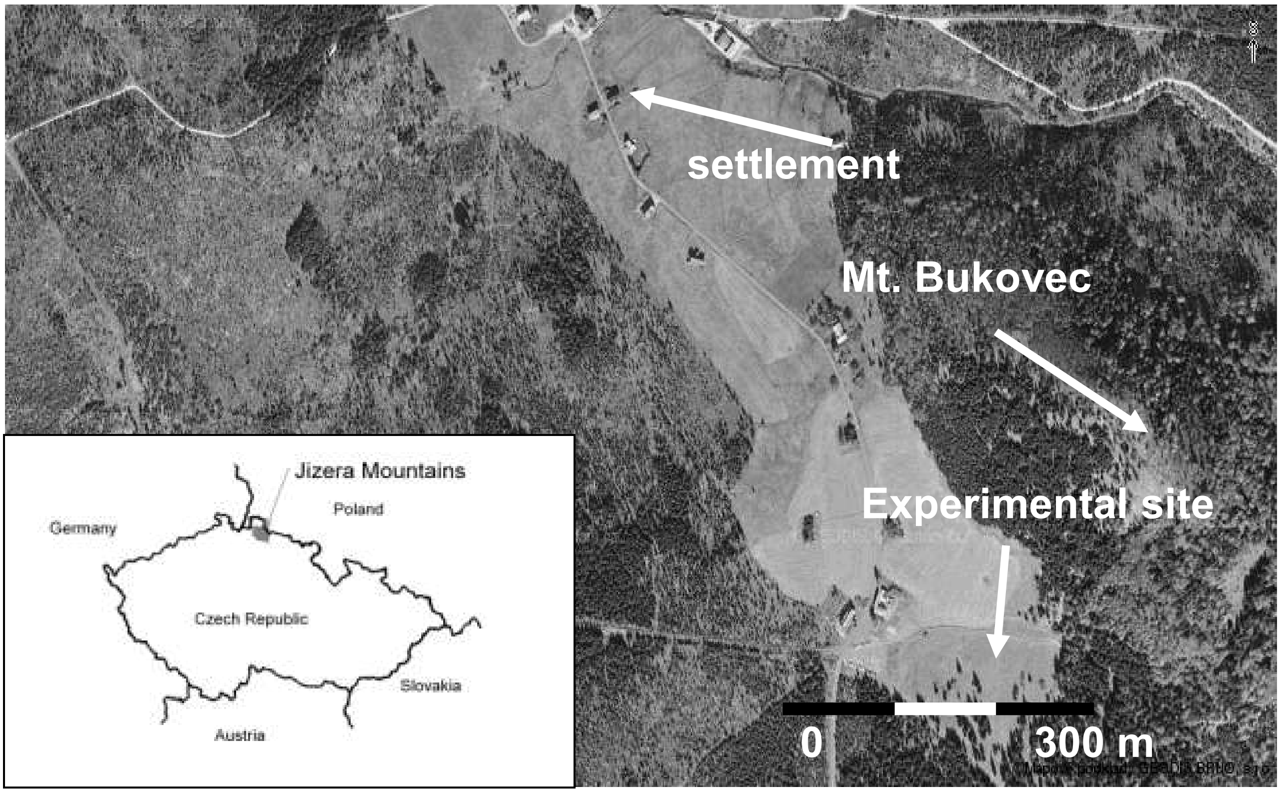

Fig. 1. Location of the Jizera Mountains within the Czech Republic and aerial view (source ©Geodis Brno s.r.o.) of the study area and its surrounding.

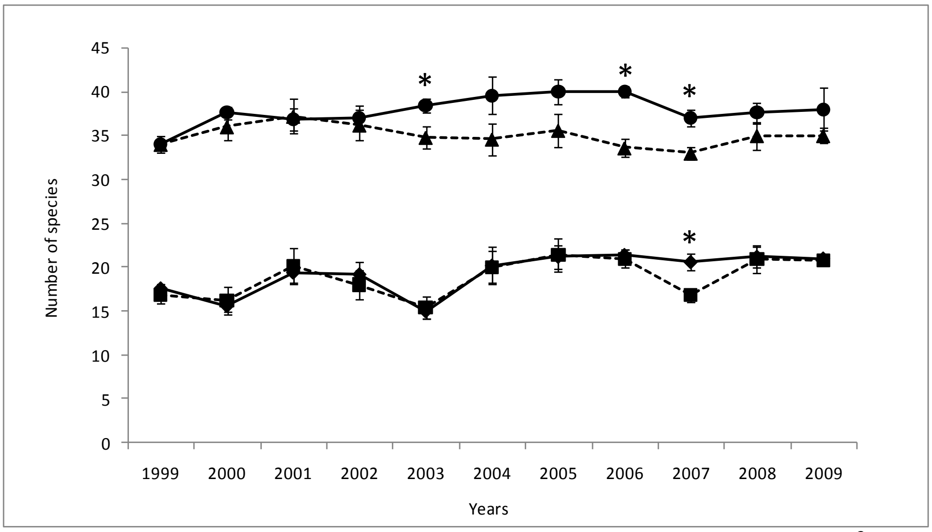

Fig. 2. Number of vascular plant species in treatment plots (per 25 m^2): total species regardless of cover for cutting (●) and unmanaged (▲) treatments, species with cover ≥1% for cutting (♦) and unmanaged (■) treatments for years 1999-2008. Standard errors are indicated by vertical lines. Significant differences between treatments within each year (P<0.05, ANOVA) are indicated by an asterisk.

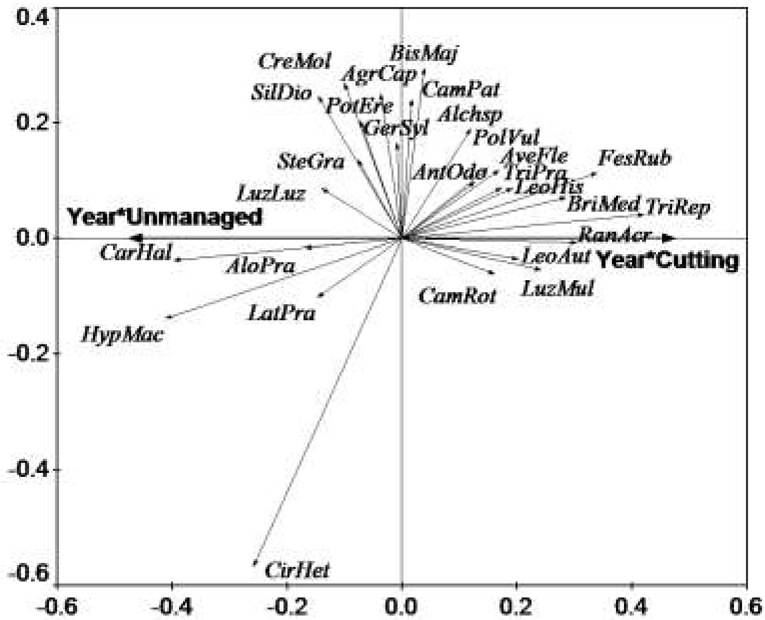

Fig. 3. Ordination diagram showing the results of the RDA. * indicates interaction of environmental variables; species abbreviations: AgrCap-*Agrostis capillaris*, Alchsp-*Alchemilla* sp., AloPra-*Alopecurus pratensis*, AntOdo-*Anthoxanthum odoratum*, AveFle-*Avenella flexuosa*, BisMaj-*Bistorta major*, BriMed-*Briza media*, CamPat-*Campanula patula*, CamRot-*Campanula rotundifolia*, CarHal-*Cardaminopsis halleri*, CirHet-*Cirsium heterophyllum*, CreMol-*Crepis mollis* subsp. *hieracioides*, FesRub-*Festuca rubra*, GerSyl-*Geranium sylvaticum*, HypMac-*Hypericum maculatum*, LatPra-*Lathyrus pratensis*, LeoAut-*Leontodon autumnalis*, LeoHis-*Leontodon hispidus*, LuzLuz-*Luzula luzuloides*, LuzMul-*Luzula multiflora*, PolVul-*Polygala vulgaris*, PotEre-*Potentilla erecta*, RanAcr-*Ranunculus acris*, RumAce-*Rumex acetosa*, SilDio-*Silene dioica*, SteGra-*Stellaria graminea*, TriFla-*Trisetum flavescens*, TriPra-*Trifolium pratense,* TriRep-*Trifolium repens.*

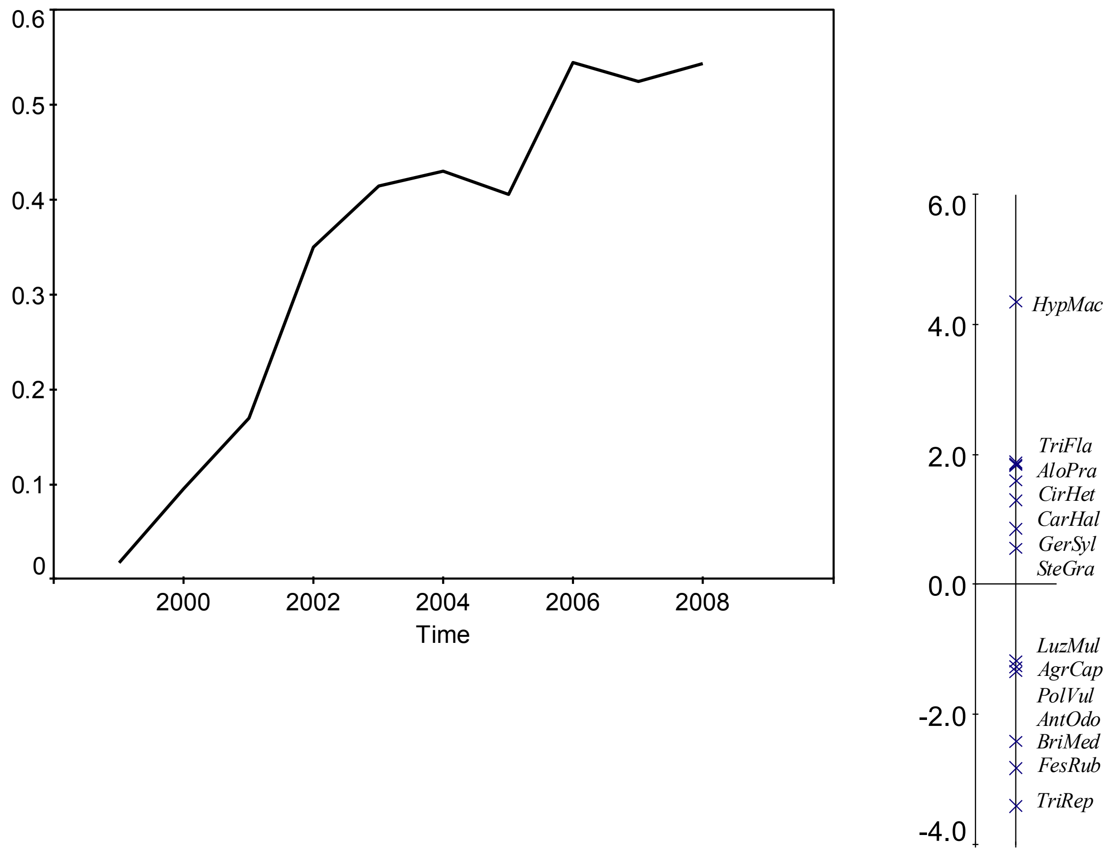

Fig. 4. Principal response curves for unmanaged treatment (—) during the experiment. The x-axis corresponds to time, and the y-axis is the first principal component, showing the main differentiation of the plant communities. The cutting treatment was taken as a reference - zero lines (thus the score for parameters in the cutting treatment is 0 in all times, and consequently, is represented with a line identical to the x-axis). The one-dimensional diagram on the right shows the species scores on the RDA axis. The species showing the best fit to the model are plotted as crosses on the line to the right of the graph. For species abbreviations see Fig. 3.

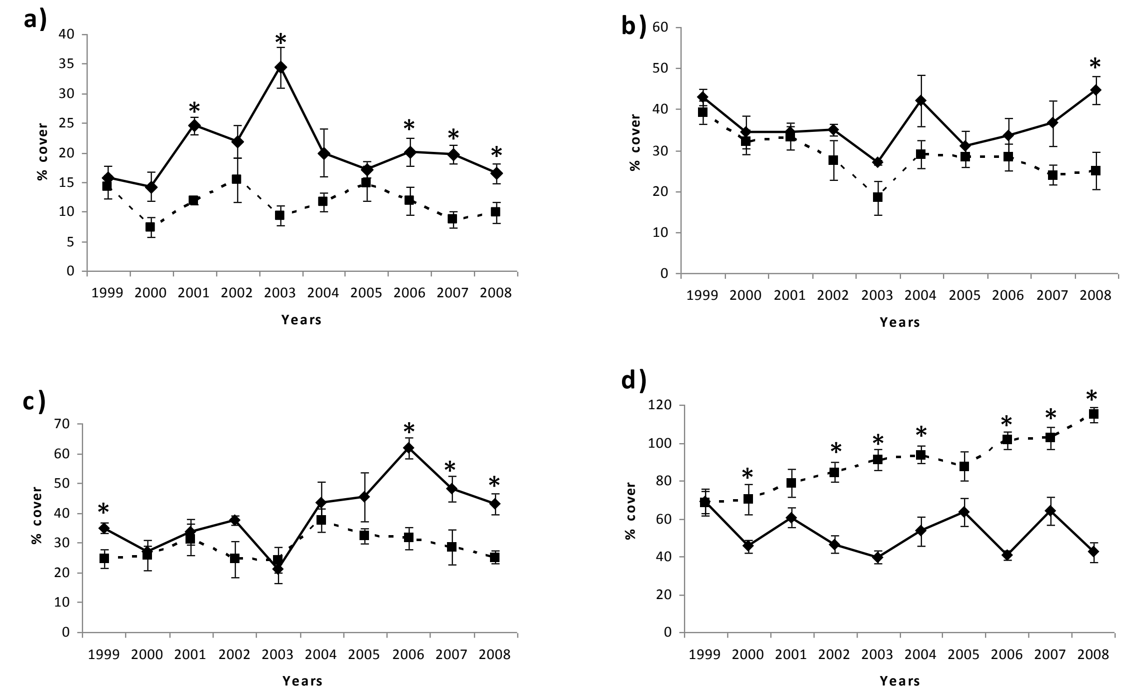

Fig. 5. Changes in cover (%) of functional groups: a) short graminoids, b) tall graminoids, c) short forbs, and d) tall forbs for cut (♦) and unmanaged (■) treatments for years 1999-2008. Standard errors are indicated by vertical lines. Significant differences between treatments within each year (*P*<0.05, ANOVA) are indicated by an asterisk.

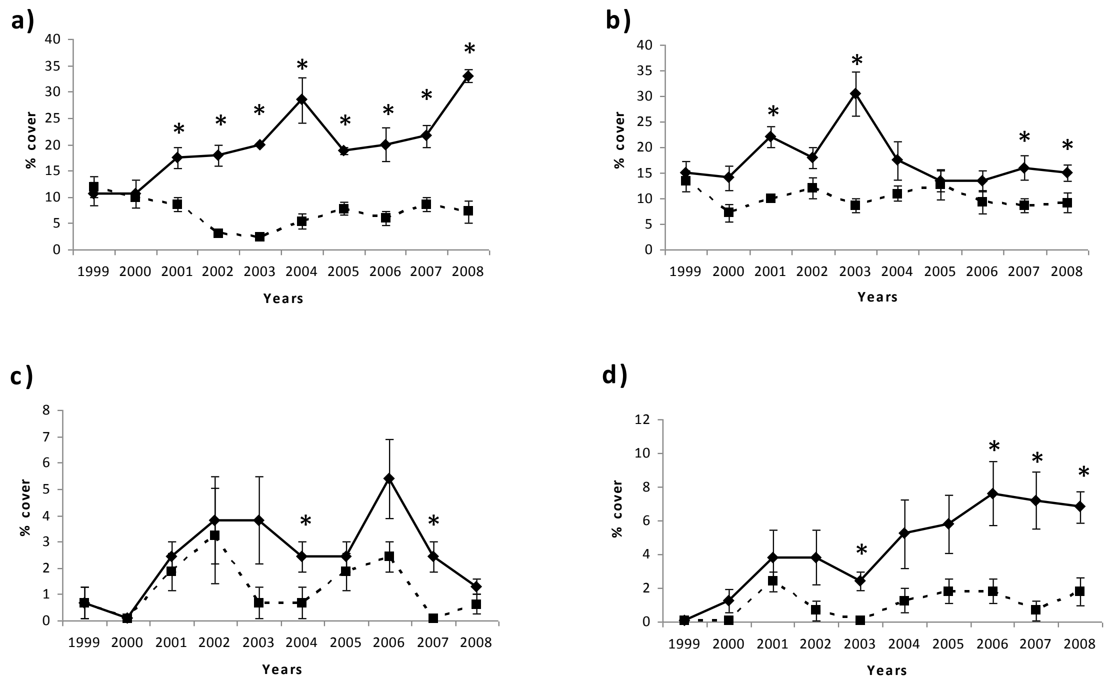

Fig. 6. Changes in cover (%) of a) *Festuca rubra* agg., b) *Agrostis capillaris*, c) *Anthoxanthum odoratum*, and d) *Briza media* for cut (♦) and unmanaged (■) treatments for years 1999-2008. Standard errors are indicated by vertical lines. Significant differences between treatments within each year (*P*<0.05, ANOVA) are indicated by an asterisk.

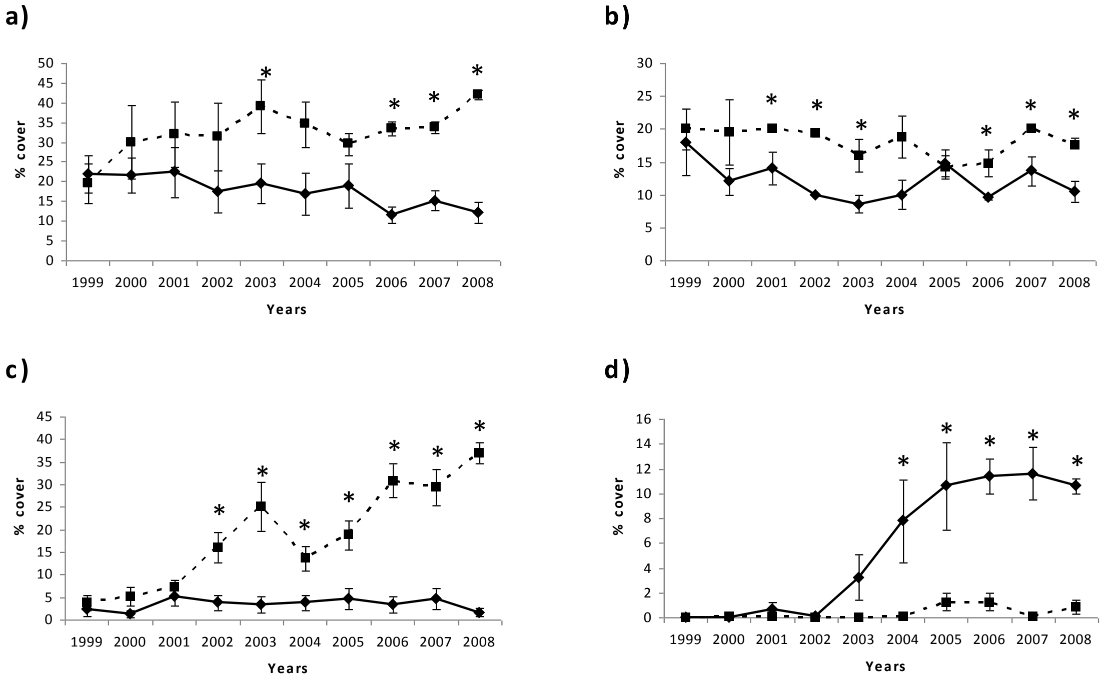

Fig. 7. Changes in cover (%) of a) *Cirsium heterophyllum*, b) *Geranium sylvaticum*, c) *Hypericum maculatum*, and d) *Trifolium repens* for cut (♦) and unmanaged (■) treatments for years 1999-2008. Standard errors are indicated by vertical lines. Significant differences between treatments within each year (*P*<0.05, ANOVA) are indicated by an asterisk.

Appendix 1. Average percentage cover of species for cutting (C) and unmanaged (U) treatments (five replicates each) for years 1999-2008. „+“ indicates cover less than 1%.

	1999		2000		2001		2002		2003		2004		2005		2006		2007		2008	
	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U
Agrostis capillaris	15	13	14	7	22	10	18	12	31	9	17	11	13	13	13	9	16	9	15	9
Achillea millefolium	5	5	+	2	+	+	+	+	+	+	2	1	2	2	2	+	4	2	2	+
Alchemilla sp.	7	7	7	9	7	9	4	7	4	9	7	11	8	11	7	7	8	9	9	5
Alopecurus pratensis	8	4	4	3	+	2	+	4	+	3	0	+	+	2	+	2	+	2	0	2
Anemone nemorosa	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthoxanthum odoratum	+	+	+	+	2	2	4	3	4	+	2	+	2	2	5	2	2	+	1	+
Arnica montana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
Arrhenatherum elatius	+	+	+	+	+	+	+	+	+	+	+	+	+	1	+	+	+	1	0	1
Avenella flexuosa	+	+	+	+	+	+	+	+	+	0	+	+	+	+	1	+	2	+	1	0
Bistorta major	5	6	3	4	6	8	3	7	2	4	7	8	7	5	4	4	5	7	2	4
Botrychium lunaria	0	0	0	0	+	+	0	0	+	0	+	+	+	+	+	0	0	0	0	0
Briza media	+	+	1	+	4	2	4	+	2	+	5	1	6	2	8	2	7	+	7	2
Campanula patula	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Campanula rhomboidalis	0	+	+	+	+	+	+	+	+	+	0	+	+	0	+	+	+	+	0	0
Campanula rotundifolia	+	+	+	+	+	+	+	+	+	+	+	+	2	1	3	+	1	+	+	+
Cardaminopsis halleri	+	+	+	+	2	1	3	3	+	1	2	2	+	2	+	2	+	3	+	3
Carex ovalis	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carex pallescens	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	0	0	+	0
Carex pilulifera	+	0	+	0	0	0	0	0	0	0	+	0	+	+	+	0	0	0	+	0
Carlina acaulis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerastium holosteoides	+	+	0	0	0	0	+	0	0	0	0	+	+	0	0	0	0	0	0	0
Cirsium heterophyllum	22	20	22	30	22	32	18	31	20	39	17	35	19	30	12	33	15	34	12	42
Crepis mollis subsp. *hieracioides*	6	4	3	2	6	3	4	2	2	2	6	9	7	7	4	6	8	6	7	7
Deschampsia cespitosa	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Elytrigia repens	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+
Epilobium montanum	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0
Euphrasia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
Festuca pratensis	+	+	+	+	+	+	+	+	+	+	0	1	0	1	0	1	+	+	+	+
Festuca rubra	11	12	11	10	17	9	18	3	20	2	28	5	19	8	20	6	22	9	33	7
Filipendula ulmaria	0	0	0	+	+	+	0	+	+	+	0	+	0	+	0	+	0	+	0	+
Galeopsis sp.	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Galium mollugo agg.	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0
Galium saxatile	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Geranium sylvaticum	18	20	12	20	14	20	10	19	9	16	10	19	15	14	10	15	14	20	10	18
Gymnadenia conopsea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0
Hieracium aurantiacum	0	0	0	0	+	+	+	0	+	+	+	0	+	+	+	0	+	0	+	+
Hieracium laevigatum	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+
Hieracium pilosella	0	0	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0
Holcus mollis	+	+	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
Hypericum maculatum	2	4	1	5	5	7	4	16	3	25	4	14	5	19	3	31	5	29	2	37
Juncus filiformis	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
Lathyrus pratensis	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+
Leontodon autumnalis	0	+	0	+	0	+	0	0	0	+	0	0	0	0	0	+	+	0	0	0
Leontodon hispidus	1	+	+	3	+	1	+	+	+	1	+	1	2	+	3	+	1	+	2	1
Leucanthemum ircutianum	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+	+
Luzula luzuloides	12	7	4	3	4	10	4	7	3	7	4	8	4	6	4	9	4	6	3	5
Luzula multiflora	+	+	+	+	+	+	+	+	+	+	+	+	1	+	1	+	1	+	+	+
Lychnis flos-cuculi	+	0	0	+	+	+	+	+	+	0	+	0	+	+	+	0	+	0	+	+
Meum athamanticum	11	7	10	5	6	6	10	6	6	5	10	7	7	6	15	11	10	7	10	7
Myosotis nemorosa	+	+	+	+	+	2	+	+	+	+	+	2	+	+	0	+	0	+	0	+
Nardus stricta	0	0	0	0	+	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
Phyteuma spicatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Plantago lanceolata	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+
Poa chaixii	0	3	0	2	0	4	0	3	0	2	0	2	0	2	+	2	+	2	0	4
Poa pratensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Poa trivialis	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Polygala vulgaris	+	+	+	+	1	+	1	0	+	+	1	0	3	+	4	0	+	+	1	0
Potentilla erecta	5	2	4	2	4	4	4	4	1	2	6	5	4	3	7	3	4	7	5	3
Ranunculus acris	2	4	2	3	4	4	4	2	2	1	4	3	3	3	2	3	10	2	5	4
Ranunculus repens	+	+	+	0	0	0	0	+	0	+	0	0	+	0	0	0	0	0	0	+
Rhinanthus minor	+	+	1	+	4	+	11	+	1	+	3	+	2	+	3	+	4	+	1	+
Rumex acetosa	4	3	+	+	+	+	+	3	+	1	1	4	2	2	+	3	+	1	+	+
Senecio nemorensis agg.	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+
Silene dioica	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stellaria graminea	+	+	0	0	0	0	+	+	+	+	+	5	+	+	0	0	0	+	+	+
Taraxacum sect. *Ruderalia*	+	0	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	0	+	0
Trifolium pratense	+	+	+	+	+	+	+	+	+	+	+	+	1	+	3	+	+	+	+	+
Trifolium repens	+	0	+	+	+	+	+	+	3	+	8	+	11	1	11	1	12	+	11	+
Trifolium spadiceum	0	0	0	0	+	0	0	0	+	0	+	0	2	0	+	0	+	+	0	0
Trisetum flavescens	11	13	14	13	7	4	7	9	+	4	3	10	1	6	+	5	2	2	+	2
Trollius altissimus	+	+	+	+	+	1	+	+	+	1	+	+	+	+	+	+	+	+	0	+
Vaccinium myrtillus	+	0	0	0	0	0	0	0	0	0	+	0	0	0	+	0	0	0	0	0
Veratrum album subsp. *lobelianum*	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	+
Veronica chamaedrys	9	6	3	4	6	4	3	2	2	3	3	2	3	3	4	3	4	1	2	3
Veronica officinalis	0	0	0	0	0	+	0	0	0	0	+	0	0	0	0	0	0	0	+	0
Vicia cracca	3	3	2	3	2	1	1	2	+	+	2	1	2	3	2	5	2	+	+	2
Viola tricolor	0	0	0	0	0	0	+	0	0	0	0	+	+	0	+	0	0	0	0	0
Seedlings																				
Acer pseudoplatanus	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	+	+
Picea abies	0	0	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	0	+

Appendix 2. Functional groups of the study sward.

Tall graminoids	Short graminoids	Tall forbs	Short forbs
Alopecurus pratensis	*Agrostis capillaris*	*Achillea millefolium*	*Alchemilla* sp.
Arrhenatherum elatius	*Anthoxanthum odoratum*	*Bistorta major*	*Anemone nemorosa*
Avenella flexuosa	*Briza media*	*Cirsium heterophyllum*	*Arnica montana*
Deschampsia cespitosa	*Carex ovalis*	*Crepis mollis* subsp. *hieracioides*	*Campanula patula*
Elytrigia repens	*Carex pallescens*	*Epilobium montanum*	*Campanula rhomboidalis*
Festuca pratensis	*Carex pilulifera*	*Filipendula ulmaria*	*Campanula rotundifolia*
Festuca rubra	*Juncus filiformis*	*Galium mollugo* agg.	*Cardaminopsis halleri*
Holcus mollis	*Luzula multiflora*	*Geranium sylvaticum*	*Carlina acaulis*
Luzula luzuloides	*Nardus stricta*	*Gymnadenia conopsea*	*Cerastium holosteoides*
Poa chaixii		*Hiearacium laevigatum*	*Euphrasia* sp.
Poa pratensis		*Hypericum maculatum*	*Galeopsis* sp.
Poa trivialis		*Lathyrus pratensis*	*Galium saxatile*
Trisetum flavescens		*Leucanthemum ircutianum*	*Hieracium aurantiacum*
		Phyteuma spicatum	*Hieracium pilosella*
		Ranunculus acris	*Leontodon autumnalis*
		Rumex acetosa	*Leontodon hispidus*
		Senecio nemorensis agg.	*Lychnis flos-cuculi*
		Trollius altissimus	*Meum athamanticum*
		Vicia cracca	*Myosotis nemorosa*
		Veratrum album subsp. *lobelianum*	*Plantago lanceolata*
			Polygala vulgaris
			Potentilla erecta
			Ranunculus repens
			Rhinanthus minor
			Silene dioica
			Stellaria graminea
			Taraxacum sect. *Ruderalia*
			Trifolium pratense
			Trifolium repens
			Trifolium spadiceum
			Vaccinium myrtillus
			Veronica chamaedrys
			Veronica officinalis
			Viola tricolor

Chapter 4

Effect of fertilisation and abandonment on plant species composition of *Festuca rubra* grassland

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Effect of fertilisation and abandonment on plant species composition of *Festuca rubra* grassland

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ABSTRACT

We asked how N, P and K application affects plant species composition and soil chemical properties of mountain grassland during managed period and whether residual after effects of fertiliser application can be detected eight years after its abandonment?

The fertiliser experiment with unfertilised control, phosphorus and potassium (PK) fertiliser treatment and treatments with low and higher level of nitrogen and PK application under three (intensive management from 1993 to 1997) and two cut management (moderate management from 1997 to 1999) was established in Jizera Mts. (Czech Republic) on *Festuca rubra* meadow and then the experiment was eight years abandoned.

Immediately **a**fter introduction of intensive management the significant diversification of plant species composition occurred and lasted under intensive or moderate management. The cover of *Alopecurus pratensis* increased >50 % and the cover of *Agrostis capillaris* decreased < 5% in the most fertilised treatments during the period with intensive management. However within eight years with no management the differences in plant species composition disappeared and *Festuca rubra* became again dominant in all treatments. The differences in biomass P concentration and plant available P and K concentrations in the soil were still detectable eight years after the last fertiliser application.

Termination of fertiliser application and cutting management resulted in dominance of only several plant species, and differences in plant species composition arising from previous management completely disappeared. Moderate application of NPK and consequent abandonment need not generate irreversible structural and as well as compositional changes in *Festuca rubra* grassland.

Keywords:

Cutting frequency; Mountain meadow; Nitrogen, Phosphorus and Potassium; Species richness; Residual effect

1. Introduction

In the second half of the twenty century, an increase in use of mineral fertilisers, amelioration, re-sowing by highly productive forage species and cutting frequency were the main factors used to intensify grassland production in Europe. This intensification resulted in creation of uniform and species poor swards with several highly productive species (Hejcman et al. 2012). The species rich unimproved grasslands have persisted on a few percentages of their former area up to now. For the economic and/or environmental reasons the large areas of formerly intensively managed grasslands through Europe were de-intensified in last twenty years (Isselstein et al. 2005). However due to a long-term after effects of grassland intensification, the biodiversity and therefore the conservation value of such grasslands is low even after several decades without any intensive management (Pavlů et al. 2011a).

Continuous long-term application of N, P and K fertilisers increases stand productivity and nutrient content in the soil and forage (e.g. Spiegelberger et al. 2006; Sammul et al. 2003; Honsová et al. 2007; Smith et al. 2008), but substantially decreases plant species richness (e.g. Galka et al. 2005; Willems and van Nieuwstadt 1996; Schellberg et al. 1999; Hejcman et al. 2007a; Semelová et al. 2008). Even short-term fertilisers application can cause long-term changes in grassland functioning especially in low productive areas (Spiegelberger et al. 2006; Hejcman et al. 2007b; Henkin et al. 2010) and some changes can even be irreversible (Dahler 1992; Hegg et al. 1992; Hejcman et al. 2007b; Semelová et al. 2008; Hejcman et al. 2010).

Therefore restoration of species rich grasslands from formerly heavily fertilised grasslands is very difficult and long-term process which must be connected with reduction of nutrients availability in the soil. According to Janssens et al. (1998), N is the main element affecting grassland productivity and species diversity but its effect on grassland functioning is controlled by P. High availability of P in the soil is therefore connected with low species richness and together with N application supports predominance of highly productive species in grasslands (Critchley et al. 2002; Hejcman et al. 2007a; Wellstein et al. 2007). On the other hand high K availability is compatible with high plant species diversity. Increasing plant species diversity by reducing nutrients availability is based on “hump-back” model by Grime (2001), where species richness peaking at moderate level of nutrients availability and the reasonable application of fertilisers can even increase plant species diversity in low productive and species-poor grasslands.

To study resilience, the ability of a grassland to recover into its original plant species composition after cessation of fertiliser application, is therefore highly important for restoration of species rich grasslands from formerly heavily fertilised ones. Majority of published studies concerning the resilience of grasslands after cessation of fertiliser application comes from low productive alpine or calcareous grasslands but only a little attention was paid to the resilience of common upland grasslands which are the most frequent in Central Europe (Královec et al. 2009; Pavlů et al. 2011a). In this study, set aside grassland fertilised experiment (Fiala 1997; Gaisler et al. 1998) abandoned for eight years was revisited to study the resilience of upland grassland. The unique feature of this study is that baseline data collected before start of the N, P and K application, data collected during application of fertilisers as well as data collected after cessation of fertiliser application and cutting management are presented together.

The aim of this study was to answer the following questions: 1) How N, P and K application affects plant species composition and soil chemical properties of upland grassland during the managed period? 2) Can be residual after effects of fertiliser application on plant species composition, soil and biomass chemical properties detected eight years after the last N, P and K application and cutting management?

2. Materials and methods

2.1 Study site

The experiment was carried out in the Jizera Mountains (50°50'N, 15°05'E) 11 km north-west from the town Liberec, Czech Republic. The altitude of the site is 420 m a. s. l., average annual temperature 7.2°C and annual precipitation 803 mm (Liberec meteorological station). The geological substratum is granite underlying cambisol. The following soil chemical properties were measured in upper 15 cm of the soil in 1993, pH/KCl=4.5, P content = 15 mg kg^{-1} (low availability), K content = 86 mg kg^{-1} (sufficient), Mg content = 42 mg kg^{-1} (sufficient); extracted by Mehlich II reagent (Fiala 1997).

The plant community of the study area was classified as *Arrhenatherion* alliance (Chytrý et al. 2007) with the dominant species *Festuca rubra*, *Alopecurus pratensis* and *Agrostis capillaris.* Before start of the experiment, the study site was cut once per year at the end of June or at the beginning of July and the mean annual dry matter yield of forage ranged from 2 to 3.5 t ha^{-1}.

2.2 Design of the experiment

The fertiliser experiment with four treatments was established in four complete randomized blocks (arranged in four rows) in the autumn 1992 (4 x 4 = 16 experimental plots together (Fiala 1997). The each experimental plot was rectangle 11.0 m × 2.7 m and plots were separated by 0.9 m wide buffer zone with no fertiliser input. Description of investigated treatments (Control (Co), PK, N1PK, N2PK) in three different periods is given in the Table 1. The experiment was terminated in 2000 and since that time, no fertiliser input and cutting management was applied for eight years. In 2007 one block experimental plots was damaged therefore all presented data are only from three blocks.

2.3 Plant species composition

The cover of all present vascular plant species rooting in the experimental plots was visually estimated directly in percentages (Whalley and Hardy, 2000) and nomenclature of species follows Kubát et al. (2002).

To avoid edge effects, data were collected within the inner 20 m^2 (10 m x 2 m) part of the each plot. Relevés collecting was performed annually in May before the first cut in managed period. Relevés were collected in years 1993, 1995, 1996, 1997, 1998, 1999, 2001 and 2007. To obtain the baseline data, the initial plant cover estimation was performed before the first experimental manipulation in spring 1993. The species diversity of all vascular plants was assessed by plant species richness, Simpson´s (D) species diversity index and Simpson´s (E) species evenness index (Begon et al. 2005).

2.4 Soil and plant sampling

Soil and biomass samples were taken in June 2007. In each plot, four separate soil samples were taken from 0 to 15 cm depth after removing plant residues, and combined to form one representative sample. Basic soil chemical analyses were performed in 2007 using the Mehlich III extraction (Mehlich, 1984) to determine content of plant-available Ca, P, K, and Mg. Total N was analysed by the Kjeldahl method and C by means of colorimetry (AOAC, 1984).

Biomass yield was collected from four random rectangles 0.25 m x 0.5 m in each plot. The mean of four replications was used for statistical calculation. Biomass was dried for 48 hours at 70 ^{o}C and weighed and then dry matter (DM) yield per hectare was calculated. The total mean aboveground biomass and biomass of dominant grass *F. rub*ra was analysed for Ca, Mg, P and K concentrations by ICP-OES (inductively coupled plasma optical emission spektrometry) in 2007.

2.5 Data analysis
GLM (General Linear Model) with repeated measures was used to evaluate the cover of the most abundant vascular plant species, species richness and diversity. One-way GLM was used to evaluate soil and biomass chemical data. The vegetation data were processed by unconstrained and constrained ordination techniques by Detrended Correspondence Analysis (DCA) and by Redundancy Analysis (RDA) in CANOCO 4.56 program (ter Braak and Šmilauer 2002). DCA was used to detect trends in vegetation development during run of the experiment. RDA was used to evaluate vegetation data in particular years. The blocks were used as covariables in all analyses to restrict permutations. Cover data were logarithmically transformed to downweight dominant species (Lepš and Šmilauer 2003) and 999 permutations were used in all performed analyses. Ordination diagram, constructed by the CANODRAW program (ter Braak and Šmilauer 2002), was used to visualize the results of the DCA analysis.

3. Results

3.1 Plant species richness and composition

In the first year when baseline data were collected (1993), the similarity of plant species composition is clearly indicated by low distance of marks for different treatments (Fig. 1). In 1995 and 1996 there were revealed the two major lines of development based on fertiliser application 1) N1PK and N2PK treatments and 2) Co (control) and PK treatments. The two years after the last cutting management and fertiliser application the differences among treatments were not detecable as the marks for all treatments were close together in 2001 and as well as in 2007. Calculated by repeated measures GLM, species richness per 20 m^2 was significantly affected by treatment, year and by their interaction (Table 2). Slight decrease in species richness of vascular plants was recorded immediately after introduction of three cuts management (Fig. 2a) and this lasted during whole intensive management period. In the period of moderate management (1997-1999) the number of plant species slightly increased. However in no management period (2000-2007) the number of plant species was successively reduced in all treatments. Richness of species with cover >5% per 20 m^2 was significantly affected only by year (Table 2). During the high and moderate management periods the highest number of plant species with cover >5% was recorded under PK treatment (Fig. 2b). After termination of cutting management and fertiliser application, substantial reduction of plant species with cover >5% was revealed in all study treatments and varied from 2.2 to 4.4 in 2007. The Simpson´s species diversity and evenness indices were significantly affected by treatment, year and by their interaction (Table 2) and they decreased in order PK> Co > N1PK> N2PK treatments especially in period with intensive management (Fig. 2c,d). During the moderate management the treatments with nitrogen fertilisers (N1PK and N2PK) started to be similar to control. Both indices substantially decreased in no management period.

Based on the results of RDA analyses, non-significant effect of treatment on plant species composition in 1993 (analysis 1993 in Table 3) and significant effect in years 1995 – 1999 (analyses 1995 – 1999 in Table 3) was recorded. The percentage of variability explained by treatments varied from 59.2 to 68.3 for the first axis and from 70.2 to 85.2 for all canonical axes in years 1995 – 1999. Differences in plant species composition created in years 1995 – 1999 were not recorded in 2001, two years after the last cutting management and fertiliser application (analysis 2001 in Table 3).

The mean cover of all recorded species in the first (1993) and last (2007) year of study is given in Appendix A. Cover of *Festuca rubra*, dominant grass at the beginning of the experiment, was significantly affected by treatment, year but not by their interaction (Table 2). *F. rubra* was strongly suppressed by the highest dose of fertilisers (N2PK) during years 1993 - 1996 (Fig. 3c). Consequent reducing of cutting frequency and dose of N supported its cover in years 1997 - 1999. However the highest dominance of *F. rubra* (cover >66%) was recorded in the period with no management.

Cover of *Alopecurus pratensis* was significantly affected by treatment, year and by their interaction (Table 2). During the intensive management period cover of *A. pratensis* increased as a positive response to fertiliser application (Co< PK< N1PK< N2PK, Fig. 3b) In N2PK treatment, the cover was over 50 % in years 1995 – 1997 (Fig. 3b).

Cover of *Agrostis capillaris* was significantly affected by treatment, year and by their interaction (Table 2). In contrast to *A. pratensis*, *A. capillaris* was suppressed by N2PK treatment and on the other hand promoted in control treatment during the high and moderate intensity periods (Fig. 3a). Reduction of N dose and cutting frequency in 1997 - 1999 resulted in decrease in the cover of *A. pratensis,* but rather supported *A. capillaris,* especially in PK treatment and control. Absence of any management from 2000 to 2007 enabled dramatic decrease of *A. pratensis* cover up to <5%, whereas cover of *A. capillaris* was affected only slightly.

Cover of *T. repens* was significantly affected by treatment, year and by their interaction (Table 2). The highest cover of *T. repens* was recorded in PK treatment in both intensive and moderate management periods but only sporadically occurred in treatments with N application (Fig. 3e). No plants of *T. repens* were recorded in any treatment during the no management period with the exception of PK treatment in 2001.

Cover of *Plantago lanceolata* was significantly affected by treatment, year and by their interaction (Table 2). *P. lanceolata* was promoted by moderate management but not in N2PK treatment (Fig. 3f). Absence of any management resulted almost in its elimination from the sward in 2007.

Cover of *Hypericum maculatum* was significantly affected only by year (Table 2). Cover of *H. maculatum* was low during the intensive and moderate management periods but it increased substantially under no management (Fig. 3e).

3.2 Standing biomass, soil chemical properties and biomass quality

In 2007 mean aboveground standing biomass was 5.05 (± 0.67 sem), 4.89 (± 0.37 sem), 4.98 (± 0.11 sem), 5.26 (± 0.28 sem) t ha^{-1} in Co, PK, N1PK and N2PK treatment, respectively. The differences among treatments were not statistically significant. Eight years after the last fertiliser application there was no significant effect of treatment on soil reaction, total N, organic C and plant available Ca and Mg concentrations (Appendix B). On the other hand, marginally significant effect on plant available P, K soil concentration was recorded. With the exception of P, no effect of treatment on biomass chemical properties was recorded (Appendix C). Effect of treatment on P concentration in total biomass was marginally significant (P=0.084) and P concentration was the lowest in control and the highest in PK treatment. The same result was recorded for *F. rubra* biomass. The plant available content in the soil and content in the aboveground biomass of *F. rubra* were significantly positively correlated (Fig. 4).

4. Discussion

During fifteen years of the study, significant alternations of essential properties of grassland sward and soil nutrient content have occurred and have reflected applied management intensity (Fiala 1997). Introducing three cut management during the intensive management period substantially decreased species richness regardless treatment. It confirms results from long-term de-intensification experiments (Hejcman et al. 2010; Pavlů et al. 2011a), that higher frequency of cuts supports plant species that are commonly connected with intensive cutting management, even under relatively low N application and low content of plant-available P in the soil. Surprisingly lower species richness was revealed in control than in fertiliser treatments during the period with moderate management. The positive effect of fertilisers on plant species richness in PK treatment could be explained by "hump-back" model by Grime (2001). In the case of low fertile grasslands enhance in nutrient availability can increase species richness as the highest number of species in the meadows is adapted on moderate fertility. Similar results were recently published from low productive meadows in Slovenia by Čop et al. (2009) or by Güsewell et al. (2005) from wet grasslands. In our experiment there was substantial reduction in number of vascular plant species in the most fertilised treatment during intensive and moderate management periods. This is in accordance with other studies, that higher dose of N under no P and K limitation resulted in substantial decline in species richness and simplified the vegetation structure (e.g. Galka et al. 2005; Willems and van Nieuwstadt 1996; Schellberg et al. 1999; Hejcman et al. 2007a; Honsová et al. 2007). We revealed substantial reductions in total species richness during the period with no management as it has been documented in other studies (e.g. Bakker 1998; Smith and Rushton 1994; Losvik 1999; Pavlů et al. 2005; Pavlů et al. 2007). This successive simplification of plant species composition was due to sharp decline of species with cover lower than 5%, however any endangered plant species extinction nor invasion of new species was not occurred. After the abandonment majority plant species which disappeared from intensively fertilised treatments returned back from the vicinity of the experiment. The differences between initial and final plants species composition was the change in abundance of some of dominant species, but there was no change in the overall plant community. It shows good resilience of upland *Festuca rubra* grasslands to short term intensively managed periods.

In the period when fertiliser application and management were ceased the thick layer of litter developed and some tall species (*F. rubra*, *Hypericum maculatum*) well adapted on competition for light start to prevail. High increase in cover of *H. maculatum* under no management has been commonly recorded in abandoned mountain grasslands in Cental Europe (Hejcman et al. 2005, 2008; Mašková et al. 2009; Pavlů et al. 2011b) Nevertheless many perennial species could survive in grassland for long-term period without any management, but they substantially reduce their cover or number of individuals (Pavlů et al. 2011b).

The positive effect of PK application on *T. repens* was in accordance with the conclusion of other authors (Dodd et al. 1994; Galka et al. 2005; Silvertown et al. 2006: Honsová et al. 2007). The positive effect of P and K on cover of legumes can be expected on P and K poor soils at the start of fertiliser application (Velich 1986) as it was in our experiment.

The cover of tall dominant grass *A. pratensis* was supported by N application during the management period however its cover decreased (<5 %) immediately after cessation of fertiliser application and cutting management. This rhizomatous grass is known for the highly positive response on N application under high P and K availability in the soil (Velich 1986; Gaisler et al. 1998; Hejcman et al. 2007a; Honsová et al. 2007; Prach 2008). Completely opposite to *A. pratensis* was the behaviour of *A. capillaries* that had the highest cover under control with no fertiliser input. It should be noted that *A. capillaris* is competitive weak species especially in unmanaged meadows (Pavlů et al. 2007).

The dominant grass species *F. rubra* behaved indifferently to applied amount of fertilisers but was suppressed in the most fertilised treatment during the intensive managed period. In the central European upland grasslands, *F. rubra* is known as highly tolerant to frequent defoliation (Gaisler et al. 2004; Pavlů et al. 2007; Pavlů et al. 2011a; Krahulec et al. 2001) and therefore it is often used as a component of sown mixtures for forage production and as well as for lawns. However in our experiment *F. rubra* prevailed in the sward under no management. It could be explained by its high plasticity to fertiliser application and/or cutting management as was revealed also in pot experiments by Duru et al. (2004) and Gastal et al. 2010. Therefore *F. rubra* is able to grow in different habitats and as well as under different types of management (Grime et al. 1988). Since two years of abandonment the differences in plant species composition aroused during the intensive and moderate management periods completely has disappeared and uniform grassland with dominance *F. rubra* was set up regardless of previous treatments. This result contradicts results of several studies where even short-term application of fertilisers caused decades-long changes in the soil and biomass chemical properties and in plant species composition (e.g. Dahler 1992; Hegg et al. 1992; Hejcman et al*.* 2007b; Spiegelberger et al. 2006; Klaudisová et al. 2009). This can be explained by relatively low application rates of NPK fertilisers used in the experiment and therefore applied nutrients were removed with increased amount of harvested biomass with higher concentrations of nutrients. For example in years 1993-1996 mean DM yield of harvested biomass was significantly affected by fertiliser application and was 3.89, 4.81, 6.33 and 7.66 t ha^{-1} in Co, PK, N1PK and N2PK treatments, respectively (Fiala 1997; Gaisler et al. 1998). In addition to removal of nutrients with harvested biomass some part of applied N was leached from the plots as was documented by increased N concentrations in lysimeter waters collected in studied plots (Fiala 1997). Only marginally significant differences among treatments in plant available P and K concentrations in the soil were detected at the end of the study. Concentration of P in total and *F. rubra* biomass well reflected soil P concentrations as the highest P concentration in the soil was connected with the highest P concentration in the biomass. It seems that P concentration in the aboveground biomass of *F. rubra* than total aboveground biomass could be a good indicator of plant available P in the soil. On the other hand there are studies from temperate grasslands (Schaffers 2002), wetlands (Koerselman and Meuleman 1996) and Mediterranean dry grasslands (Henkin et al. 2010) demonstrating that simple relationship between soil and P tissue concentration does not exist. It must be noted, that effect of treatment on P concentrations in the soil on the total aboveground biomass was only marginally significant especially because of low P application rates and increased removal of nutrients with the biomass during intensive and moderate management periods, but as well as because of only three analyzed replicates and therefore lower power of the statistical tests as the fourth block of the experiment was lost. Our results contrasts with substantially more pronounced residual effects recorded by other authors which used higher P application rates or applied P for a longer time (Silvertown et al. 1994; Niinemets and Kull 2005; Chiarucci and Maccherini 2007; Semelová et al. 2008; Smits et al. 2008; Klaudisová et al. 2009). Higher plant available (Mehlich III) K concentration in PK treatment is consistent with results published by Hrevušová et al. (2009) and can be probably explained by increased K release from the soil substratum.

The majority of vegetation changes occurred within the first two years after the abandonment of the meadow.

5. Conclusions

Immediately after introduction of intensive management significant diversification of plant species composition occurred in different fertiliser treatments and the differences lasted for seven years with management and fertiliser application. Consequent termination of the cutting management and fertiliser application resulted in dominance of only several plant species, and differences in plant species composition arising from previous management completely disappeared. However the differences in biomass quality and soil available nutrients arose by previous management were still detectable in case of P. Lower plant available P in soil and content P in total plant of above ground biomass were recorded in formerly unfertilised treatment. Especially content of P in aboveground biomass of *F. rubra* seems to be good indicator of plant available P in the soil. The main message of this study is that moderate application of NPK need not generate irreversible structural and as well as compositional changes in upland *Festuca rubra* grassland.

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Table 1 Description of investigated treatments

Study period	Management intensity	Number of cuts per year	Treatment abbreviations and amount of applied N, P and K (kg ha^{-1}) per year			
			Co (control)	PK	N1PK	N2PK
1993-1996	intensive	three	-	30 kg P + 60 kg K	90 kg N + 30 kg P + 60 kg K	180 kg N + 30 kg P + 60 kg K
1997-1999	moderate	two	-	30 kg P + 60 kg K	60 kg N + 30 kg P + 60 kg K	120 kg N + 30 kg P + 60 kg K
2000-2007	no	no	-	-	-	-

The following fertilisers were used: limestone ammonium nitrate (KAS 27, NH_4NO_3 + $CaCO_3$, 27.5% N, 10% Ca); potassium chloride (KCl, 50% K, 47% Cl) and super-phosphate ($Ca(H_2PO_4)_2$ + $CaSO_4$, 8.5% P, 20% Ca, 10% S)

Table 3 Results of the RDA analyses of cover estimates performed separately for each year. Co (control), PK, N1PK, N2PK = treatments abbreviations, see Table 1; % expl. var. = species variability explained by one (all) ordination axis (measure of explanatory power of the explanatory variables); F – ratio = F statistics for the test of particular analysis; P - value = corresponding probability value obtained by the Monte Carlo permutation test. Tested hypothesis: Is there any effect of treatment on plant species composition in particular year?

Year	Type of management	Explanatory variables	Covari-ables	% expl. var.1st axis (all axes)	*F*- ratio1st axis (all axes)	*P*-value 1st axis (all axes)
1993	baseline data	Co, PK, N1PK,N2PK	blocks	19.7 (45.4)	1.5 (1.7)	0.379 (0.069)
1995	intensive management	Co, PK, N1PK,N2PK	blocks	68.3 (70.2)	12.9 (4.7)	0.025 (0.031)
1996	intensive management	Co, PK, N1PK,N2PK	blocks	73.7 (82.4)	16.8 (9.3)	0.004 (0.001)
1997	intensive management	Co, PK, N1PK,N2PK	blocks	63.0 (76.5)	10.2 (6.5)	0.028 (0.020)
1998	moderate management	Co, PK, N1PK,N2PK	blocks	57.5 (72.4)	8.1 (5.2)	0.001 (0.004)
1999	moderate management	Co, PK, N1PK,N2PK	blocks	59.2 (85.2)	8.7 (11.5)	0.001 (0.001)
2001	no management	Co, PK, N1PK,N2PK	blocks	31.0 (37.2)	2.6 (1.2)	0.352 (0.341)
2007	no management	Co, PK, N1PK,N2PK	blocks	9.6 (16.8)	0.6 (0.4)	0.900 (0.888)

Table 2 Results of GLM analyses (effects Treatment, Year and Treatment*Year interaction) of dominant species, number of plant vascular plant species and Simpson´s species diversity and evenness index. *F*-ratio represents a value derived from *F* test in repeated measurements, *df* represents degree of freedom and *P*-value represents related probability value

	Effect	*df*	*F*-ratio	*P*-value
Agrostis capillaris	Treatment	3	14.35	<0.001
	Year	7	13.16	<0.001
	Treatment*Year	21	2.81	<0.001
Alopecurus pratensis	Treatment	3	68.25	<0.001
	Year	7	35.44	<0.001
	Treatment*Year	21	6.38	<0.001
Festuca rubra	Treatment	3	5.13	0.003
	Year	7	22.38	<0.001
	Treatment*Year	21	1.54	0.096
Hypericum maculatum	Treatment	3	0.75	0.526
	Year	7	16.78	<0.001
	Treatment*Year	18	0.40	0.989
Plantago lanceolata	Treatment	3	9.44	<0.001
	Year	7	31.95	<0.001
	Treatment*Year	21	2.37	0.004
Trifolium repens	Treatment	3	98.24	<0.001
	Year	7	12.18	<0.001
	Treatment*Year	21	15.36	<0.001
Total number of plant species	Treatment	3	4.42	0.007
	Year	7	26.21	<0.001
	Treatment*Year	21	2.20	0.008
Number of plant species >5%	Treatment	3	2.07	0.113
	Year	7	6.02	<0.001
	Treatment*Year	21	1.48	0.117
Simpson´s species diversity index	Treatment	3	4.53	0.006
	Year	7	27.08	<0.001
	Treatment*Year	21	2.54	0.002
Simpson´s species evenness index	Treatment	3	5.54	0.001
	Year	7	15.35	<0.001
	Treatment*Year	21	2.43	0.003

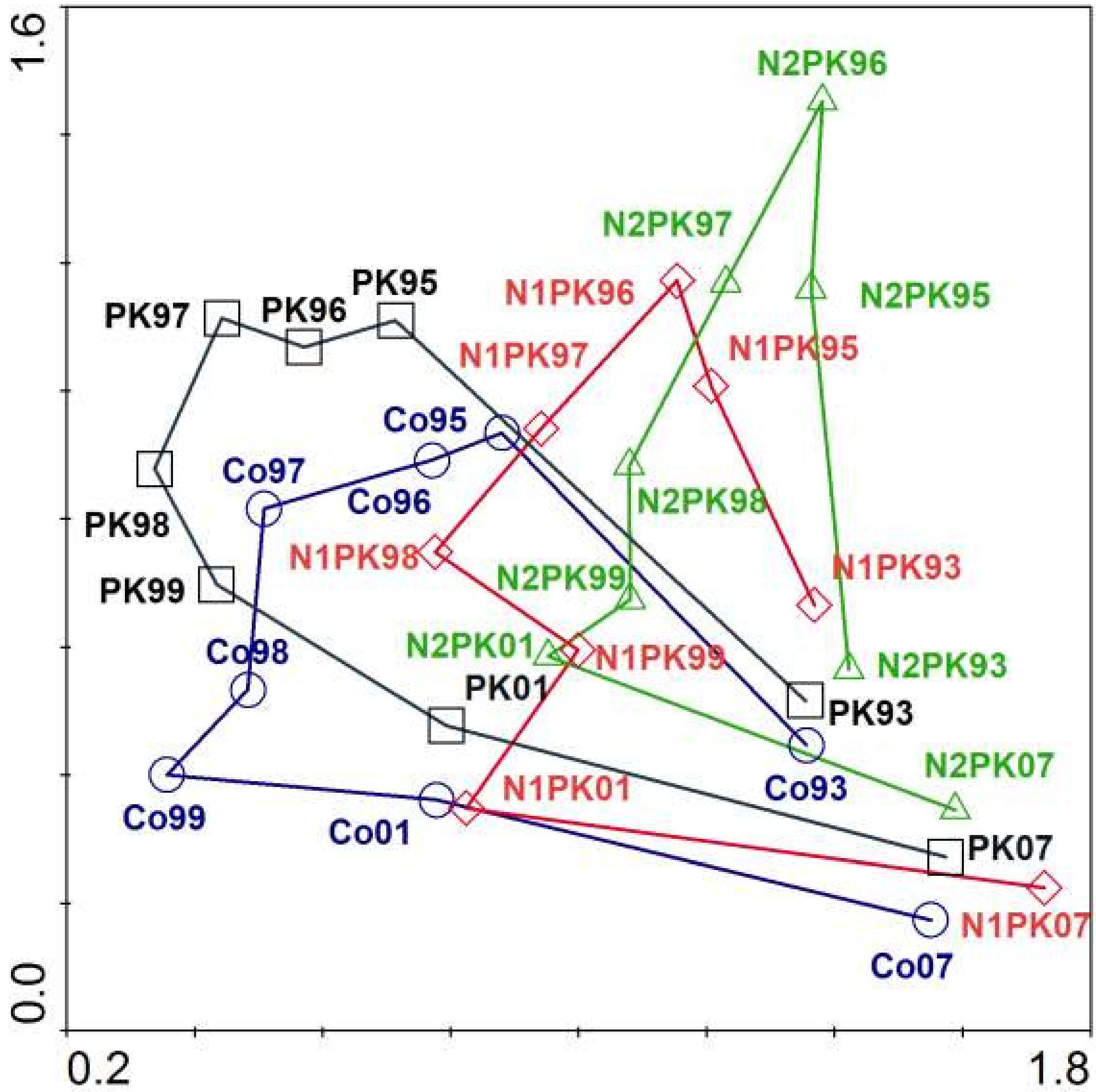

Fig. 1. Detrended Correspondence Analysis (DCA) ordination of vegetation samples for treatments Co-control (○), PK (□), N1PK (◊), N2PK (Δ) in the years 1993-2007 (last two numbers represent year). Periods: 1993-base line, 1993-1996 intensive management, 1997-1999 moderate management, 2000-2007 no management. Centroids, representing replicate averages, are used.

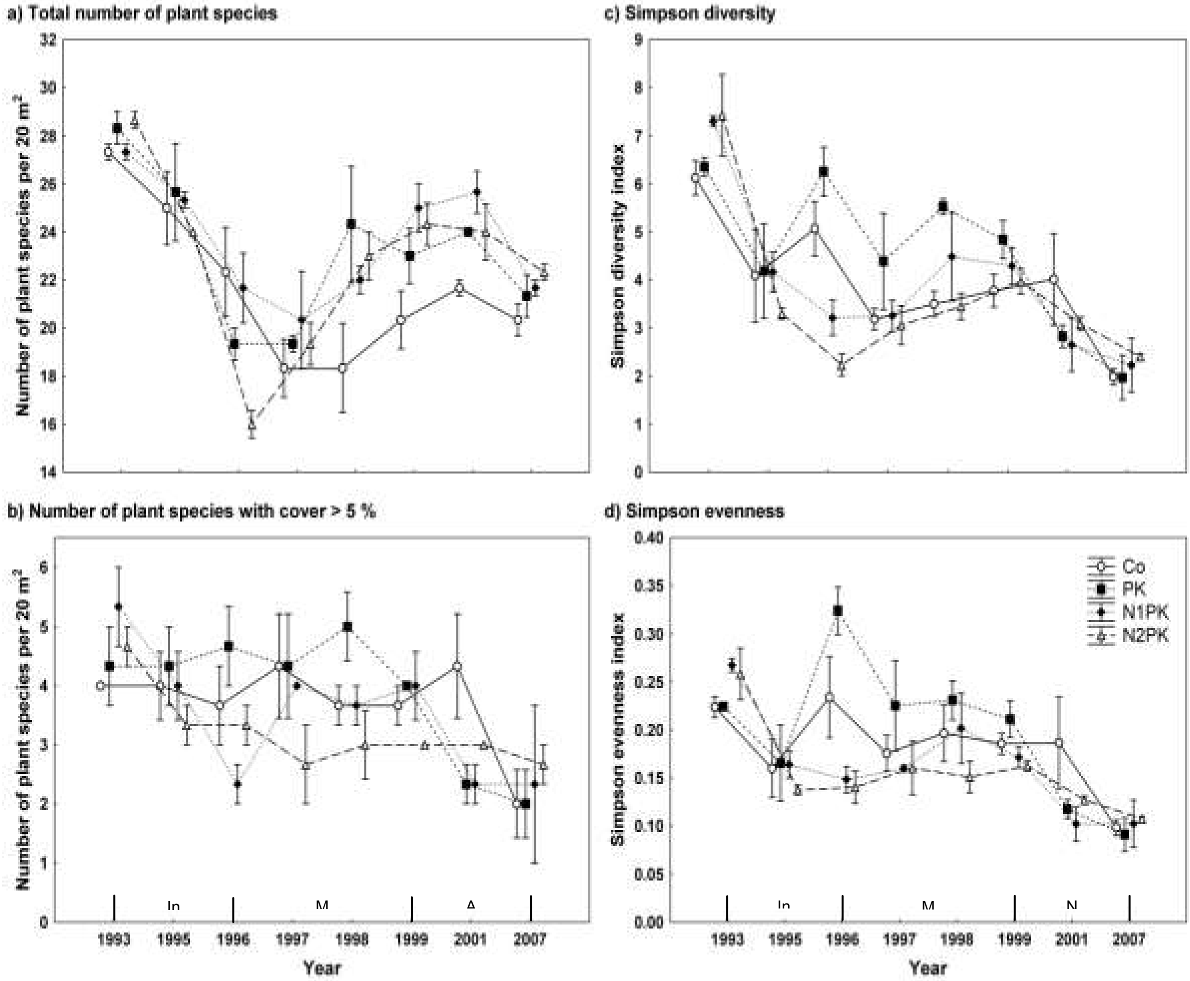

Fig. 2. Changes in number of vascular plant species (a,b) and Simpson´s species diversity index (c) and Simpson´s species evenness index (d) per 20 m^2 for treatments Co-control, PK, N1PK, N2PK in the years 1993-2007. Periods: 1993-base line, 1993-1996 intensive management (Int), 1997-1999 moderate management (Mo), 2000-2007 no management (No). Standard errors are indicated by the vertical lines.

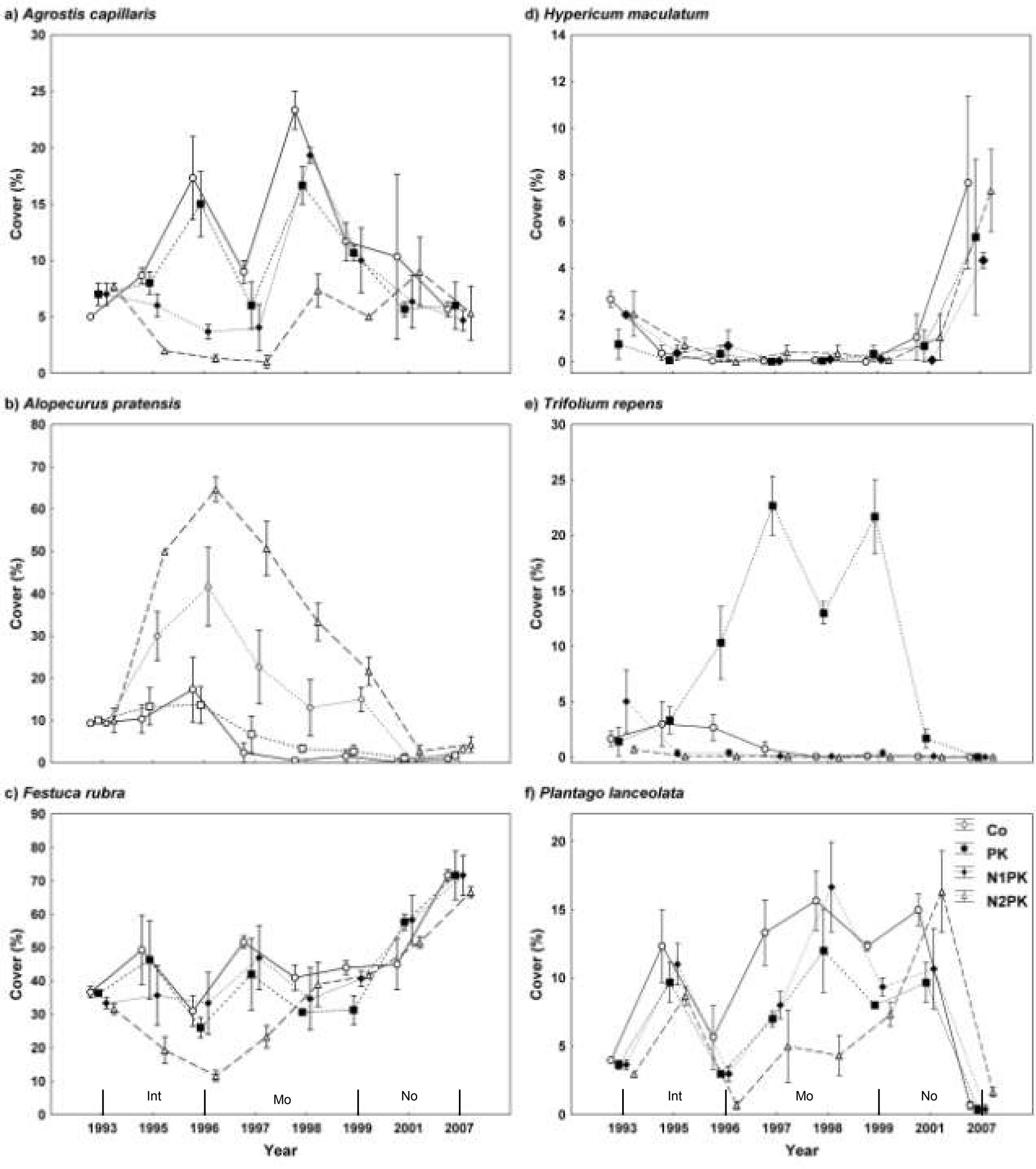

Fig. 3. Changes in coverage (%) of dominant species per 20 m^2 for for treatments Co-control, PK, N1PK, N2PK in the years 1993-2007. Periods: 1993-base line, 1993-1996 intensive management (Int), 1997-1999 moderate management (Mo), 2000-2007 no management (No). Standard errors are indicated by the vertical lines.

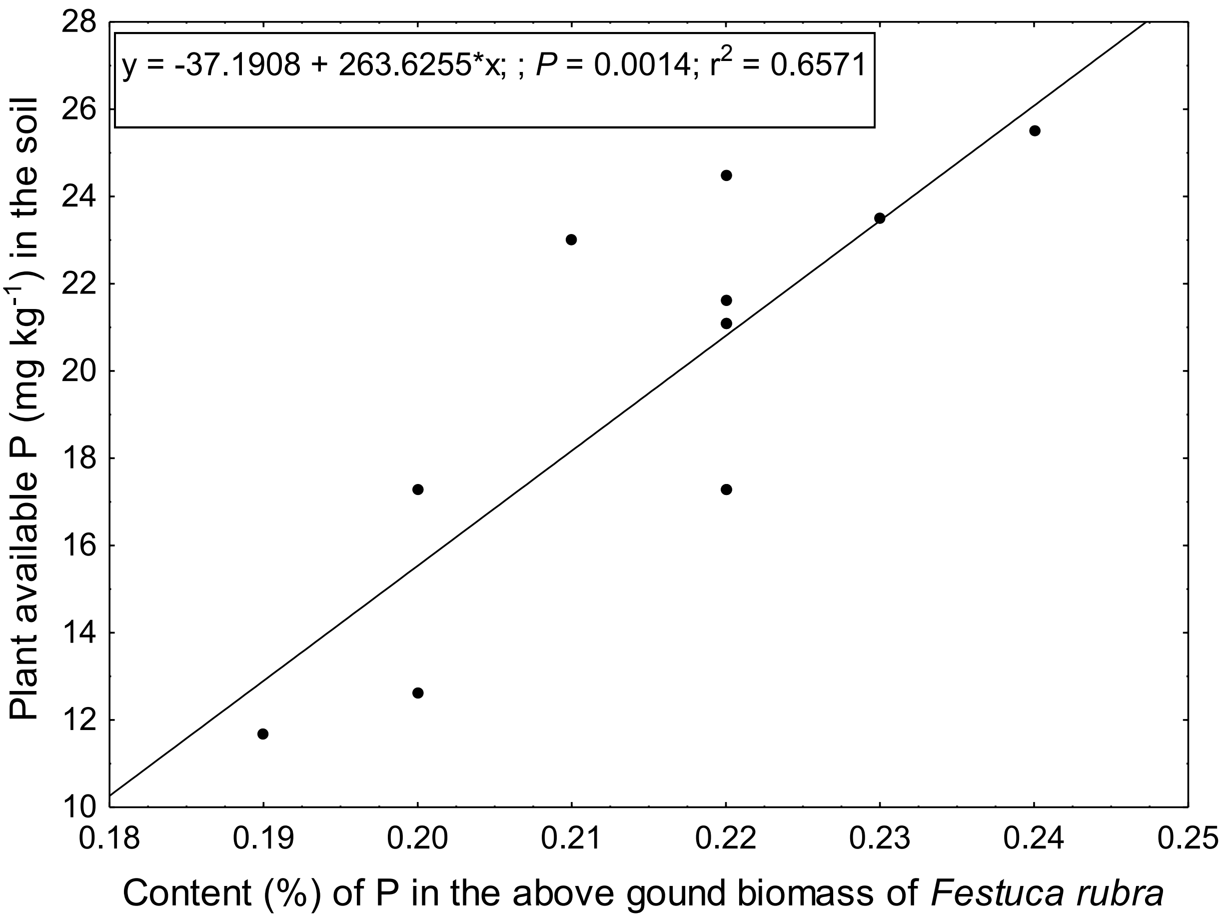

Fig. 4. Relationship between plant available P (mg kg^{-1}) in the soil and P % content in the above ground dry matter biomass of *Festuca rubra.*

Appendix A. Average percentage cover of species for study treatments in 1983 and 2007. "+" indicates cover less than 1%. Treatment abbreviations are given in Table 1.

	Co		PK		N1PK		N2PK	
	1993	2007	1993	2007	1993	2007	1993	2007
Agrostis capillaris	5	6	7	6	7	5	8	5
Alopecurus pratensis	9	1	10	2	9	3	10	4
Anthoxanthum odoratum	2	0	3	0	2	0	2	0
Arrhenatherum elatius	2	1	2	2	2	4	3	2
Dactylis glomerata	2	2	2	3	2	3	2	2
Festuca rubra	37	72	36	72	33	72	32	67
Holcus lanatus	3	1	2	+	2	+	3	1
Holcus mollis	+	+	1	+	1	+	1	+
Luzula campestris	1	+	2	0	+	+	1	+
Phleum pratense	0	0	0	+	0	+	0	0
Poa pratensis	8	+	5	+	7	+	6	+
Trisetum flavescens	7	0	6	0	6	+	7	0
Trifolium pratense	+	0	+	0	+	0	+	0
Trifolium repens	2	0	1	0	5	0	1	+
Vicia cracca	1	+	+	+	1	+	1	+
Aegopodium podagraria	+	0	4	1	2	1	2	1
Achillea millefolium	1	+	2	+	+	1	1	+
Alchemilla sp.	3	+	6	+	6	+	6	+
Angelica sylvestris	0	0	0	0	0	1	0	+
Anthriscus sylvestris	0	+	+	+	0	0	1	+
Campanula patula	+	+	+	+	+	+	+	+
Campanula rotundifolia	0	+	+	+	0	+	0	+
Cardamine pratensis	+	+	+	+	+	+	+	+
Galium album	1	3	+	+	1	3	+	3
Galium saxatile	0	0	0	+	0	+	0	0
Heracleum sphondylium	+	0	+	+	1	0	+	0
Hypericum maculatum	3	8	1	5	2	4	2	7
Knautia arvensis	0	+	0	+	0	+	0	+
Pimpinella saxifraga	2	3	2	2	2	3	2	3
Plantago lanceolata	4	1	4	+	4	+	3	2
Ranunculus acris	4	3	4	2	5	2	4	4
Rumex acetosa	1	2	+	1	+	1	1	1
Scorzoneroides autumnalis	0	0	0	0	0	+	0	+
Stellaria graminea	+	0	+	+	+	+	+	0
Thlaspi alpestre	0	0	0	0	0	0	0	+
Veronica chamaedrys	3	+	3	1	3	2	5	2
Tree Seedlings								
Quercus robur	0	0	0	0	0	0	0	+

Appendix B. Effect of former fertiliser treatment on soil reaction, content of total N (N-tot), organic C (C-org) and plant available (Mehlich III) concentrations of P, K, Ca and Mg in upper 10 cm soil layer analysed 8 years after the last fertiliser application. Numbers represent average of three replicates, ± values represent standard error of the mean (SE). n.s. – non-significant, m.s. – marginally significant (0.05-0.10) results of one-way GLM. Treatment abbreviations are given in Table 1.

Treatment	pH/KCl	N-tot (%)	C-org (%)	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
Co	4.38±0.02	0.56±0.02	8.61±0.27	15.13±2.45	87.1±7.2	847±35	45.2±6.6
PK	4.27±0.05	0.59±0.02	8.66±0.21	21.47±1.93	103.7±3.9	680±83	39.0±6.9
N1PK	4.31±0.02	0.56±0.03	7.83±0.38	21.27±1.62	96.4±6.4	752±17	35.8±1.1
N2PK	4.40±0.08	0.57±0.01	8.94±0.28	20.97±1.70	86.3±4.6	919±156	41.0±3.6
	n.s.	n.s.	n.s.	m.s.	m.s.	n.s.	n.s.

Appendix C. Effect of former fertiliser treatment on total aboveground and *Festuca rubra* biomass chemical properties analysed 8 years after the last fertiliser application. Numbers represent average of three replicates, ± values represent standard error of the mean (SE). n.s. – non-significant, m.s. – marginally significant (0.05-0.10) results of one-way GLM. Treatment abbreviations are given in Table 1.

Treat-ment				Total biomass					
	N-tot (%)	P (%)	K (%)	Ca (%)	Mg (%)	N/P	N/K	K/P	Ca/P
Co	1.63±0.11	0.26±0.02	1.34±0.11	0.57±0.02	0.16±0.00	6.35±0.72	1.23±0.02	5.20±0.67	2.18±0.11
PK	1.73±0.05	0.31±0.01	1.59±0.06	0.62±0.07	0.14±0.01	5.65±0.26	1.09±0.01	5.19±0.28	2.02±0.21
N1PK	1.58±0.04	0.30±0.00	1.45±0.10	0.56±0.01	0.13±0.00	5.31±0.14	1.10±0.05	4.88±0.31	1.90±0.03
N2PK	1.58±0.06	0.30±0.01	1.28±0.09	0.63±0.01	0.15±0.01	5.29±0.17	1.26±0.10	4.27±0.36	2.11±0.01
	n.s.	m.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
					Festuca rubra				
Co	1.85±0.10	0.20±0.01	0.76±0.05	0.54±0.03	0.15±0.00	9.16±0.65	2.46±0.17	3.73±0.15	2.67±0.06
PK	1.81±0.01	0.23±0.01	0.86±0.05	0.56±0.05	0.14±0.00	8.00±0.21	2.12±0.11	3.79±0.14	2.48±0.23
N1PK	1.80±0.03	0.22±0.00	0.82±0.07	0.52±0.01	0.14±0.00	8.19±0.20	2.24±0.23	3.74±0.29	2.37±0.05
N2PK	1.87±0.05	0.21±0.01	0.71±0.01	0.56±0.04	0.14±0.01	8.76±0.11	2.62±0.04	3.35±0.04	2.62±0.18
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Chapter 5

Restoration of grazing management and its effect on vegetation in an upland grassland

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Restoration of grazing management and its effect on vegetation in an upland grassland

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Abstract
Question: How are plant species and functional group composition, and potential sward height affected by implementation of different grazing regimes on previously abandoned semi-natural grassland?
Location: The Jizerské mountains, northern Czech Republic.
Methods: We established a randomized block experiment with the following treatments: unmanaged control (U), intensive (IG) and extensive (EG) continuous grazing, first cut followed by intensive (ICG) and first cut followed by extensive (ECG) continuous grazing for the rest of the growing season. The percentage cover of all vascular plant species was recorded in 40 permanent plots.
Results: Total plant species richness increased in all managed treatments, whereas species number was reduced in U at the end of the experiment. Tall forbs (*Aegopodium podagraria, Galium album, Anthriscus sylvestris, Cirsium arvense*) as well as tall grasses (*Elytrigia repens* and *Alopecurus pratensis*) were more abundant in U. Species associated with both grazing treatments (IG, EG) were *Dactylis glomerata, Festuca rubra* agg. and *Phleum pratense. Agrostis capillaris, Taraxacum* spp., *Trifolium repens*, *Ranunculus repens* and *Cirsium vulgare* were promoted by ECG and ICG. Abundance of tall grasses and tall forbs reflected the intensity of management in the order U>EG, ECG>IG, ICG. Prostrate forbs, on the other hand, increased their cover with increasing intensity: ICG>IG>ECG>EG.
Conclusions: Plant species composition of semi-natural grasslands is affected by the defoliation regime. Continuous grazing on abandoned grassland alters the sward structure towards a permanent pasture with short, light-sensitive grasses and prostrate forbs. To maintain or enhance plant species richness in semi-natural grasslands, understanding the effects of different grazing regimes on plant species composition is necessary.
Keywords: Abandoned semi-natural grassland; Grazing intensity; Pasture; Principal response curve; Vegetation trait.
Nomenclature: Kubát et al. (2002).
Abbreviations: ECG = Extensive grazing after first cut; EG = Extensive grazing; ICG = Intensive grazing after first cut; IG = Intensive grazing; PRC = Principal response curve; RDA = Redundancy Analysis; U = Unmanaged control.

Introduction

During the last two decades considerable changes in the utilization of grassland in Europe have occurred. The most remarkable changes occurred in eastern and central European countries, which had to build up a market economy in the 1990s. Continued intensification of cattle production by introducing highly digestible forages from arable land and concentrates will probably further decrease demand for grassland areas in the future (Isselstein et al. 2005).

Absence of grassland defoliation frequently leads to a decrease in plant species diversity (Bakker 1989; Smith & Rushton 1994; Pecháčková & Krahulec 1995; Losvik 1999; Tasser & Tappeiner 2002; Pykälä 2004; Pavlů et al. 2005). Extensive grazing is characterized by a strongly variable sward height and species composition (Correll et al. 2003). The main mechanism by which grazing livestock maintains high biodiversity in pastures is the creation and maintenance of sward structure heterogeneity (Schlapfer et al. 1998; Rook et al. 2004). Patchiness created by extensive grazing is particularly interesting for nature conservation. Therefore, extensive grazing is frequently recommended as an alternative way of managing semi-natural hay meadows (Bakker 1998; Adler et al. 2001; Bakker et al. 2004), but not in all type of grasslands (Fisher & Wipf 2002). In practice, there is an urgent need to find a type of management acceptable for both farmer's profitability and nature conservation targets (Watkinson & Ormerod 2001).

The impact of grazing management on vegetation dynamics has been widely studied in western Europe during the last two decades (e.g. Bakker 1989; WallisDeVries et al. 1998; Marriott et al. 2002). However, research on livestock grazing in central Europe has concentrated on a small number of case studies although the use of grazing management has dramatically increased due to socio-economical changes in the rural economy since the 1990s. Several experiments describe attempts to restore abandoned mountain grasslands via livestock grazing (Krahulec et al. 2001; Matějková et al. 2003) but, so far, with little attention paid to the restoration of upland grasslands, although they are one of the most frequent of all grassland types. This is the reason why we set up a grazing experiment on abandoned upland grassland to determine the long-term changes in sward structure following the introduction of continuous extensive and intensive grazing or first cut followed by both grazing intensities. Although the use of single species response gives us important information, a functional analysis of vegetation may help to understand and predict the impact of management in more general ways (de Bello et al. 2005; Louault et al. 2005). Therefore, the aim of our study was to answer the following questions: 1. How are plant species composition, plant species diversity, proportion of plant functional groups and potential sward height affected by implementation of different grazing regimes on previously abandoned semi-natural grassland? 2. When do changes in sward structure under different grazing regimes occur?

Material and Methods

Study site

The experiment was undertaken in the Jizerské mountains in the northern Czech Republic, 10 km north of the township of Liberec (50°50' N, 15°06' E). The site is underlain by granite bedrock and medium deep brown soil (cambisol) with the following attributes: pH/KCl = 5.1, C_{ox} =3.9%, available P content = 64 mg.kg^{-1}, available K content = 95 mg.kg^{-1} and available Mg content = 92 mg.kg^{-1}. The altitude is 420 m a.s.l., the mean annual precipitation is 803 mm and the mean annual temperature is 7.2 °C (Liberec meteorological station). The experimental area was drained, ploughed and reseeded with a highly productive grass/clover mixture in the 1980s and was intensively managed by cutting and grazing. In the early 1990s mulching was applied once a year only (in August) and then the grassland was abandoned. There was no agricultural management in the five years before the start of the experiment in 1998. According to the phytosociological nomenclature (Moravec 1995) the vegetation before introduction of experimental treatments was classified as upland hay meadow

(*Arrhenatherion*). The dominant species of the unmanaged sward were *Agrostis capillaris*, *Alopecurus pratensis*, *Festuca rubra* agg., *Aegopodium podagraria* and *Galium album.*

Experimental design

The experiment was established in the spring of 1998. Treatments applied were: intensive grazing (IG), first cut followed by intensive grazing (ICG), extensive grazing (EG), first cut followed by extensive grazing (ECG) and unmanaged grassland (U) as the control. Each grazed paddock was ca. 0.35 ha and the unmanaged control was 0.12 ha. Experimental paddocks were replicated twice in a randomized block design. Descriptions of the treatments applied are shown in Table 1.

Sward height was measured weekly by the first contact method (Pavlů 1997) between 1998-2001 and by the rising-plate meter method (Castle 1976) between 2002-2004. To maintain the target sward height, an additional non-sampling area with the requested sward height was added during the course of the grazing season. All treatments were grazed continuously by young heifers with initial live weights of 150-220 kg in each grazing season. Grazing was applied from early May to late October and the mean productivity of the pasture varied from 2 to 4 $t.ha^{-1}.yr^{-1}$ dry matter.

Plant species composition

Permanent 1 m × 1 m plots were analysed using a continuous grid of nine 0.33 m × 0.33 m subplots in four replications in each paddock. We recorded the proportional cover of all vascular species. Plant cover values in each subplots were visually estimated before the treatments started in early May each year from 1998 to 2004. The mean of nine subplots was used for statistical evaluation. An initial estimate was conducted before the first experimental manipulation to provide baseline data for each plot. Based on descriptions of vascular plants in the regional flora (Kubát et al. 2002), all plant species within the study area were *a priori* categorized according to their main traits. We recognized four categories: short grasses, tall grasses, prostrate forbs (dicot perennial species with creeping or prostrate growth) and tall forbs (Table 2).

Potential height of sward

To reveal the expected relationship between mean height of a particular species and their response to treatments, the mean height of species (Kubát et al. 2002) were weighted according to their cover in a particular relevé. Potential height of sward was defined to generalize reaction of all sward components together and to test the effect of grazing systems on replacement of sward dominants according to their heights. Potential height of sward (instead of actual heights – which define only intensity of grazing) enables us to briefly reveal changes in species composition of sward in relation to grazing treatments.

Data analysis

Repeated measures ANOVA was used to evaluate species diversity and functional group data. The community response was analysed by constrained ordinations. The redundancy analysis (RDA, Lepš & Šmilauer 2003) in the CANOCO package (ter Braak & Šmilauer 2002) followed by a Monte Carlo permutation test was used to evaluate trends in plant species composition. A split plot design was used in the permutation type to cope with repeated measures. We used 999 permutations in all performed analyses. Our data form used repeated observations with the baseline data (measurements performed before the introduction of grazing), thus the interaction of treatments and year were the most important variables. The mean of nine subplots was used for statistical evaluation. A standard biplot ordination diagram as well as principal response curves (PRC) constructed by the CanoDraw program (ter Braak & Šmilauer 2002) was used to visualize the results of the CANOCO analyses. The resulting response curve shows us the extent and directions of development of grassland vegetation under different experimental treatments, compared with the control treatment (Lepš & Šmilauer 2003). The vertical scores of PRC curves are based on the scores of environmental variables from an RDA, the sampling time indicators are used as covariables and the interaction between the treatment levels and sampling times stand as environmental variables. No differences were found between blocks, therefore the effect of block was not taken in account data analysis.

Results

Potential sward height

Before the start of the experiment the mean potential heights of the sward were 64, 71, 63, 71 and 70 cm under the EG, ECG, IG, ICG and U treatments, respectively (Fig. 1). During the first four years of the study, the potential sward height was similar under all studied treatments. Then a gradual decrease under IG, ICG and ECG treatments was recorded whereas under EG and U a slight decrease (P = 0.006, interaction time and treatment). At the end of the experiment, the mean potential heights of the sward were 49, 43, 40, 40 and 56 cm under the EG, ECG, IG, ICG and U treatments respectively.

Plant species richness and composition

Significant differences in the number of plant species as a function of management were recorded (P = 0.011, interaction time and treatment). Similar numbers of species (16.4, 17.5, 17.8, 15.9 and 15.5 per plot in the ECG, EG, ICG, IG and U treatment respectively) were present initially in all treatments (Fig. 2). The total plant species richness increased to at least 20 in all managed treatments over six years. There were significant differences between treatments, as well as successional development, independent of the treatments. (see Table 3, analysis A1 and A2 for details).

Tall forbs (*Aegopodium podagraria, Galium album, Anthriscus sylvestris, Cirsium arvense, Senecio ovatus*) as well as tall grasses (*Elytrigia repens, Alopecurus pratensis*) had higher abundance in the U treatment (Fig. 3). Species associated with both grazed only treatments (IG, EG) were *Dactylis glomerata, Festuca rubra* agg. and *Phleum pratense*. *Agrostis capillaris, Taraxacum* spp.*, Trifolium repens*, *Ranunculus acris* and *Cirsium vulgare*, on the other hand, were supported by both cut (ECG and ICG) treatments. Results of repeated measurements ANOVA analyses showed a significant effect of treatment*year on cover of tall grasses (P = 0.0002), short grasses (P = 0.011), tall forbs ($P < 0.001$) and prostrate forbs ($P < 0.001$).

Decreased abundance of tall grasses, represented mainly by *A. pratensis*, was recorded in all treatments during the run of the experiment (Fig. 4a). The highest decrease was revealed in the IG treatment and the lowest in U treatment. Increased abundance of tall forbs was recorded in the unmanaged control, an opposite trend (Fig. 4b). Substantial cover reduction was revealed in all managed treatments, particularly in both intensively managed plots (IG and ICG). Tall forbs were represented by two dominants, *A. podagraria* and *G. album*. Short grasses, mainly *A. capillaris*, were promoted by all managed treatments, especially by intensive grazing, whereas they decreased in cover in treatment U (Fig. 4c). Prostrate herbs were suppressed by no defoliation management in the control, on the other hand they increased under all defoliation regimes (Fig. 4d). *T. repens* increased cover as a function of defoliation intensity (ICG > IG > ECG > EG), whereas *Taraxacum* spp. was strongly promoted by the first cut followed by both grazing intensities (ICG, ECG). In general, there was a shift from tall to short plant species in all managed treatments, which indicated the change in grassland community. PRC analyses based on RDA shows that diversification in plant species composition created by different defoliation occurred in the fourth year of the study (Fig. 5). All management treatments with negative PRC scores have higher abundance of *Taraxacum* spp., *A. capillaris* and *T. repens*, whereas unmanaged plots become dominated by tall species (*Aegopodium podagraria, Galium album, Alopecurus pratensis, Holcus mollis, Vicia cracca, Cirsium arvense, Hypericum maculatum* and *Urtica dioica)*.

Discussion

Potential sward height

Potential sward height reflected the intensity of management and was an indicator of the replacement of tall dominants by lower growing species. A decrease of potential sward height in intensively defoliated plots indicates more suitable conditions for the development of a pasture community characterized by a high proportion of prostrate forbs and short grasses. Several studies have shown that plant height seems to be the best single predictor of species reaction to intensive defoliation in regularly grazed species (Díaz et al. 2001; Pavlů et al. 2003; Pykälä 2004).

Plant species richness and composition

Seven years of grazing management did not lead to the development of a very species-rich grassland in our study site; however an increase in the number of plant species at a scale of 1 m2 in all managed treatments occurred. Increases in plant species number at a fine scale were probably due mainly to a change in plant distribution within the experimental grassland. The reduction in sward height of the previously unmanaged grassland by the defoliation treatments enabled the spread of species surviving frequently in less productive and disturbed sites only. The increase in plant species richness in managed plots was moderate and potentially due to the limited number of species present in the regional species pool and also due to the relatively short duration of the experiment. The diversification of grassland vegetation has been shown to occur as in Hellströms study (Hellström et al. 2003) after only three years of grazing. However, changes in both cover of presented plant species and in plant densities of all grassland components, especially grass tillers, were revealed after the introduction of grazing management (Pavlů et al. 2006c). The positive effect of grazing on species richness compared to unmanaged grassland is well documented in many other studies (e.g. Bakker 1989; Smith & Rushton 1994; Tasser & Tappeiner 2002; Pykälä 2005; Pavlů et al. 2006b). According to Olff & Ritchie (1998), this is a general effect of large herbivore grazers in temperate grasslands. The response of plant species richness to defoliation was shown in all the managed treatments and this increase was higher than in the unmanaged control. These results indicate the necessity of some kind of defoliation to enable coexistence of the many plant species in semi-natural grasslands.

Plant species composition and functional group

The change in grassland community from *Arrhena-therion* to *Cynosurion* (*sensu* Moravec 1995) manifested mostly as an exchange of dominant species. The dominant tall grass in the unmanaged meadows, *Alopecurus pratensis,* was generally replaced by the short grass species *Agrostis capillaris,* on account of defoliation in all the managed treatments. This is in accordance with several previous studies showing that short grass *A. capillaris* (CRS strategy, Grime 1987) is often promoted by grazing in low productive grasslands (e.g. Hellström et al. 2003; Louault et al. 2005; Pavlů et al. 2006a). The most abundant prostrate dicotyledonous species (*T. repens* and *Taraxacum* spp.) increased in cover in all managed treatments, as in other studies testing intensity or grazing itself (Bullock et al. 2001; Pavlů et al. 2003; Pykälä 2005; Louault et al. 2005). However, this study suggests different strategies of *Taraxacum* spp. and *T. repens.* The common pasture legume *T. repens* was more frequent under both intensively grazed treatments (ICG>IG) at the end of the experiment. The clonal growth of *T. repens* enables it to avoid the defoliation of the majority of the above-ground biomass and to quickly colonize bare ground (Thorhallsdottir 1990). In contrast to *T. repens*, *Taraxacum* spp. became more dominant under both first cut treatments than under grazed only. Postponing cutting to late May or early June allowed *Taraxacum* to reproduce and consequently spread its seeds in both first cut treatments. Higher abundance of *Taraxacum* spp. in ICG and ECG can, therefore, probably be ascribed to higher diaspore production. In this study, both tall dominant forbs (*A. podagraria* and *G. album*) were reduced by grazing and they increased in abandoned grassland.

Conclusion

This study has shown that restoration of grazing management on abandoned mesic grassland altered the plant species composition toward increased proportions of short grasses and prostrate forbs. At the conclusion of the experiment, higher numbers of plant species in all managed treatments in comparison to the unmanaged control indicates the necessity of defoliation management to enable co-existence of many plant species in semi-natural grasslands. Similarly with other experiments studying different grazing intensities (Bullock et al. 2001; Scimone et al. 2004), the effect of defoliation intensity on species richness have been found not to be so straightforward. Potential sward height, a parameter based on plant height according to local flora and cover of present species, is useful to reveal the replacement of tall dominants by short species under defoliation. Simple vegetation traits can predict responses to the different managements. However, they are strongly dependent on the several dominant species, sometimes with miscellaneous responses.

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Table 1. Detailed description of the investigated treatments.

Treatment	Description	Target sward height during grazing	Date of 1st cut	Start of grazing
U	unmanaged control	no grazing	no cut	no grazing
IG	intensive grazing	5 cm	no cut	beginning of May
ICG	1st cut followed by intensive grazing	5 cm	start of June	middle of June
EG	extensive grazing	10 cm	no cut	middle of May
ECG	1st cut followed by extensive grazing	10 cm	start of June	end of June

Table 2. Functional groups of the study sward.

Short grasses	Tall grasses	Prostate forbs	Tall forbs	Others
Agrostis capillaris	*Alopecurus pratensis*	*Alchemilla* spp.	*Achillea millefolium*	*Cardamine pratensis*
Anthoxanthum odoratum	*Dactylis glomerata*	*Anemone nemorosa*	*Aegopodium podagraria*	*Cerastium holosteoides*
Lolium perenne	*Deschampsia cespitosa*	*Campanula patula*	*Anthriscus sylvestris*	*Glechoma hederacea*
Poa annua	*Elytrigia repens*	*Campanula rotundifolia*	*Cirsium arvense*	*Lychnis flos-cuculi*
Poa pratensis	*Festuca pratensis*	*Capsela bursa-pastoris*	*Cirsium palustre*	*Stellaria graminea*
	Festuca rubra	*Leontodon autumnalis*	*Cirsium vulgare*	*Veronica arvensis*
	Holcus lanatus	*Plantago lanceolata*	*Galium album*	*Veronica chamaedrys*
	Holcus mollis	*Plantago major*	*Heracleum sphondylium*	
	Phleum pratense	*Ranunculus acris*	*Hypericum maculatum*	
	Poa trivialis	*Ranunculus repens*	*Lathyrus pratensis*	
	Trisetum flavescens	*Taraxacum* spp.	*Leucanthemum vulgare*	
		Trifolium dubium	*Lotus uliginosus*	
		Trifolium pratense	*Rumex acetosa*	
		Trifolium repens	*Rumex obtusifolius*	
		Veronica serpyllifolia	*Senecio ovatus*	
			Urtica dioica	
			Vicia cracca	

Table 3. Results of the RDA analyses of cover estimates. IG, ICG, EG, ECG, U = treatments abbreviations, see Tab. 1; PlotID = plot identifier; % expl. variability = species variability explained by one (all) ordination axes (measure of explanatory power of the explanatory variables); F – ratio = F statistics for the test of particular analysis; P - value = corresponding probability value obtained by the Monte Carlo permutation test; Int =grazing intensity. Tested null hypotheses: A1 There are no directional changes in time in the species composition, that are common to all the treatments or specific for particular treatments; A2 The temporal trend in the species composition is independent of all treatments.

Analysis	Explanatory variables	Covariables	% expl. 1^{st} (all) axis	F ratio 1^{st} (all) axis	P 1^{st} (all) axis
A1	Year,Year*IG, Year*EG, Year*ICG, Year*ECG, Year*U	Plot ID	31.4 (37.7)	107.59 (28.89)	0.001 (0.001)
A2	Year*IG, Year*EG, Year*ICG, Year*ECG, Year*U	Year, Plot ID	11.7 (17.2)	31.28 (12.19)	0.001 (0.001)

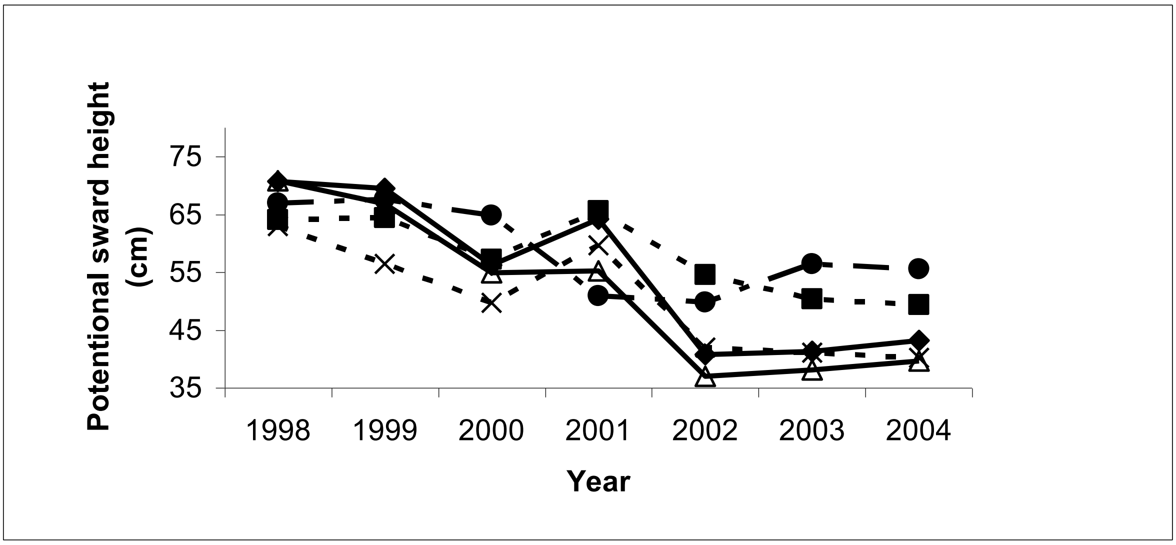


Fig. 1. Potential sward height for different treatments ECG (◆),EG (■), ICG (△), IG (X), U (●) between 1998-2004. Standard errors are indicated by the vertical lines.

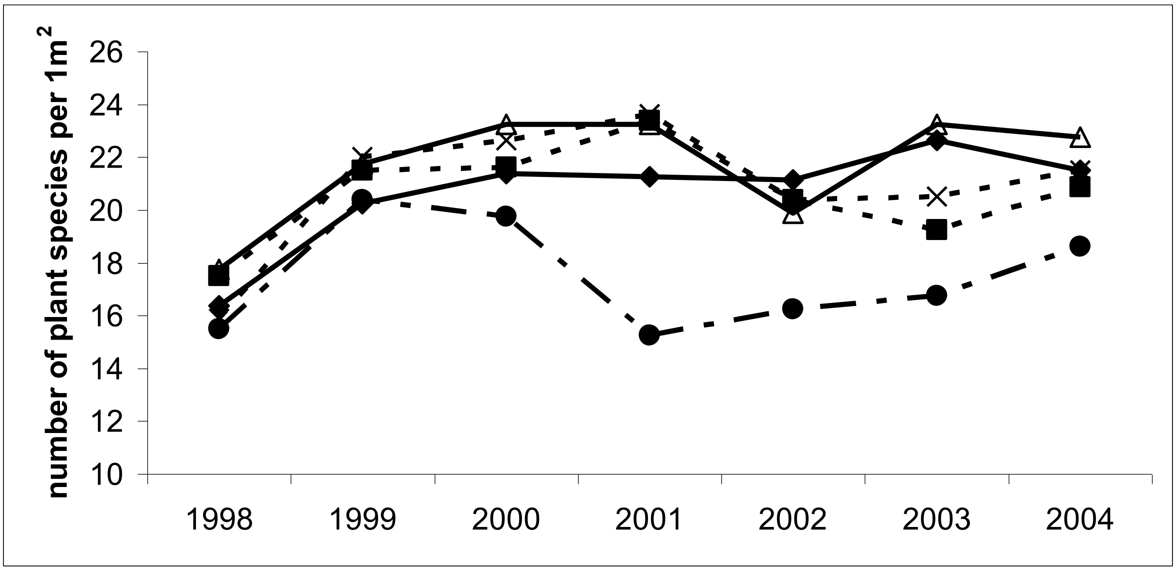


Fig. 2. Number of plant species per 1m ^{2} for different treatments ECG (◆),EG (■), ICG (△), IG (X), U (●) between 1998-2004. Standard errors are indicated by the vertical lines.

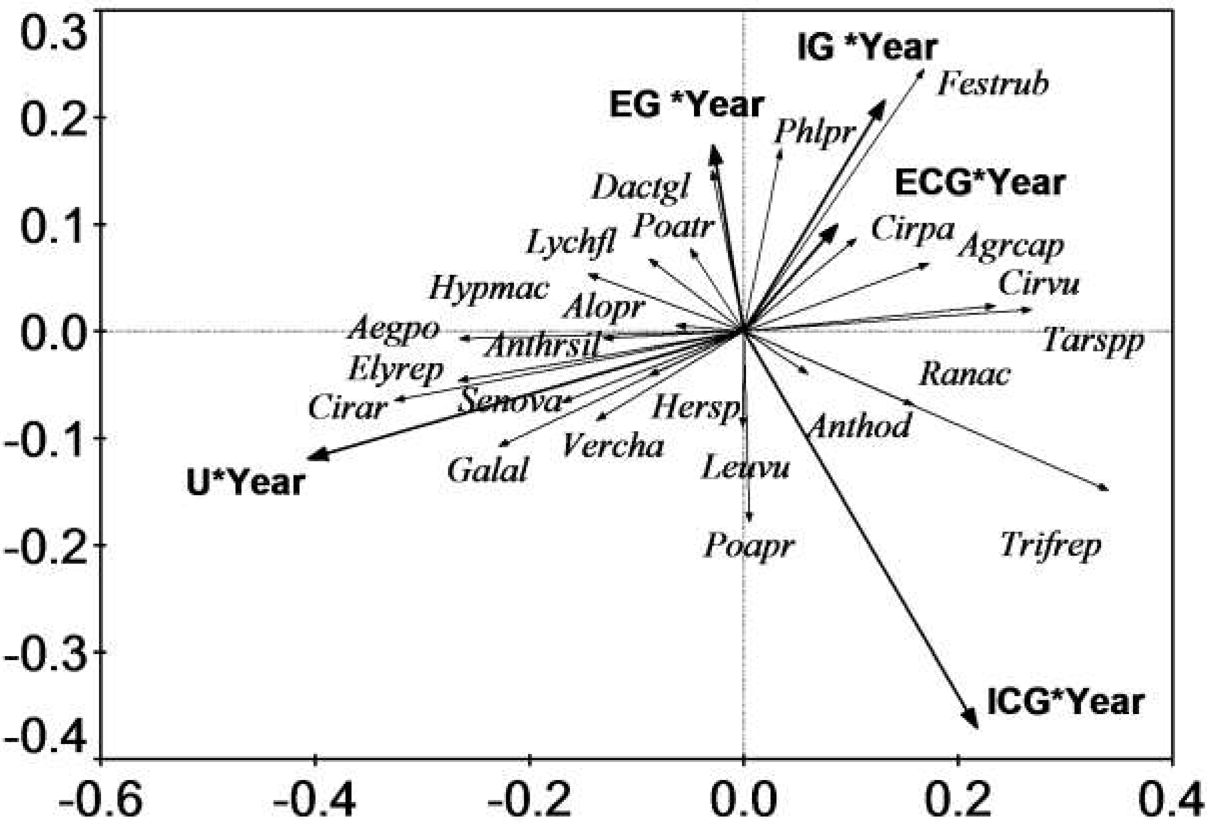
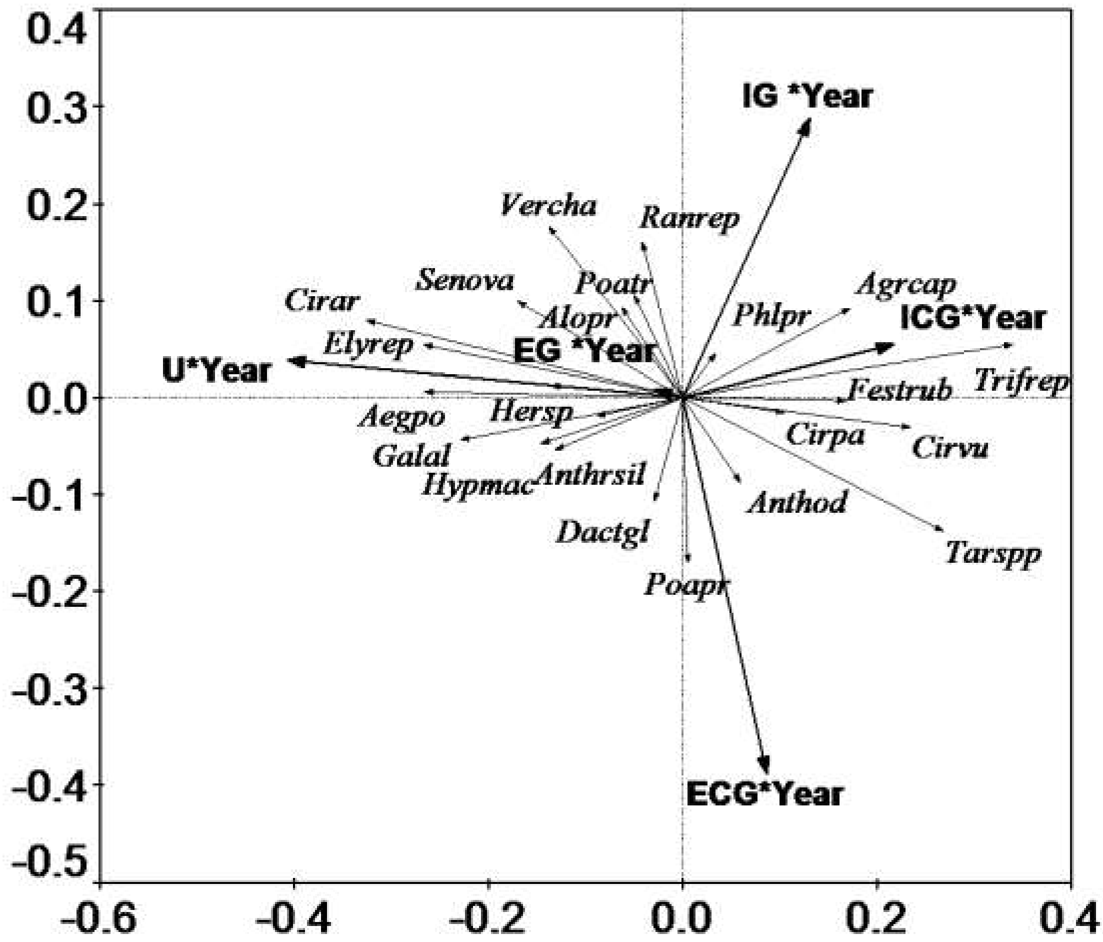


Fig. 3. Ordination diagram showing the result of RDA analysis

a) first and second axis

Abbreviations: IG, ICG, EG, ECG and U treatments (see Tab. 1); * indicates interaction of environmental variables; Aegpo-*Aegopodium podagraria*, Agrcap- *Agrostis capillaris*, Alopr-*Alopecurus pratensis*, Anthod-*Anthoxantum odoratum*, Anthrsil-*Anthriscus silvestris*, Cirar-*Cirsium arvense*, Cirpa-*Cirsium palustre*, Cirvu-*Cirsium vulgare*, Dactgl-*Dactylis glomerata*, Elyrep-*Elytrigia repens*, Festrub-*Festuca rubra*, Galal-*Galium album*, Hersp- *Heracleum sphondylium,* Hypmac-H*ypericum maculatum,*Leuvu- *Leucanthemum vulgare*, Lychfl- *Lychnis flos-cuculi*, Phlpr-*Phleum pratense*, Poapr-*Poa pratensis*, Poatr-*Poa trivialis*, Ranac-*Ranunculus acris*, Ranrep-*Ranunculus repens*, Senova-*Senecio ovatus*, Tarspp-*Taraxacum* spp., Trifrep-*Trifolium repens*, Vecha-V*eronica chamaedrys*.



b) first and third axis

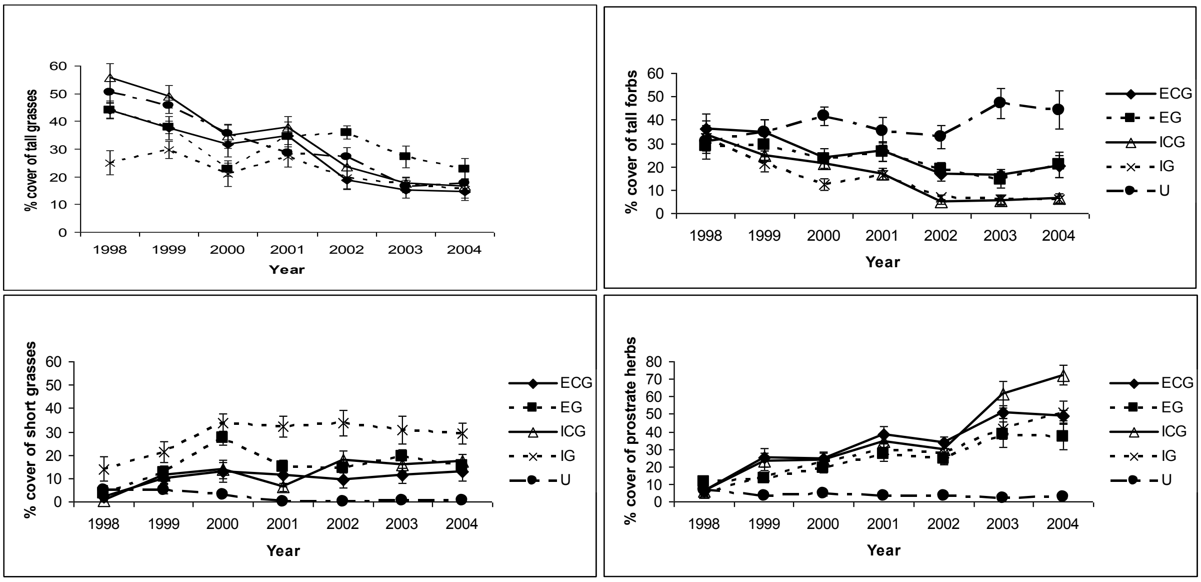


Fig. 4. Changes in cover (%) of tall grasses, short grasses, tall forbs and prostrate forbs for different treatments ECG (◆),EG (■), ICG (△), IG (✕), U (●)between 1998-2004. Standard errors are indicated by the vertical lines.

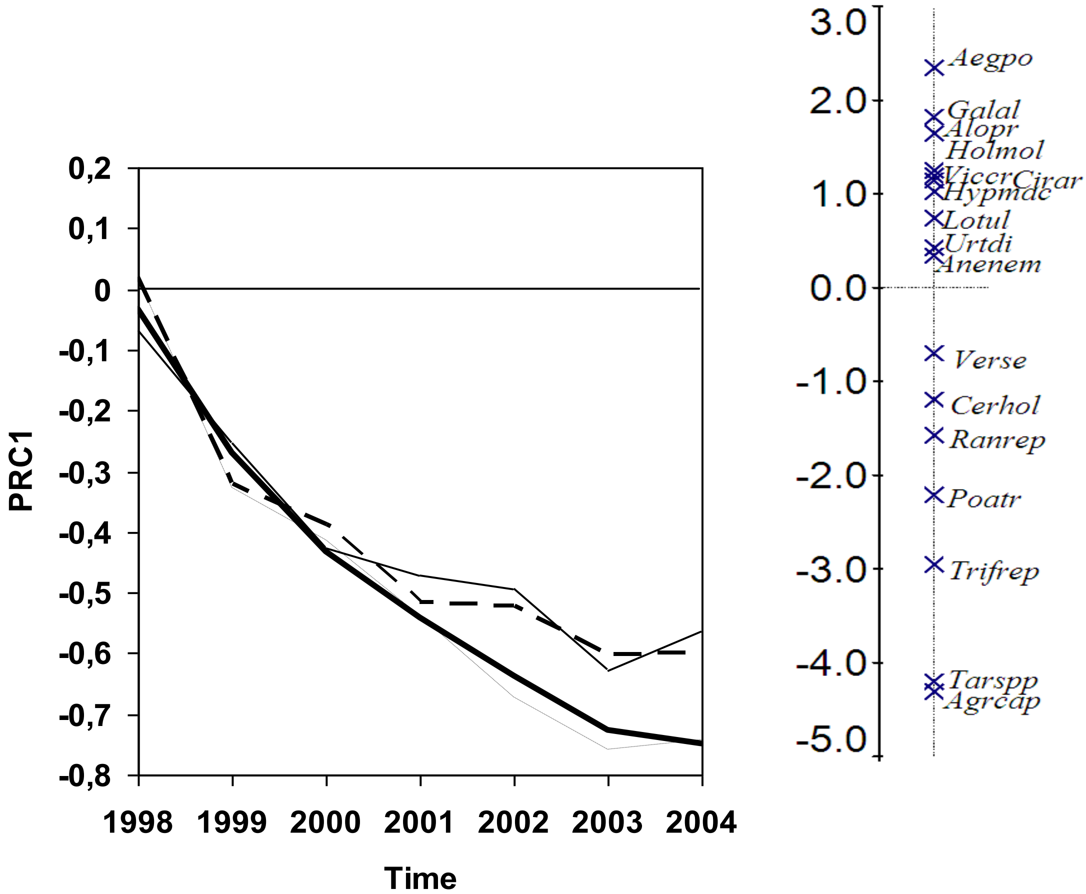


Fig. 5. Principal response curves for different treatments ECG (_ _ _ _),EG (____), ICG (..........), IG (**____**) during the experiment. The unmanaged treatment (U) was taken as a reference (zero control) treatment.

Chapter 6

Effect of different defoliation practices on weeds in an upland meadow

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Urtica dioica in abandoned plot (left) and cut meadow (right)

Effect of different defoliation practices on weeds in an upland meadow
Effekt unterschiedlichen Managements auf einige Unkrautarten in Hochlandwiesen

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Summary
The effect of different defoliation management on the occurrence of weed species was studied in upland meadow in the Jizerské Mountains from 2000 to 2007. The following treatments were applied: cutting twice a year with biomass removal, mulching from 1 to 3 times a year and no management. The semi-natural grassland had been occasionally grazed or cut before the start of the experiment. During the experiment, tall dicotyledonous species such as *Anthriscus sylvestris*, *Cirsium arvense* and *Urtica dioica* as well as the grass weed *Elytrigia repens* were promoted by no management and mulching once a year. On the other hand, prostrate species, namely *Taraxacum* spp., responded positively to a higher frequency of defoliation and disappeared under no management. *Aegopodium podagraria* spread in plots mulched twice or three times a year. Plant species number increased in treatments consisting of cutting twice and mulching twice or three times; it decreased where no management was applied. Mulching performed at least twice a year is suitable for non-chemical restriction of undesirable weeds such as *Cirsium arvense*, *Urtica dioica* or *Elytrigia repens*, but supports an increase of *Aegopodium podagraria*.

Key words: *Cirsium arvense*, *Elytrigia repens*, grassland, redundancy analysis, *Urtica dioica*, weed control

Zusammenfassung

Der Einfluss eines unterschiedlichen Managements auf die Verbreitung von Unkrautarten wurde in Hochlandwiesen im Jizerskégebirge von 2000 bis 2007 untersucht. Die folgenden Behandlungen wurden ausgeführt: zweifache Schnittnutzung pro Jahr mit Entfernen der Biomasse, 1- bis 3-maliges jährliches Mulchen und Nutzungsaufgabe. Vor Beginn der Studie war das halbnatürliche Grasland gelegentlich beweidet oder gemäht worden. Im Laufe des Experiments wurden hohe Krautartige wie *Anthriscus sylvestris*, *Cirsium arvense* und *Urtica dioica* sowie das Ungras *Elytrigia repens* durch Nutzungsaufgabe und einmaliges Mulchen gefördert. Andererseits reagierten niedrige Arten, vor allem *Taraxacum* spp., positiv auf höhere Entblätterungsfrequenzen und verschwanden auf ungenutzten Flächen. *Aegopodium podagraria* verschwand von Flächen, die zwei- bis dreimal jährlich gemulcht wurden. Die Artenanzahl steigerte sich auf Flächen, die zweimal gemäht oder zwei- oder dreimal gemulcht wurden und verringerte sich auf ungenutzten Flächen. Mindestens zweimaliges Mulchen pro Jahr eignet sich, um ohne Herbizideinsatz ungewünschte Unkräuter wie *Cirsium arvense*, *Urtica dioica* oder *Elytrigia repens* zu verdrängen, aber es förderte die Ausbreitung von *Aegopodium podagraria*.

Stichwörter: *Cirsium arvense*, *Elytrigia repens*, Wiesen, Redundanz Analysis, Unkrautkontrolle, *Urtica dioica*

I Introduction

In marginal areas of the Czech Republic, the importance of semi-natural grasslands for forage production decreased due to reduction of cattle in the last decade of the 20th century. Large areas of the meadows and pastures were abandoned. Dicotyledonous species, namely undesirable perennial weeds such us *Cirsium arvense, Elytrigia repens, Urtica dioica* or *Rumex obtusifolius*, often start to predominate in mesic unmanaged grassland (KNEIFELOVÁ and MIKULKA 2003; PAVLŮ et al. 2005).
Mulching is a simple and low-cost procedure that can help maintain many grassland locations and fallow farm land (PROCHNOW et al. 2000). However, rapid decomposition of the mulch is important, because a long cover and shading of the sward will cause unwanted changes in grassland composition. ZELENÝ et al. (2001) reported that mulching can avoid predomination of undesirable tall herbs. GAISLER et al. (2004) compared the effect of different mulching and cutting regimes on vegetation of an upland meadow and concluded that mulching is not able to restrict highly competitive dominant herbs if performed once per year only in late summer or in autumn. The effect of different management including mulching on the plant functional traits is described in the study of KAHMEN et al. (2002). After 25 years of management, the treatments mowing and mulching resulted in a species composition similar to that from grazing by sheep. MOOG´S et al. (2002) concluded that mulching twice a year was found to be suitable for the conservation of unimproved, species-rich grasslands.
The aim of this paper was to describe the effect of mulching with different frequency on the occurrence of weedy species. Mulched treatments were compared with the traditional two cut system and with no management.

2 Materials and methods

2.I Study site and design of experiment

The experiment was carried out in the Jizerské Mountains (50°51’N, 15°02’E) 12 km north-west from the town Liberec, Czech Republic. The altitude of the study site is 450 m ASL, mean annual temperature is 7.2 °C and mean annual precipitation is 803 mm (Liberec meteorological station). The geological substratum is granit. The grassland had been occasionally grazed or moved during five years before the start of the experiment. The mean forage yield of the meadow ranged from 3-6 t ha^{-1} of dry matter per year over the experimental period 2000-2007), no fertilisers had been applied on the site. The plant community of the study site was classified as *Arrhenatherion*. Dominant plant species before establishment of the experiment were *Dactylis glomerata, Festuca pratensis, Elytrigia repens, Aegopodium podagraria* and *Veronica chamaedrys*.
In spring 2000, the experiment was established in four complete randomised blocks with the following treatments:

- **2C** Two cuts a year with removal of biomass performed in 1st decade of June and in the mid of August
- **U** Unmanaged control treatment
- **1M** Mulching once a year in July
- **2M** Mulching twice a year in June and August
- **3M** Mulching three times a year in May, July and September

2.2 Data collection and analyses

Individual plot size was 10 x 5 m, but only the central 8.0 x 3.0 m plot was used for data collection to avoid edge effect. The cover of all plant species present was estimated visually directly in percentages. Data collection was performed annually in May before application of any defoliation management. To obtain baseline data, the initial estimation of plant cover was performed before the first experimental manipulation in 2000. Nomenclature of plant species follows KUBÁT et al. (2002). Redundancy analysis (RDA) with the CANOCO program (TER BRAAK and ŠMILAUER 1998) was used to evaluate the multivariate vegetation data. Redundancy analysis is a direct gradient analysis method based on the assumption of a linear response and it was used because the data set was relatively homogeneous. In all analyses we used centering by species. Blocks were defined by covariables. In direct gradient analysis, various combinations of environmental variables, covariables and their interactions can be used with an appropriate Monte Carlo permutation test to test a wide range of hypotheses (TER BRAAK and ŠMILAUER 1998). Our data are in the form of repeated measures; using the permutation scheme adjusted to the repeated measures design provided an opportunity to test particular effects in a way directly comparable to repeated measures ANOVA. We used a split plot design (main plot factor is the permanent plot repeated in time. Subplot factor is the plot at a given year) and permutations were performed within each block of plots. Whole plots were records of one permanent plot repeated in time and were permuted completely at random. Split plots were not permuted. The significant effect of time and treatment interaction indicates divergent temporal development of plots under different management. The biplot ordination diagram, constructed by the CANODRAW program (TER BRAAK and ŠMILAUER 1998), was used to visualize the results of the analysis.

3 Results

During the eight years of the experiment, significant changes in plant species composition were recorded under different treatment regimes (Table 1).

Several species and functional groups responded differently to application of defoliation management. The proportion of grasses decreased in all the treatments, from roughly 60 % to 40 %, most in twice cut (2C), twice mulched (2M) and three times mulched (3M) (Fig. 1). No management (U) and the mulching performed once a year (1M) resulted in the development of the tall forbs functional group. In contrast to tall forbs, species with prostrate growth were supported by treatments where the defoliation was performed twice or three times a year (Fig. 1). *Aegopodium podagraria, Anthriscus sylvestris, Cirsium arvense, Galium album* and *Urtica dioica* were weedy plant species representing the functional group of tall forbs. Prostrate herbs were represented mainly by *Taraxacum* sp., *Trifolium repens*, *Ranunculus repens*, *Plantago lanceolata* and *Cerastium holosteoides*.

The effect of different treatments on successional changes of the vegetation is visible from the ordination diagram (Fig. 2). No management (U) most promoted tall weed species such as *Urtica dioica*, *Cirsium arvense* and *Anthriscus sylvestris*. On the other hand, prostrate species *Taraxacum* sp. and *Ranunculus repens* reached highest cover in 2M a 3M treatments.

Cover of *Elytrigia repens* decreased in all treatments, most in 2C, 2M and 3M. This grass weed temporarily spread again in 2005, most in U and 1M treatments. Cover of *Cirsium arvense* increased from 2 % to 10 % and 6 % in U and 1M treatments respectively and in the last year its abundance decreased to 3%. On the contrary, cover of *C. arvense* continuously decreased in 2C treatment, where this species almost disappeared (Fig. 3). Cover of this species was relatively constant in 2M and 3M treatments.

Cover of *Aegopodium podagraria* was lowest in the 2C treatment, whereas this species spread in all mulching variants and in the U treatment. We further recorded an increase in cover of *Anthriscus sylvestris* in the U, 1M and 2M treatments, while its cover was almost negligible in 2C and 3M treatments.

Cover of *Galium album* increased from 3 to 16 % in the 2C treatment during the experiment. High cover of *G. album* was recorded also in once mulched treatment, where it reached 18 % in 2005. *Urtica dioica* spread in unmanaged grassland from 1 to 7 % whereas in other treatments its cover was low.

Prostrate herbs had increased cover in all treatments with higher frequency of defoliation, while their cover was low in the 1M and U treatments. The cover of *Taraxacum* sp. increased from 2 to 7 % in the 2M and even from 2 to 13 % in the 3M treatment. A slight decrease of its cover was found in the U treatment.

4 Discussion

Weed species with tall growth form such as *Cirsium arvense, Urtica dioica* and *Elytrigia repens* are highly competitive in a sward without regular defoliation and these species seem to be sensitive to frequent defoliation. Cutting or mulching frequency is a more important factor than nutrient removal, although the above mentioned species are well known for their high demands for nutrients (KNEIFELOVÁ and MIKULKA 2004). In the present study, *Cirsium arvense* spread in the unmanaged treatment. This result accords with the findings of KASPERCZYK and SZEWCZYK (1999) who recorded an increase in cover of *C. arvense* on the abandoned meadow.

In the treatment mulched once a year, we found similar trends in cover changes of most of the tall weeds as in unmanaged plots. Yet, mulching once a year would not be suitable at all for the conservation of a traditional cultural landscape with high biodiversity; this management is appropriate to prevent shrubs and forest formation only (BRIEMLE 2005).

PŘIKRYLOVÁ (2006) reported a high effect of a double removal of aboveground biomass on restriction of *Aegopodium podagraria*. This is consistent with the results of this study where a decrease in cover of *A. podagraria* was recorded in the 2C treatment. On the other hand, we found the highest cover on twice and three-times mulched plots. This was caused probably by high disturbance; wild boars entered and grouted on these plots in 2005.

Cutting or mulching with higher frequency promoted prostrate herbs as is evident from the enhancement of *Taraxacum* sp. This is in accordance with results of GAISLER et al. (2004), where an increase of prostrate herbs as a function of defoliation frequency was described on a low productive upland meadow. Cutting and mulching have positive effects on the species richness in comparison with no management due to restriction of competition from tall dominants, often undesirable weedy plant species.

Acknowledgements

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Tab. 1: Results of the RDA analyses of cover estimates.

Tab. 1: Ergebnisse der RDA-Analyse der Deckungsgradschätzungen.

Analysis	Explanatory variables	Covariables	% expl. 1st (all) axis	F-ratio 1st axis (all axes)	P 1st axes (all axes)
A1	Year, Year*2C, Year*U, Year*1M, Year*2M, Year*3M	Plot ID	7.9 (13.6)	23.882 (9.390)	0.0320 (0.001)
A2	Year*2C, Year*U, Year*1M, Year*2M, Year*3M	Year, Plot ID	3.9 (5.8)	12.806 (4.983)	0.001 (0.001)

1 2C, U, 1M, 2M, 3M = treatments abbreviation, see chapter Material and methods;

* interaction.

2 Plot ID = plot identifier.

3 % expl. = species variability explained by one (all) ordination axis (axes) (measure of explanatory power of the explanatory variables).

4 F-ratio = F statistics for the test of particular analyses; P = corresponding probability value obtained by the Monte Carlo permutation test.

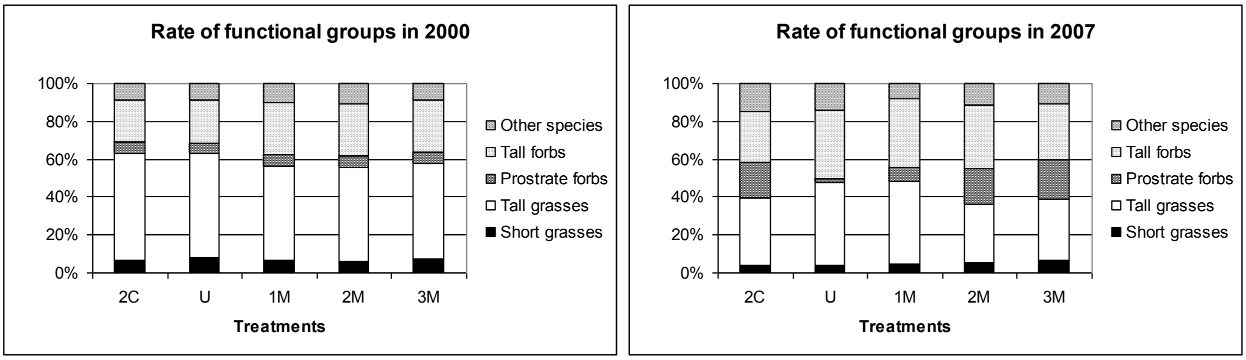

Fig. 1: Cover of functional groups in 2000 (a) and 2007 (b).

Abb. 1: Deckungsgrad der Funktionsgruppen in 2000 (a) und 2007 (b)

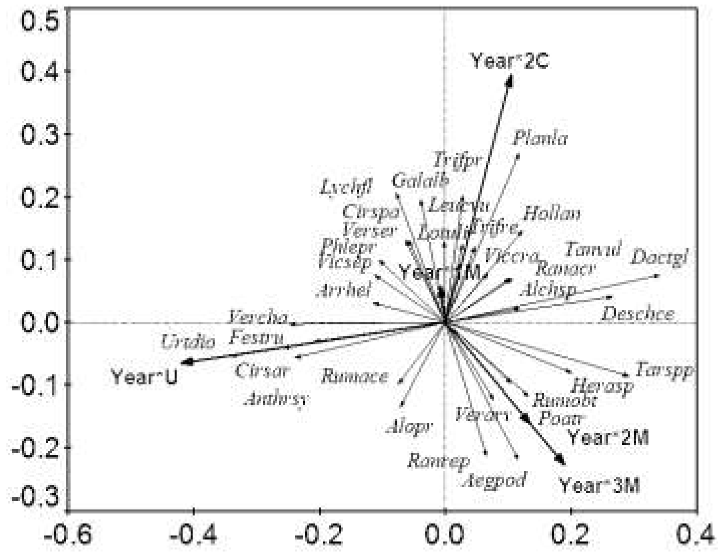

Fig. 2: Ordination diagram showing the results of the redundancy analysis. First and second axis.
Abb. 2: Ordinationsdiagramm der Ergebnisse der RDA-Analyse. Erste und zweite Achse.
Abbreviations: 2C, U, 1M, 2M, 3M treatments (see Materials and methods); * indicates interaction of environmental variables; AEOPO = *Aegopodium podagraria*, ALCSS = *Alchemilla* spp., ALOPR = *Alopecurus pratensis*, ANRSY = *Anthriscus sylvestris*, ARREL = *Arrhenatherum elatius*, CHYLE = *Leucanthemum vulgare*, CHYVU = *Tanacetum vulgare*, CIRAR = *Cirsium arvense*, CIRPA = *Cirsium palustre*, DACGL = *Dactylis glomerata*, DECCA = *Deschampsia cespitosa*, FESRU = *Festuca rubra*, GALAL = *Galium album*, HERSP = *Heracleum sphondylium*, HOLLA = *Holcus lanatus*, LOTUL = *Lotus uliginosus*, LYHFC = *Lychnis flos-cuculi*, PHLPR = *Phleum pratense*, PLALA = *Plantago lanceolata*, POATR = *Poa trivialis*, RANAC = *Ranunculus acris*, RANRE = *Ranunculus repens*, RUMAC = *Rumex acetosa*, RUMOB = *Rumex obtusifolius*, TARSS = *Taraxacum* sp., TRFPR = *Trifolium pratense*, TRFRE = *Trifolium repens*, URTDI = *Urtica dioica*, VERAR = *Veronica arvensis*, VERCH = *Veronica chamaedrys*, VICCR = *Vicia cracca*, VICSE = *Vicia sepium,* 1VERG = *Veronica serphyllifolia.*

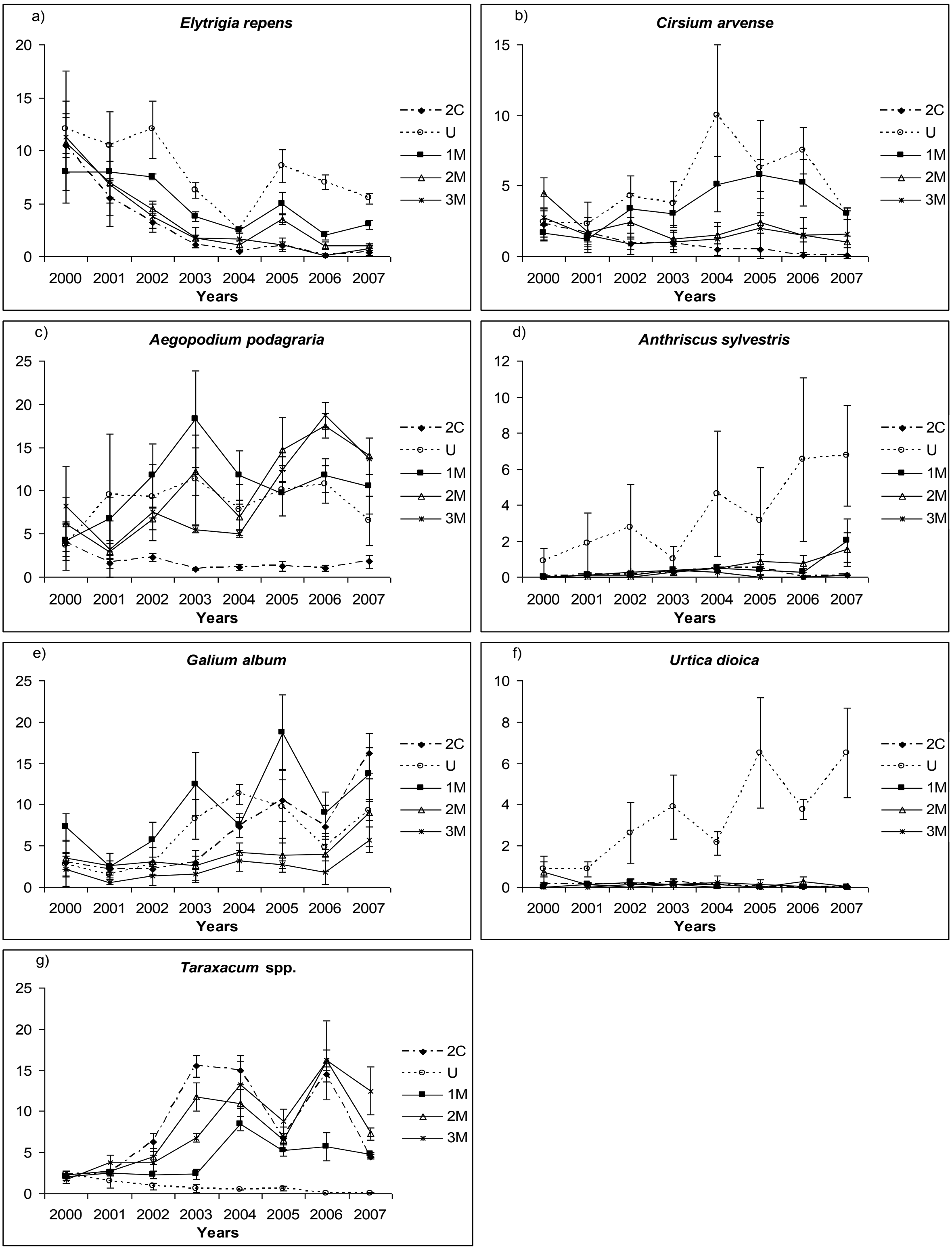

Fig. 3: Cover (%) of seven weed species over the 2000-2007 period under different treatments: a) Elytrigia repens*, b)* Cirsium arvense, *c)* Aegopodium podagraria, *d)* Anthriscus sylvestris, *e)* Galium album, *f)* Urtica dioica *and g)* Taraxacum spp. *Vertical bars represent SE.*

Abb. 3: Deckungsgrad (%) von Sieben Unkrautarten in unterschiedlichen Behandlungen (2000-2007): a) Elytrigia repens, *b)* Cirsium arvense, *c)* Aegopodium podagraria, *d)* Anthriscus sylvestris, *e)* Galium album, *f)* Urtica dioica *und g)* Taraxacum spp.

Chapter 7

Rare species of waxcaps (*Hygrocybe*) in Liberec region

Jan Gaisler, Vilém Pavlů & Lenka Pavlů

The manuscript

Hygrocybe spadicea

Hygrocybe citrinovirens

Rare species of waxcaps (*Hygrocybe*) in Liberec region

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Abstract
Thirty four members of genus *Hygrocybe* were found and identified predominantly in the grasslands of Liberec region during the study period (2007-2012). Apart from common species we found also ten waxcaps which are recorded in Red list of fungi (macromycetes) of the Czech Republic in the category of critically endangered: *Hygrocybe collemaniana*, *H. fornicata*, *H. ingrata*, *H. intermedia*, *H. laeta*, *H. mucronella*, *H. nitrata*, *H. ovina*, *H. reidii* and *H. spadicea*. In case of *H. spadicea* it is only recent record in Czech Republic nowadays. As well as there were recorded five species in the category of endangered, two species in the category of near threatened and five species in the category of fungi with data deficient. Furthermore there were found some waxcap species which are rare but has not been recorded in current red list yet, namely *Hygrocybe splendidissima*, *H. citrinovirens*, *H. flavipes*, *H. quieta* and *H. subpapillata*. Because of number and rarity of some waxcap species the meadow of the Liberec region belongs to very important localities in the Czech Republic.
Keywords: meadows; mosses; fungi; waxcaps; red list; biodiversity

Abstrakt
Během průzkumu v letech 2007 - 2012 bylo v převážně travinných porostech Libereckého kraje nalezeno a určeno 34 taxonů rodu voskovka (*Hygrocybe*). Kromě běžnějších druhů zde bylo zaznamenáno deset voskovek, které jsou zapsány v Červeném seznamu hub (makromycetů) České republiky v kategorii kriticky ohrožených, a to *Hygrocybe collemaniana*, *H. fornicata*, *H. ingrata*, *H. intermedia*, *H. laeta*, *H. mucronella*, *H. nitrata*, *H. ovina*, *H. reidii* a *H. spadicea*. U *H. spadicea* se v současnosti jedná o jediný recentní nález v ČR. Dále zde bylo zaznamenáno pět druhů z kategorie ohrožených, dva druhy z kategorie hub téměř ohrožených a pět druhů z kategorie hub, o kterých je nedostatek údajů. Také zde byly nalezeny druhy, které jsou rovněž vzácné, ale nejsou zapsány v dosud platném červeném seznamu, a to *Hygrocybe splendidissima*, *H. citrinovirens*, *H. flavipes, H. quieta* a *H. subpapillata*. Některé voskové louky v Libereckém kraji patří z hlediska počtu, popř. vzácnosti druhů mezi velmi významné lokality v rámci celé České republiky.
Klíčová slova: louky; mechy; houby; voskovky; červený seznam; biodiverzita

Introduction

Showy fungi of rather large genus wax-caps (*Hygrocybe*) can grow on the unfertilised semi-natural meadows and pastures under specific conditions. Several tens of species with conspicuously coloured caps or whole fruitbodies belong to the genus. The most frequent colours are red, orange or yellow. These fungi are not only decoration of our meadows and pastures but their presence implies high quality of semi-natural grasslands without negative effect of previous intensive management. In the second half of 20th century majority of semi-natural grasslands was affected by agriculture intensification which consisted especially of drainage of wet meadows or fertilisation of oligotrophic meadows by the inorganic fertilizers. As well as grasslands were often ploughed and reseeded by high productive grass-legume mixtures (Gaisler et al., 2008). Generally the waxcaps have the high sensitivity to any intensification treatment and its effect can last many decades. On the other hand *Hygrocybe conica* and *H. virginea* belong to most spread waxcaps because of their low sensitivity to previous intensive grassland management. These species can have be revealed on previously intensively managed sites already after ten to fifteen years. However the majority of *Hygrocybe* genus are very intolerant to high content of plant available nutrients in soil, especially phosphorus (McHugh et al., 2001) therefore they need quite long period focused on nutrient depletion from soil by biomass removal. For example *H. splendidissima*, *H. intermedia* and *H. spadicea* belong to these most sensitive waxcaps. Not only intensive management but as well as massive abandonment of meadows and pastures management as consequence with reduction of cattle herds after 1990 (Gaisler et al., 2013) cause decrease of potentially suitable stands. The high proportion of grasslands remained without any management and was overgrown by shrubs and trees and therefore a lot of waxcaps species are nowadays very rare in nature of The Czech Republic. A lot of *Hygrocybe* were categorized in Red list of fungi (macromycetes) of the Czech Republic (Holec et Beran, 2006). In many foreign countries the distribution of waxcaps and other grassland fungi are intensively studied. There are a lot of studies focusing on mapping of fungi species indicating preservation value of stands from fungi biodiversity point of view especially on sites with presence of waxcaps (*Hygrocybe*), pinkgills (*Entoloma*), clavarioid fungi (*Clavariaceae*) and earth-tongues (*Geoglossaceae*) (e.g. Nitare 1988, McHugh et al. 2001, Newton et al. 2003, Adamčík et Kautmanová, 2005) The ecology and diversity of Waxcap fungi were summarized in review of Griffith et al (2002). However in the Czech Republic monitoring and protection of grassland fungi are very extensive and recent studies are very rare (Antonín et al. 2010, Holec et al. 2008, Egertová et Kříž 2009, Holec et Adamčík 2008).

Relatively high number of *Hygrocybe* members is still present in Jizerské hory Mts. and some places of the Liberec region, especially meadows around Jizerka settlement belong to richest ones in Czech Republic. Thefore there was carried out an intentional study during the period 2007 to 2012. The aim of the study was the monitoring of fungi species indicating well-preserved state of grassland ecosystems. The paper is focused especially on waxcap species occurring in Liberec region listed in current red list as critical endangered and on the other hand rare species which is missing in the list.

Material and methods

Monitoring was carried out on the selected semi-natural grasslands in Liberec region during period 2008 to 2012 (Fig. 1). The study was based on repeated visits of selected sites in irregular intervals (according weather conditions after 2-4 weeks) from July to November every year. Grasslands were systematic reviewed and all presented fungi species from indicating groups (*Hygrocybe*, *Entoloma*, *Clavariaceae*, *Geoglossaceae*) were recorded. The specimens of problematic species were collected for the determination by the macro- and micro-characteristics. Drying specimens are deposited in mycological collections of National Museum in Prague or private herbarium of corresponding author. Nomenclature follows Index Fungorum (2013).

List of localities with occurrence of critically endangered waxcaps:

1. Jizerka - cut meadows in Nature preserve “Bukovec” on the slight S slope with altitude 890 to 920 m. Bedrock is nefelinit (basalt), in lower parts of locality granit, some parts are overlapped by the clayish-stony sediments. Study area includes annually cut meadows and also grasslands with alternating management (cut every second to third year). Grasslands are formed by the different communities and associations. On the parts with lower soil moisture are some association *Polygono-Trisetion*, for example *Geranio-Trisetetum* and *Meo athamantici-Cirsietum heterophylli*. Also *Violion caninae* is occurred and on the wet stands we can differ *Junco filiformi – Polygonetum*.
2. Rašovka - cut meadows east of village on the SE slope with altitude 605 to 615 m. Bedrock is bazaltandezit. Vegetation is formed by the *Arrhenatherion* meadow with lower moss coverage.
3. Rašovka - cut meadows west of village on the W slope with altitude 575 to 600 m. Bedrock is bazaltandezit. Vegetation is formed by the *Arrhenatherion* meadow with well developed moss floor, which cover is almost 100 % on some places.
4. Sychrov – extensively managed plain lawn in castle park with altitude 381 m. Bedrock is loess-clay. There were dominated prostrate members of *Asteraceae* as *Leontodon hispidus*, *Hieracium pilosella*, *Hypochoeris radicata* and among grasses especially *Agrostis capillaris*, on some places moss floor is well developed.
5. Bredovská zahrada - extensively managed lawn with altitude 361 m. Bedrock is sediment - till. There were dominated prostrate species as *Plantago lanceolata*, *Leontodon hispidus*, *Hypochoeris radicata* among grasses especially *Agrostis capillaris*, on some places moss floor is well developed.
6. Kryštofovo údolí “Rokytnice”- (east part of village, near margin of National natural preserve “Karlovské bučiny”, on the right stream bank with altitude 350 m. There is cut meadow with dominancy of *Plantago lanceolata*, *Leontodon hispidus*, *Dactylis glomerata* and *Cynosurus cristatus*, moss floor is well developed. Bedrock is sand-clay sediment, slope on the both sides of stream is formed by the crystalline limestone.
7. Panenská hůrka - NW part of Ještěd ridge between “Buk Republiky” and “Roimund”, mixed forest on NE slope with altitude 600 m. Bedrock is phyllite slate.

Results and discussion

On selected localities in Liberec region we found and determined ten *Hygrocybe* species registered in Red list of macromycetes (Holec et Beran 2006) as critically endangered (CR) during the study period 2007-2012. We recorded also four species from category endangered (EN), two species from category near threatened (NT) and five species with data deficient (DD) (Tab. 1).

Except commonly spread waxcaps as *H. virginea*, *H. conica* or *H. psittacina* we found also species, which are rare but they are not recorded in current red list. They are especially *H. splendidissima*, *H. citrinovirens*, *H. quieta* and *H. flavipes*. List of observed localities is in Tab. 2, where stands with occurrence only one of commonest species such as H. conica and H. virginea are not mentioned. The majority of localities are cut meadows with well developed moss floor that is in accordance with review of Griffith et al. (2002). We recorded some indication fungi also in forest stands, but except locality “Hamrštejn” there were sporadic findings only.

Species of CR category

There are mentioned all determined species from category CR in following overview, there are also description of found fruitbodies, data of finding, distribution in past and present, advice for prepared red list of the Czech Republic.

Toasted Waxcap (voskovka Colemannova) – *Hygrocybe colemanniana* (A. Bloxam) P.D. Orton & Watling (1969)

The waxcap was found on cut meadow near Kryštofovo údolí in part “Rokytnice” (leg. M. Kříž). Fruitbodies were upto 5 cm high, light brown caps were with relatively darker radial stripes on light margins and gills were deep convergent. In the past this species was recorded on seven localities in the Czech Republic, in the last period two findings exist from 2007 in Bílé Karpaty Mts. and next data are known from Vyšenské kopce and Orlické hory Mts. Due to low number of localities in the Czech Republic we should recommend this waxcap keep in red list in CR category.

Bitter Waxcap (voskovka hořká) – *Hygrocybe mucronella* (Fr.) P. Karst. (1879)
This species was found in the garden of "Bredovský letohrádek" near castle Lvová on unfertilised cut grassland with well developed moss floor (Egertová et Kříž 2009). This smaller waxcap is characteristic of dry red-orange cap, bright widely connected gills, smooth orange stem, irregular form of spores and bitter taste. In the past the *Hygrocybe* was not almost recorded, one specimen in National museum in Prague was from Vodňany in 1937. Since 2000 it was found already on nine localities, among these two localities are in Orlické hory Mts., another two localities in České Středohoří and localities in Hrubšice, Mohelno, Chřiby, Kokořín and Bredovský letohrádek. The waxcap will be found probably also on another places during more intensive monitoring. We should recommend change its status to EN category.

Earthy Waxcap (voskovka výstřední) – *Hygrocybe fornicata* var. *fornicata* (Fr.) Singer 1951
We found the species on two places of S hillside of the Bukovec in 2011, its three pieces grown on unimproved cut *Polygono-Trisetion* meadow. The fruitbodies was up 7 cm high, with light brown to 5 cm wide caps, whitish slightly convergent gills and light yellowish stem, which was covered by the darker scales on its bottom part. The waxcap was rare in the past. Before 1960 it was recorded only on two localities in the Czech Republic. However in last fifteen years the waxcap was found and determined near Albrechtičky, in České středohoří, Český kras and in South Bohemia. Other localities exist in Bílé Karpaty Mts., Vyšenské kopce and surroundings of Letonice. We should recommend change its status from point of view of localities number increase to EN category.

Nitrous Waxcap (voskovka ledková) – *Hygrocybe nitrata* (Pers.) Wünsche (1877)
We recorded this species in number of several tens of pieces on the extensively managed lawn in park of the Sychrov castle in 2011 and 2012. There was almost 100% cover of mosses and minimal occurrence of vascular plants. Fruitbodies was up to 8 cm high with brown-gray gently squamose 5 - 8 cm wide caps, gills were gray connected with gray stem by the small tooth. The waxcap is characterised by the unpleasant nitrous odour. Twenty five its specimens were registered in collections of National Museum in Prague before 1970. In present this species is very rare, one specimen is from Dobroslavice near Ostrava (1995), two another records are from Orlické hory Mts. (2008 and 2010). Due to low number of localities we should recommend this waxcap keep in red list in CR category.

Blushing Waxcap (voskovka ovčí) – *Hygrocybe ovina* (Bull.) Kühner (1926)
We found this *Hygrocybe* in number 3 to 20 fruitbodies on the E margin of Rašovka village in 2009, 2010 and 2011. Four pieces were recorded also on lawn in park of the Sychrov castle in 2013. The species grown on cut unfertilised meadow and friutbodies were rather huge up to 15 cm high with dark brown mostly rough caps 6 to 10 cm wide. Gills were gray-brown with reddish tint, stem was coloured as upper part of cap. In Czech Republic the waxcap was known from eight localities in the past, in 90th of last century it was found in Bílé Karpaty Mts. In last period it except of our findigs was recorded exclude on at least six other localities (České Středohoří, Bílé Karpaty Mts., Orlické hory Mts., Český kras and urban park in Choceň). One locality is also recorded on the border between Liberec and Ústí nad Labem regions in Lužické hory Mts. in 2011. We expect that the species can grow in other places of the Czech Republic and we should recommend change its status to EN category.

Honey Waxcap (voskovka Reidova) – *Hygrocybe reidii* Kühner (1976)
This species was recorded in NW part of Ještěd ridge near Panenská Hůrka in mixed forest in 2010 (leg. Z. Egertová et M. Sochor) and Slavíček (2011) referred the species in National natural reserve "Karlovské bučiny". Fruitbodies are usually up to 6 cm high with orange-red smooth cap, orange-yellow gills and stem. The waxcap is characteristic of honey smell. In the past this species was confused *H. marchii*. Since 2000 it was found already on more than twenty localities, in the last period especially in Bílé Karpaty Mts., Českomoravská vysočina and Orlické hory Mts. We should recommend change its status to VU category.

Spindle-shank Waxcap (voskovka nemilá) - *Hygrocybe ingrata* J.P. Jensen & F.H. Møller (1945)
We found one fruitbody in drier part of cut meadow (*Violion caninae*) under Bukovec in Jizerské hory Mts. in 2012 and about ten fruitbodies in park of Sychrov castle on extensively managed lawn in 2011 and 2012. Fruitbodies were 6 to 8 cm high with light orange-brown smooth slimy 2 to 3 cm wide caps, a bit more light gills and stem. It is very rare species in the Czech Republic. In the past it was found only on two localities near Turnov – "Farářství" (1944) and "Ve Struhách" (1946) and another tally is from "Velká Horka" near Mnichovo Hradiště (Herink 1958) and "Kozelské polesí" near Plzeň (1983). In the last period the *Hygrocybe* was found except of Liberec region only in Bílé Karpaty Mts. in 2013. Another record does not exist and therefore we should recommend this waxcap keep in red list in CR category.

Fibrous Waxcap (voskovka prostřední) - *Hygrocybe intermedia* (Pass.) Fayod (1889)
We found several fruitbodies of the *Hygrocybe* in two places on S hillside of Bukovec in 2011 and 2012, there are cut *Polygono-Trisetion* and *Nardion* meadows. We also found one piece on cut *Arrhenatherion* meadow near Rašovka village on SE hillside of Ještěd ridge in 2011. The fruitbodies were up to 10 cm high with flaming red-orange fibrous caps, which were 5 to 10 cm wide. In the Czech Republic the waxcap is rare, there were documented only six localities in the past - Ještěd (1920), "Divoká Šárka" in Prague (1938), Žarošice in Ždánický les (1947), "Velká Horka" near Mnichovo Hradiště (published in Herink 1958), next ones in Trutnov (1981) and Černovice u Kunštátu (1997). During the last ten years it was found on at least ten localities, especially in Bílé Karpaty Mts. and in surrounding of Vsetín. We should recommend change its status to EN category.

Heath Waxcap (voskovka veselá) - *Hygrocybe laeta* var. *laeta* (Pers.) P. Kumm. (1871)
We recorded this waxcap on three localities in Liberec region. During the period 2008-2012 it was found regularly in number a lot tens of pieces in castle park Sychrov on the extensively managed lawn. We recorded up to ten pieces near Rašovka village on cut meadow in 2011 a 2012 and also six pieces on S hillside of Bukovec on occasionally cut *Nardion* meadow in 2012. There was moos floor well developed on the all sites with minimal vascular plant coverage. The fruitbodies were up to 7 cm high with orange-brown slimy 2 to 3 cm wide caps. Stems were orange-brown or orange-green with violet tones in upper part. In the Czech Republic the species was found relatively often in the past, but since 70th of last century it became rare and it was listed in CR category (Holec et Beran, 2006). Nevertheless in last period except of our findings it was found on more than fifteen localities in different parts of the country. In point of view of the site number increase we should recommend change its status to VU category.

Date Waxcap (voskovka osmahlá) - (*Hygrocybe spadicea*) (Fr.) P. Karst. (1879)
We recorded eight and five pieces in 2011 and 2012, respectively. They grown on dryer site of annually cut *Nardion* meadow on S hillside of Bukovec. The fruitbodies were up to 12 cm high with dark brown 3 to 6 cm wide caps, yellow gills and yellow stems. It is one of the rarest fungi in the Czech Republic similarly to other European countries. In the history only six records were known here, since 90th of the last century this waxcap was found in reserve "Nad řekami" near Hrubšice in Moravia and also on one site in České středohoří, where it was not confirmed again during several

years. In the present our stand in Jizerské hory Mts. is only one recent locality in Czech Republic and we should recommend keep it resolutely in CR category.
The locality on Bukovec in Jizerské hory Mts. is exceptionally rich for waxcaps species. There were found twenty members of genus *Hygrocybe* (Tab. 2 and 3), among others five critically endangered (*H. fornicata*, *H. ingrata*, *H. intermedia*, *H. laeta* and *H. spadicea*) and two very rare species, what are not listed (*H. splendidissima* and *H. citrinovirens*).

Rare species not listed in Red list of macromycetes of Czech Republic
There are mentioned following species unregistered, nevertheless we are confident that they should be recorded in next prepared red list certainly.

Splendid Waxcap (voskovka nádherná) - *Hygrocybe splendidissima* (P.D. Orton) M.M. Moser (1967)
We found on the meadow on S hillside of Bukovec up to 12 cm high fruitbodies of the waxcap with red smooth to 8 cm wide caps, orange-yellow gills and red-orange smooth stem, the specimens were characteristic of honey smell during drying. We found twelve pieces together on more dry part of *Polygono-Trisetion* a *Violion caninae* meadow during the autumnal months of 2012. In the Czech Republic (as well as across Europe) it is very rare *Hygrocybe* with only several recent localities (except Bukovec also Orlické hory, Bílé Karpaty and Hostýnské vrchy). The waxcap should be listed in next red list what mentioned Holec et al. (2008), we should recommend record it in the CR category.

Citrine Waxcap (voskovka sírožlutá) - *Hygrocybe citrinovirens* (J.E. Lange) Jul. Schäff. (1947)
Also this species was discovered on the meadows near Bukovec. The fruitbodies were up to 12 cm high with yellow to light green-yellow 4 to 7 cm wide conical caps. Whitish gills were freely connected to stem which was similarly coloured as upper part of caps with lengthways denting. We found fifteen (2011) and nine (2012) fruitbodies on several drier places of *Polygono-Trisetion* and *Violion caninae* meadows. Data about its distribution in the Czech Republic did almost absent in historical records. Herink (1958) referred it on “Velká Horka” near Mnichovo Hradiště. This *Hygrocybe* was found in Orlické hory Mts. (2007), other three localities are in Beskydy Mts. - Huslenky (2010) and surrounding of Velké Karlovice (2006 and 2009). Because absence of data from richest waxcap meadows in Bílé Karpaty Mts. we should recommend record this species in the CR category, possibly EN category in case of next findings.

Oily Waxcap (voskovka klidná) - *Hygrocybe quieta* (Kühner) Singer (1951)
We found six pieces of the waxcap in central part of *Polygono-Trisetion* meadow on hillside of Bukovec in 2012. The fruitbodies were up to 10 cm high with yellow-orange caps wide to 6 cm, closely connected gills were orange coloured as well as stem and obvious bad smell. Data about its distribution in the Czech Republic is missing in the past. It was recorded on two localities in Liberec region (second site is near Mníšek u Liberce) and five other data exist (Orlické hory, Ochoz u Brna, Bílé Karpaty and unclear record from Borkovice). We should recommend record this species in the CR category, possibly EN category in case of next findings.

Yellow Foot Waxcap (voskovka žlutonohá) - *Hygrocybe flavipes* (Britzelm.) Arnolds (1989)
The waxcap was found on four localities in Liberec region. We recorded this species in number 5 to 12 pieces in park of Sychrov castle on extensively managed cut lawn in 2010 to 2012 period. Five and eight fruitbodies were found also in *Nardion* meadow on hillside of Bukovec in 2011 and 2012, respectively. Other site is in the garden of “Bredovský letohrádek” near castle Lvová on unfertilised cut grassland with well developed moss floor (Egertová et Kříž 2009). Fourth locality is in Mníšek u Liberce on our experimental site where 4 and 1 fruitbodies were found on frequently mulched grassland in 2012 and 2013, respectively. The fruitbodies were usually 6 to 9 cm high with light violet

smooth 7 cm wide caps. Gills were light brownish as well as stem which had yellow base. This species was described recently and data about its distribution in the Czech Republic is missing in the past. We collected available data - this waxcap was found in Orlické hory Mts and Choltická obora in 2007 and near Albrechtice u Sušice in 2009. We expect that the species can grow in other places of the Czech Republic and recommend record this species in the EN category.

Pale Waxcap (voskovka luční bílá) - *Hygrocybe pratensis* var. *pallida* (Cooke) Arnolds (1985)
In the period 2007-2012 the species was found at least on six localities on extensively managed grasslands. Fruitbodies were 6 to 12 cm high with light cream-coloured to almost white, 4 to 8 cm wide caps. Gills were deep convergent similarly coloured as upper part of caps and stem too. More detailed data about its distribution in the Czech Republic is missing however we can expect that it belongs to more common waxcaps. We should recommend record this species in the NT category.

Papilla Waxcap (voskovka bradavkatá) – *Hygrocybe subpapillata* Kühner (1979)
This small waxcap was found in number to 20 pieces on occasionally grazed meadow between villages Rašovka and Bystrá in 2012. In the same year several fruitbodies were found also on cut lawn in town Rychnov u Jablonce n.N. and on extensive pasture in Oldřichov v Hájích. One record is from private garden in Jablonné v Podještědí. The moss floor was well developed at the all stands. The fruitbodies were to 6 cm high with up to 2 cm wide red-orange caps. Gills were orange-yellow, dry stems were similarly coloured as caps. Obvious papilla on the top of cap is characteristic for the species. Because data about its distribution in the Czech Republic is missing, we should recommend record this species in the DD category.

Conclusions

A lot of members of genus *Hygrocybe* and other indication fungi were recorded during the study of grasslands in the Liberec region. Ten of waxcaps are listed in current Red list of macromycets of Czech Republic as critically endangered (CR). From the collected data about their distribution is evident that some species are more spread and we should recommend change its status to EN. There are especially *Hygrocybe mucronella*, *H. fornicata* var. *fornicata*, *H. ovina* and *H. intermedia*. We should recommend change status to VU for more common waxcaps *H. reidii* a *H. laeta* var. *laeta*. On the base of total distribution of some not listed species we should recommend record *H. splendidissima* and *H. citrinovirens* to CR category, *H. flavipes* and *H. quieta* to EN category, *H. pratensis* var. *pallida* to category NT and *H. subpapillata* to DD category in the next prepared red list. The most of found waxcaps preferred localities with cut meadows where is well developed moss floor. The waxcaps grown especially on long-term cut or grazed grasslands with minimal soil disturbance and without previous intensive fertilisation. Some low sensitive species can grow on mulched plots and on unmanaged grasslands, however most of species was found on regularly cut areas.

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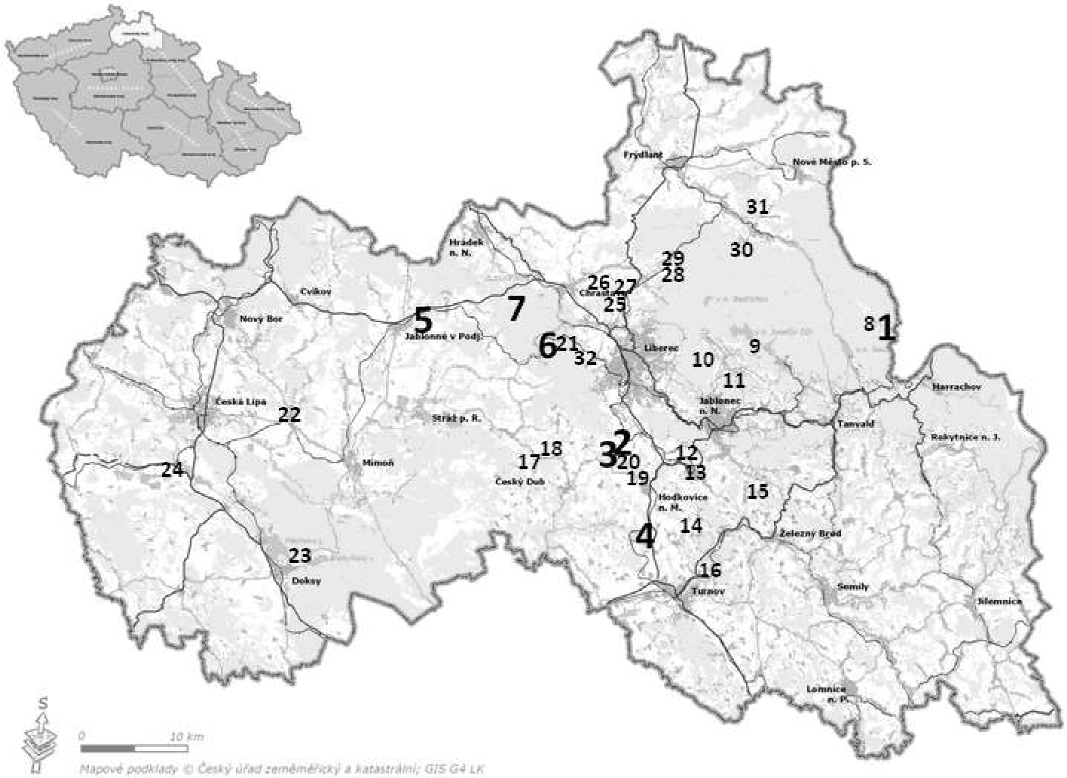

Figure 1 Map of the localities with critically endangered waxcaps (large font) and other sites (small font). List of the localities is in table 2.

kategory CR	**kategory EN**	**kategory NT**	**kategory DD**	**Not listed**
H. colemanniana	*H. coccinea*	*H. chlorophana*	*H. cantharellus*	*H. acutoconica*
H. fornicata	*H. coccineocrenata*	*H. pratensis*	*H. ceracea*	*H. berkeleyi*
H. ingrata	*H. helobia*		*H. glutinipes*	*H. citrinovirens*
H. intermedia	*H. irrigata*		*H. insipida*	*H. conica*
H. laeta var. *laeta*	*H. punicea*		*H. virginea.* var. *fuscescens*	*H. flavipes*
H. mucronella				*H. miniata*
H. nitrata				*H. quieta*
H. ovina				*H. pratensis* var. *pallida*
H. reidii				*H. psittacina*
H. spadicea				*H. splendidissima*
				H. subpapillata
				H. virginea var. *virginea*

Table 1 Species of genus *Hygrocybe* recorded in Liberec region
CR - critically endangered, EN - endangered, NT - near threatened, DD - data deficient

Localities	Vegetation	Moss cover	*H*	*E*	*C*	*G*
1. Bukovec	cut meadow/marsh	well	20	6	8	3
2. Rašovka E	cut meadow	poor-well	11	3	9	1
3. Rašovka SW	cut meadow	well	10	3	4	1
4. Sychrov	cut lawn	well	14	5	8	6
5. Bredovská zahrada	extensive lawn	poor-well	13	2	6	2
6. Kryštofovo údolí	cut meadow	well	1	0	0	0
7. Panenská hůrka	forest	not	1	0	0	0
8. Jizerka	cut marsh/meadow	well	5	1	2	1
9. Josefův Důl - hráz	cut meadow	well	5	2	3	0
10. Hraničná	cut marsh/meadow	well	6	2	4	4
11. Horní Maxov	cut marsh	well	4	2	3	2
12. Rychnov u Jablonce n.N.	cut meadow	well	4	1	4	2
13. Rychnov u Jablonce n.N.	cut urban lawn	poor-well	4	2	3	0
14. Frýdštejn	cut meadow	well	8	4	4	2
15. Huť	cut meadow	well	8	3	4	1
16. Rakousy	unmanaged orchard	well	3	5	5	1
17. Smržov	cut meadow	well	5	2	3	2
18. Sobákov	pasture	poor	6	2	3	1
19. Bystrá	cut/grazed meadow	well	9	2	4	0
20. Rašovka pastvina	cut/grazed meadow	well	6	4	7	1
21. Hamrštejn	forest	not	5	3	9	8
22. Zákupy	cut park lawn	well	5	4	4	2
23. Doksy - Máchovo jezero	marsh	well	2	2	0	0
24. Zahrádky u České Lípy	marsh	well	2	0	0	0
25. Mníšek - experiment	cut/mulched meadow	well/poor	9	4	6	2
26. Mníšek - Amerika	cut meadow	well	6	2	8	2
27. Mníšek - sad	unmanaged orchard/forest	not	3	1	3	1
28. Betlém	intensive pasture	well	7	2	3	1
29. Betlém	extensive pasture	well	6	3	7	3
30. Ferdinandov	forest	not	3	0	3	1
31. Lázně Libverda	park lawn	well	4	2	3	1
32. Karlov	cut lawn	well	5	2	3	0

Table 2. List of observed localities with indication fungi, locality numbers are corresponding with maps in Fig. 1. *H – Hygrocybe*, *E – Entoloma*, *C – Clavariaceae*, *G - Geoglossaceae*

Critically endangered Waxcaps species	Localities
H. colemanniana	6
H. fornicata	1
H. ingrata	1, 4
H. intermedia	1, 2
H. laeta var. *laeta*	1, 3, 4
H. mucronella	5
H. nitrata	4
H. ovina	2, 4
H. reidii	7
H. spadicea	1

Table 3 Overview of critically endangered waxcaps and their occurrence on localities in Liberec region

Chapter 8

Principal conclusions of the thesis

The PhD. thesis is focused on the study of management and biodiversity of upland and mountain grasslands in the Jizerské hory Mts. In the end of twenty century due to massive reduction agricultural production especially in marginal areas the significant changes in management systems on grassland occurred In the Czech Republic. There were applied more extensive systems of management such as extensive grazing, alternate cutting, mulching or abandoning. In the thesis, the effects of different defoliation practices on grassland vegetation and fungi species diversity were evaluated. First part is foccused on general information concerning different management systems, weed control in grassland, plants and fungi diversity of meadow and pastures. In this part, directions of scientific research of grasslands managements in Czech Republic and in foreign countries were discussed. In the other parts of thesis results obtained during our long-term experiments carried out on upland and mountain grasslands were described. There are introduced studies concerning changes of botanical composition, weed plants occurence and fungi diversity under different management treatments.

The main goal of the thesis was the comparison of different alternative treatments or abandonment with regular systems of management. The different long term alternative managements and their effects on grassland biodiversity were discussed.

Chapter 2: After 12 years of the manipulative experiment, four groups of treatments were found that had a similar response of plant species composition to long-term defoliation management: i) unmanaged control and grassland mulched once a year in September; ii) grassland mulched once a year in May and in July; iii), grassland mulched twice a year in June and August and grassland mulched three times a year in May, July and September; iv) grassland cut twice a year with biomass removal in June and August. The main finding of this study is that the mulching at least twice a year can substitute for cutting management in low productive *Festuca rubra* grassland, without substantial losses of plant species richness and diversity. Mulching carried out once a year in September affected plant species composition of the grassland in a similar way as no management. Mulching performed in September is only useful to prevent grassland encroachment by shrubs and trees. The majority of changes in plant species composition developed during the first five years of the experiment, therefore, experiments designed to study the effects of defoliation management on plant species composition of grasslands must not be performed as short tems.

Chapter 3: The study described the effect of the cutting management cessation on the plant species richness and the botanical composition of vegetation in a mountain hay meadow (*Polygono-Trisetion*). The total number of plant species was higher in the cut treatment after the first four years of the study. However the number of species with a cover ≥ 1% was the same in both treatments throughout the experiment. Abundance of some of the dominant species (e.g. *Festuca rubra*, *Cirsium heterophylum, Geranium sylvaticum, Hypericum maculatum*) was changed on abandoned plots, but it did not change in the overall plant community. Contrasting management resulted in relatively small changes of plant species composition after ten years, but these changes are still in progress. The main practical message of the study is that some types of mountain grasslands can be left without management for several years and significant changes in vegetation do not occur. Because of the low importance of *Polygono-Trisetion* grasslands for forage and of the high price of grassland management, cutting management combined with several years of abandonment may be usable way for the maintenance of *Polygono-Trisetion* grasslands.

Chapter 4: Immediately after introduction of intensive grassland management significant diversification of plant species composition occurred in different fertiliser treatments and the differences lasted for seven years with management and fertiliser application. Consequent termination of the cutting management and fertiliser application resulted in dominance of only several plant species, and differences in plant species composition arising from previous management completely disappeared. However the differences in biomass quality and soil available nutrients arose by previous management were still detectable in case of P. Lower plant available P in soil and content P in total plant of above ground biomass were recorded in formerly unfertilised treatment. Especially content of P in aboveground biomass of *F. rubra* seems to be good indicator of plant available P in the soil. The main message of this study is that moderate application of NPK need not generate irreversible structural and as well as compositional changes in upland *Festuca rubra* grassland.

Chapter 5: Restoration of grazing management on abandoned mesic grassland altered the plant species composition toward increased proportions of short grasses and prostrate forbs. At the conclusion of the experiment, higher numbers of plant species in all managed treatments in comparison to the unmanaged control indicates the necessity of defoliation management to enable co-existence of many plant species in semi-natural grasslands. Similarly with other experiments studying different grazing intensities, the effect of defoliation intensity on species richness have been found not to be so straightforward. Potential sward height, a parameter based on plant height according to local flora and cover of present species, is useful to reveal the replacement of tall dominants by short species under defoliation. Simple vegetation traits can predict responses to the different managements. However, they are strongly dependent on the several dominant species, sometimes with miscellaneous responses.

Chapter 6: Weed species with tall growth form such as *Cirsium arvense, Urtica dioica, Anthriscus sylvestris* and *Elytrigia repens* are highly competitive in a sward without regular defoliation and no management most promoted these species. In the treatment mulched once a year, we found similar trends in cover changes of most of the tall weeds as in unmanaged plots. Yet, mulching once a year would not be suitable at all for the conservation of a traditional cultural landscape with high biodiversity; this management is appropriate to prevent shrubs and forest formation only. Tall weed species seem to be sensitive to frequent defoliation (cutting or mulching twice or three times a year). On the other hand, prostrate species *Taraxacum* sp. and *Ranunculus repens* reached highest cover in twice and three times a year mulched treatments.

Chapter 7: A lot of members of genus *Hygrocybe* and fungi of genus *Entoloma* and families *Clavariaceae* and *Geoglossaceae* were recorded during the study of grasslands in the Liberec region. Ten of waxcap species are listed in current Red list of macromycets of Czech Republic as critically endangered (CR). Majority localities of their high abundance are cut meadows with of biomass removal where the moss floor is well developed. The waxcaps grown on long-term cut or grazed grasslands with minimal soil disturbance and under low plant available nutrients due to their high sensitivity to phosphorus. Some low sensitive waxcap species can grow on mulched plots as well as on unmanaged grasslands, however majority of waxcap species was found on regularly cut areas. The localities with highest waxcap species abundance with national or international importance were meadows on hillside Bukovec in Jizerské hory Mts., extensively managed lawn in park of Sychrov castle and meadows on Ještěd ridge near Rašovka village. Some waxcaps seem to be more distributed in Czech landscape than it was known. On the base of obtained data, recent records of museums or findings of eminent mycologists from the whole Czech Republic we recomended to shift some species with higher occurence in to category with lower endangering.

Chapter 9

Recommendation for further research

With respect to lack of knowledge of management effect on species diversity in upland and mountain grasslands, further research schould be focused on following issues:

Long-term observation of treatment effect (cutting, mulching, grazing, abandoment) on insects species

Effect of soil nutrients concentration on fungi occurence under different management

Monitoring of fungi communities succession on former arable land or temporary grasslands transformed to permanent meadows

Chapter 10

Souhrn (Summary in Czech)

Disertační práce je zaměřena na studium obhospodařování a druhové diverzity podhorských a horských travních porostů v Jizerských horách. V České republice, zejména v marginálních oblastech, jsme zaznamenali změny v systémech obhospodařování travních porostů z důvodu velkého poklesu zemědělské výroby. Byly zde aplikovány náhradní systémy hospodaření, jako je extenzivní pastva, střídavé sečení, mulčování a ponechávání ladem. V této práci byly vyhodnoceny vlivy různých způsobů defoliace na diverzitu rostlin a hub v travních porostech. První část je zaměřena na obecné publikované informace o různých systémech hospodaření, regulaci plevelných druhů, druhové diverzitě rostlin a hub na loukách a pastvinách. Kapitola obsahuje směry zkoumání travních porostů v České republice i v zahraničí. Další kapitoly popisují výsledky dosažené při dlouhodobých experimentech v podhorských a horských travních porostech. Byly zde studovány změny botanického složení, výskyt plevelů a diverzita indikačních lučních hub při různých způsobech obhospodařování.
Hlavním cílem disertační práce bylo porovnání alternativních způsobů s tradičním hospodařením a na druhé straně s plochami ponechanými ladem z hlediska ovlivnění biodiverzity a možností její trvalé udržitelnosti.

Kapitola 2: Po 12 letech trvání experimentu bylo možné odlišit čtyři skupiny variant hospodaření s podobnými účinky na botanické složení travního porostu: i) ponechání ladem a mulčování jednou ročně na konci vegetace v září; ii) mulčování jednou ročně v květnu a jednou ročně v červenci; iii) mulčování dvakrát ročně v červnu a srpnu a mulčování třikrát ročně v květnu, červenci a září; iv) sečení a odstraňování biomasy v červnu a srpnu. Hlavním zjištěním studia je, že mulčování prováděné alespoň dvakrát ročně může v méně produktivních travních porostech s dominancí kostřavy červené nahradit sečení s odklízením biomasy bez podstatných ztrát počtu druhů a druhové rozmanitosti rostlin. Mulčování pouze jednou ročně na podzim ovlivňuje druhové složení obdobným způsobem, jako kdyby byl porost ponechán ladem. Mulčování prováděné v září může být nanejvýš použito jako způsob zabraňující zarůstání travních porostů náletovými dřevinami. K největším změnám botanického složení docházelo v průběhu prvních pěti let experimentu, z toho vyplývá, že experimentální práce zaměřené na sledování vlivu různých způsobů obhospodařování na strukturu porostu by měly trvat minimálně tuto dobu.

Kapitola 3: Studie popisuje vliv nesečení na druhovou diverzitu rostlin a botanické složení porostu v druhově bohaté horké louce společenstva *Polygono-Trisetion*. Celkový počet rostlinných druhů bez ohledu na jejich pokryvnost byl po prvních čtyřech letech pokusu vyšší na sečených plochách. Avšak počet druhů s pokryvností 1% a vyšší byl stejný na obou variantách po celou dobu pokusu. Ponechání porostu ladem v našem experimentu ovlivnilo botanické složení porostu zejména změnou výskytu dominantních (např. *Festuca rubra*, *Cirsium heterophylum, Geranium sylvaticum, Hypericum maculatum*), ale nevedlo ke změnám ve společenstvu. Kontrastní způsoby managementu vedly k relativně malým změnám v botanickém složení, avšak tyto změny stále pokračují a proto je zapotřebí pozorovat účinky managementu opravdu dlouhodobě. Mezi hlavní poznatky studie patří zjištění, že některé typy horských travních porostů mohou zůstat ladem po dobu několika let bez podstatných změn druhového složení. Z důvodu malého významu horských luk pro výrobu píce a vysokých nákladů na jejich údržbu může být v současné době periodické sečení, kombinované s několika lety ponechání porostů ladem, vhodnou strategií pro zachování luk svazu *Polygono-Trisetion*.

Kapitola 4: Bezprostředně po zavedení intenzivního obhospodařování na travním porostu se na různě hnojených variantách projevilo rozrůznění botanického složení a rozdílypřetrvávaly po dobu sedmi let trvání hospodaření a hnojení. Ukončení hospodaření a aplikace hnojiv se v travním porostu projevilo převahou pouze několika dominantních druhů rostlin a rozdíly způsobené předcházejícím managementem úplně vymizely. Avšak rozdíly v kvalitě nadzemní biomasy a v půdní zásobě přístupných živin byly stále detekovatelné v případě fosforu. Nižší koncentrace přijatelného fosforu v půdě a celkového fosforu v nadzemní biomase byly zaznamenány na plochách, které byly během dřívějšího pokusu nehnojené. Zejména obsah fosforu v nadzemní biomase *Festuca rubra* se zdá být dobrým indikátorem zásoby přístupného fosforu v půdě. Nejdůležitějším poznatkem této studie je, že přiměřené dávky hnojení NPK nemusí nutně vést k nevratným změnám struktury a botanického složení podhorských kostřavových porostů.

Kapitola 5: Obnovení pastvy na opuštěném mezofilním travním porostu způsobilo změny v druhovém složení zvýšením podílu nízkých druhů trav a prostrátních dvouděložných. Vyšší počet rostlinných druhů ve všech obhospodařovaných variantách v porovnání s neobhospodařovanými kontrolními plochami ukazuje nezbytnost defoliačních zásahů k zachování koexistence mnoha rostlinných druhů v polopřirozených travních porostech. Podobně jako v dalších experimentálních studiích zkoumajících vliv různé intenzity pastvy na druhovou rozmanitost, vliv intenzity defoliace na počet druhů není zcela jasný. Potenciální výška porostu jako parametr, zjištěný na základě lokální flóry a pokryvnosti přítomných druhů, je použitelná pro určení míry nahrazení vysokých dominant nižšími druhy při defoliaci. Základní znaky porostu mohou předurčovat odezvu na různý management. Simple vegetation traits can predict responses to the different managements. Avšak to záleží na několika dominantních druzích, v některých případech s různou odezvou.

Kapitola 6: Plevelné druhy rostlin s vysokým vzrůstem, jako je *Cirsium arvense, Urtica dioica, Anthriscus sylvestris* a *Elytrigia repens*, jsou velmi konkurenceschopné v travních porostech bez pravidelné defoliace, a proto ponechání porostů ladem podporuje rozšiřování těchto druhů. Na plochách mulčovaných jednou ročně jsme zaznamenali podobné trendy ve změnách pokryvnosti vysokých plevelných druhů jako na plochách ponechaných ladem. Z toho důvodu není mulčování jednou ročně vhodné pro ošetřování a udržování tradiční kulturní krajiny s vysokou biodiversitou porostů; tento způsob může být použit nanejvýš pro prevenci proti zarůstání ploch náletovými dřevinami. Vysoké plevelné druhy jsou citlivé na častější defoliaci (sečení nebo mulčování vícekrát ročně). Na druhé straně rostlinné druhy s prostrátním růstem jako je *Taraxacum* spp. a *Ranunculus repens* dosahovali největšího rozšíření právě na plochách mulčovaných dvakrát a třikrát ročně.

Kapitola 7: Mnoho zástupců rodu voskovka (*Hygrocybe*) a hub rodu *Entoloma* a čeledí *Clavariaceae* a *Geoglossaceae*, které indikují zachovalost lučního ekosystému, bylo zaznamenáno v rámci studia prováděného na travních porostech v Libereckém kraji. Z toho deset druhů voskovek je zařazeno v platném Čeveném seznamu hub České republiky jako kriticky ohrožené (kategorie CR). Naprostá většina druhově bohatších lokalit se nachází na sečených loukách, odkud je nadzemní biomasa odvážena a kde je bohatě rozvinuto mechové patro. Voskovky rostly zejména na dlouhodobě sečených, popř. pasených travních porostech s minimálním narušováním drnu a kde nebyly po dlouhou dobu aplikovány anorganická hnojiva, protože většina druhů voskovek je velmi citlivá na vyšší koncentrace fosforu v půdě. Některé málo citlivé druhy byly nalezeny i v mulčovaných a neobhospodařovaných plochách, avšak naprostá většina druhů byla nalezena na pravidelně sečených loukách a trávnících. Nejbohatší lokality s národním, popř. mezinárodním významem, se nacházejí na úpatí Bukovce v Jizerských horách, dále na extenzivně obhospodařovaném trávníku v zámeckém parku v Sychrově a na sečené louce poblíž obce Rašovka na Ještědském hřebeni. Některé druhy voskovky se zdají být více rozšířené, než bylo doposud známo. Na základě získaných dat, recentních sběrů v muzeích a nálezů předních mykologů a odborníků z ČR jsme pro připravovaný červený seznam navrhli některé druhy s větším rozšířením přeřadit do kategorie s nižším stupněm ohrožení.

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