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**Changes of the vegetation of wet meadows  
depending on management**

Ph.D. Thesis

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### **Annotation**

Central Europe wet meadows are characterized by considerable species richness. The biodiversity maintenance of the wet meadows is connected with regular management (i.e. grazing or mowing). As their area drastically decreased due to changes in land use in the last century, they have become the object of interest among scientists, conservation biologists.

This thesis is composed of three original studies which are focused on describing diversity patterns of the whole spectra of wet meadows at landscape level and dynamic of their changes depending on different management regimes (mowing/abandonment, fertilizing/unfertilizing). The synthesis of these studies reveals the description of the processes underlying the wet meadows species loss depending on land use changes and proposes the principles for sustainable conservation management.

## DECLARATION [IN CZECH]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejich internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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Jan Horník

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## List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

Klimesšová, J., Janeček, Š., **Horník, J.**, & Doležal, J. (2011). Effect of the method of assessing and weighting abundance on the interpretation of the relationship between plant clonal traits and meadow management. *Preslia*, 83(3), 437-453. (IF= 2.521).  
*Jan Horník participated in experiment preparation, data collection in the field and revision of the manuscript.*

**Horník, J.**, Janeček, Š., Klimesšová, J., Doležal, J., Janečková, P., Jiráská, Š., & Lanta, V. (2012). Species-area curves revisited: the effects of model choice on parameter sensitivity to environmental, community, and individual plant characteristics. *Plant Ecology*, 213(10), 1675-1686. (IF= 1.534).  
*Jan Horník participated in experiment preparation and data collection in the field and was responsible for data assembly, partially for statistical analysis and for writing the manuscript.*

Janeček, Š., Bello, F., **Horník, J.**, Bartoš, M., Černý, T., Doležal, J., Dvorský, M., Fajmon, K., Janečková, P., Jiráská, Š., Mudrák O. & Klimesšová, J. (2013). Effects of land-use changes on plant functional and taxonomic diversity along a productivity gradient in wet meadows. *Journal of Vegetation Science*, 24(5), 898-909. (IF=3.372).  
*Jan Horník participated in experiment preparation, data collection in the field and revision of the manuscript.*

## Contents

<b>Chapter I</b>	<b>General introduction</b>	<b>1</b>
<b>Chapter II</b>	<b>Species-area curves revisited: the effects of model choice on parameter sensitivity to environmental, community, and individual plant characteristics.</b> Horník, J., Janeček, Š., Klimešová, J., Doležal, J., Janečková, P., Jiráská, Š., & Lanta, V. (2012). <i>Plant Ecology</i> , 213(10), 1675-1686	<b>19</b>
<b>Chapter III</b>	<b>Effect of the method of assessing and weighting abundance on the interpretation of the relationship between plant clonal traits and meadow management.</b> Klimešová, J., Janeček, Š., Horník, J., & Doležal, J. (2011). <i>Preslia</i> , 83(3), 437-453.	<b>37</b>
<b>Chapter IV</b>	<b>Effects of land-use changes on plant functional and taxonomic diversity along a productivity gradient in wet meadows.</b> Janeček, Š., Bello, F., Horník, J., Bartoš, M., Černý, T., Doležal, J., Dvorský, M., Fajmon, K., Janečková, P., Jiráská, Š., Mudrák O. & Klimešová, J. (2013). <i>Journal of Vegetation Science</i> , 24(5), 898-909.	<b>57</b>
	<b>Summary</b>	<b>77</b>

## **Chapter I**

### **General introduction**





## **Grasslands, their history, present and perspectives**

Temperate semi-natural grasslands became an object of the interest among the scientists and conservation biologists in the last decades. This is at least for two reasons. The first reason is that grasslands are hosting extraordinary species diversity on small scales (Kull & Zobel 1991, Klimeš 1999, Willson et al. 2012). Exploring the processes which allows small-scale co-existence of many species is a great challenge for contemporary vegetation science (Wilson 2011). The second reason is more dismal and is connected with steep decrease of semi-natural habitats in contemporary landscape (Jansen et al. 2000). The vegetation of the temperate semi-natural grasslands was formed and maintained by human extensive agricultural activities for millennia from Neolithic period (Hejzman et al. 2013) and their existence directly depends on regular management (Křenová & Lepš 1996).

Rapid decrease of the area of the grasslands is connected with changes in land use during the last century. Large areas of the grasslands were transformed to arable land. Another yet considerable area of grasslands has been left abandoned during the agriculture intensification because of their inconvenience for heavy-machine farming (e.g. the habitats on wet stands or extreme slopes, Jensen & Schrautzer 1999). Wet meadows habitats moreover declined due to modification of hydrological conditions (Prach 2008). There is evidence in the Czech Republic that 10 870 km<sup>2</sup> of arable land (e.g. nearly 14% of total area) were affected by large-scale drainage. In England, remnants of wet meadows reach less than 20% of their historic area (Treweek et al. 1997). Similarly, in Hungary, area of wet meadows decreased about 30% from 1950 to 1990 (Joyce & Wade 1998).

Agricultural intensification is characterized by intensive mowing, fertilizing and sowing of species poor seed mixtures, leading to conversion of semi-natural species rich grasslands into monotonic species poor swards with dominance of several fodder grasses and legumes (Hejzman et al. 2013). Massive application of commercial fertilizers accelerates eutrophication, expansion of species from nutrient-rich habitats (Berendse et al. 1992, Wahlman & Milberg 2002), and species richness decline (Hautier et al. 2009, Kleijn et al. 2009). Wesche et al. (2012) documented 30-50% species loss in Northern Germany floodplain grasslands, which was accompanied by increased nutrient availability. Nitrogen is supposed to become one of the three most important drivers of global biodiversity change (Sala et al. 2000).

As a result of these dramatic changes semi-natural temperate grasslands appeared one of the most endangered habitats in the world (Hoekstra et al. 2005). On the conservation field, much effort has been spent to create the national nets of natural reserves in the last century. The European grasslands conservation is also embedded in the European Union laws. Most of grassland habitats are included in the Annex 1 of Council Directive 92/43/EEC and Sites of Community Importance in Natura 2000 network are designated for their conservation and ensuring longtime sustainability of

European nature heritage. These obligations are also connected with appropriate maintenance of target habitats on one hand and with regular monitoring and reporting of actual condition. Appropriate methods of assessing the habitats diversity and its changes dependent on management at landscape level are needed.

## **Determinants of grasslands diversity**

### **Management**

Management is supposed to be major factor, determining small-scale vegetation composition (Klimek et al. 2007, Cousins et al. 2009). Grasslands are dynamic non-equilibrium communities (De Angelis & Waterhouse 1987); their maintenance is dependent on regular disturbance management of moderate intensity. Appropriate disturbance regime (e.g. mowing and grazing in Central Europe) protect grassland communities against secondary succession towards shrubby or nitrophilous species- poor vegetation (Lepš 1999, Galváneek & Lepš 2008, Galváneek & Lepš 2012, Lepš 2014).

Exact mechanism enabling co-existence of numerous species on grassland is not fully agreed on (Palmer 1994) but it is clear that management reduces competitive ability of tall plants and creates regeneration niches for seedling establishment. It is caused by fact that the effect of the management differs among the species. Tall, competitive strong species lose higher proportion of resources invested, compared to smaller competitive weak species (Grime 1973, Palmer 1994, Huston 1999, Klimeš & Klimešová 2002). Removal of biomass causes export of nutrients from the site and enhances seedling recruitment due to better light accessibility in the ground layer (Hejzman et al. 2011). Seedling recruitment is furthermore supported by small disturbances (gaps), made by grazing animals or by mowing tools (Špačková et al. 1998, Kotorová & Lepš 1999, Lepš 1999, Chaloupecká & Lepš 2004). The positive effect of mowing on the species richness is well documented from various types of grasslands, i.e. alkaline peat bogs (Fossati & Pautou 1985), calcareous fens (Güsewell et al. 1998), and wet meadows of *Molinion caeruleae* alliance (Lepš 1999, 2004, 2014). Abandonment, on the other hand, increases asymmetry and intensity of aboveground competition resulting in dominance of tall-stature species and in exclusion of small-stature species (Weiner 1990, Shwinning & Weiner 1998, Lepš 1999). Litter accumulation and lack of small-scale disturbances hinders seedling establishment of many plant species (Špačková et al. 1998, Janeček & Lepš 2005, Galváneek & Lepš 2012). Absence of gaps contributes to the spatial homogenization of a community and consequently to the niche loss followed by species extinction (van der Maarel & Sykes 1993).

## Abiotic conditions

Local abiotic conditions define the boundaries for the occurrence of species according to their ecophysiological requirements. In Central Europe, soil moisture, soil reaction and soil nutrients; respectively are considered as most important abiotic determinants of the grasslands communities structure (Merunková & Chytrý 2012). The soil moisture affects the water supply; the limitation of productivity by low water availability is not so important in Central Europe as nutrient availability (but see other regions like semi-deserts, Lauenroth & Sala 1992). In temperate grasslands soil moisture moderates the nutrient accessibility (Araya et al. 2013), for example, due to affecting litter decomposition (Galváneš & Lepš 2012). Permanently high groundwater table in fen meadows hinder microbial processes and decreasing level of groundwater table induces mineralization of top soil horizons followed by increasing availability of nutrients (Vermeer & Berendse 1983, Grootjans et al. 1985). In consequence low- productive habitats (e.g. fens and mires) are transformed to more productive habitats (e.g. wet meadows of *Calthion* alliance; Graf et al. 2010).

The species diversity is usually positively correlated with soil reaction in Central Europe; this relationship has origin in evolutionary history of the central Europe region, when base rich substrates were prevailing during the Pleistocene and thus larger species pool of calciphilous species has been evolved in the region (Pärtel 2002, Schuster & Diekmann 2003). On larger ranges of the pH, the soil reaction – species diversity relationship was found unimodal with highest species richness in moderate pH levels (Chytrý et al. 2007). Extremely low or high pH conditions are stressful for most of species and the communities developed under such extreme conditions are species poor, containing only specialists. Soil reaction also affects the diversity indirectly by moderating the nutrients accessibility. Especially in wet low productive habitats (e.g. fens), the soil reaction gradient is one of the most important determinants of the large scale community structure (Hájková et al. 2004, Rozbrojová & Hájek 2008).

Nutrient supply, in general, is linked to community productivity, which is very important driver of the species richness in grassland communities (Lepš 1999). Higher levels of nutrient input is followed by productivity increase, which consequently accelerates interspecific competition and favors tall fast growing plant species that outcompete small species adapted to nutrient poor conditions (Bakelaar & Odum 1978, Tilman 1987, Carson & Barrett 1988, Baker 1989, Pyšek & Lepš 1991, Tilman 1993, van Duren et al. 1997, Foster & Gross 1998, Lepš 1999, Hautier et al. 2009). Although mowing cause nutrient export in hay biomass, it may not be sufficient for meadow restoration (Venterink et al. 2002) or it may be compensated by airborne nitrogen deposition (Stevens et al. 2010).

Plant growth is generally (co-)limited by accessibility of three major nutrients: nitrogen, phosphorus, and potassium, respectively. Different types of nutrient limitation may produce different community composition and they are of different importance

along the productivity gradient (Venterink et al. 2001, 2003) In contrast to nitrogen, which is considered as major limiting nutrient on whole productivity gradient up to 1500 g/m<sup>2</sup>, potassium limitation was observed only from low to moderate productivity levels, while phosphorus is limiting for growth on low productive sites only, e.g. fens and mires (Venterink et al. 2003), or sub-alpine *Nardo-Caricion rigidae* grasslands (Hejman et al. 2007). Recent studies revealed that phosphorus limited communities are hosting considerably higher proportion of endangered species compared to other types of nutrient-limited communities (Wassen et al. 2005, Fujita et al. 2014). This difference is probably caused by faster area decline of phosphorus limited in comparison with nitrogen limited communities. Acidification of substrate which can happen due to air pollution or fertilization runoff may be a reason for release from phosphorus limitation, thanks to decreasing pH. Shift in species composition and increased productivity persist for longer period after phosphorus input, compared with nitrogen fertilization (Willems & Nieuwstadt 1996, Hoek et al. 2004) and restoring phosphorus limiting conditions is more difficult than restoring nitrogen limiting conditions.

### **The role of the productivity**

The relationship between species richness and productivity in grasslands is one of most studied patterns of modern vegetation science. There is, however, no agreement whether the productivity controls or is controlled by species richness (Waide et al. 1999, Loreau et al. 2001). Olff & Bakker (1991) pointed out the problem that data from various vegetation types in different sites are affected by many correlated factors (vegetation history, management, soil conditions etc.) that co-vary with the productivity. The shape of the relationship may also be determined by type of the nutrient limitation. For example, Venterink et al. (2003) found the negative species richness - productivity relationship on potassium (co-)limited communities, while nitrogen and phosphorus limitation revealed unimodal relationship.

There are theories based on empirical investigations that support three types of relationships: monotonic both positive and negative or unimodal (Waide et al. 1999, Mittelbach et al. 2001). In unimodal species richness-productivity relationship the highest species richness is observed at moderate productivity level and is declining both towards low productivity and high productivity ends of the productivity gradient. On the low productivity end, the decline is due to stressful conditions, where only small number adapted species is able to thrive (Tilman & Pacala 1993) while at the highest productivity levels, low species richness is caused by increasing competition for light, where competitive species are in advantage (Lepš 1999). Low productive sites are less prone to changes in species composition after land use changes in comparison with high productivity sites (Huston 1979, Aerts et al. 2003, Galvánek & Lepš 2012).

The type of the relationship between productivity and diversity, moreover, depends on the spatial scale (Maranon & Garcia 1997). While positive relationship is usually

proposed on global scale across biomes (Currie & Paquin 1987, Adams & Woodward 1989, Lepš 2005), the unimodal relationship is accepted as typical for local scales (Grime 1973, Gough et al. 2000, Grytnes 2000, Safford et al. 2001, Fraser et al. 2015), although both negative (Güsewell et al. 1999, Hector et al. 1999), or no relationship (Adler et al. 2011) was also found.

### **Methodological issues of measuring diversity**

Although evaluation of diversity changes in meadows under different management regimes has long tradition in Europe, there is no trivial solution to question which methods should be used. Moreover, the species richness is not the only measure of community quality; recently diversity of functional traits is being evaluated as well.

### **Measures of species diversity**

Many indices were proposed for diversity evaluations (Smith & Wilson 1996). In ecological studies, most used are two: Shannon-Weiner index (Shannon and & Weaver 1949) and Simpson index (Simpson 1949) or its variants (Lepš 2005). Up-to-date concepts divide diversity into two components: species richness (the number of species in the sample) and species evenness (Pielou 1977, Smith & Wilson 1996, Stirling & Wilsey 2001, Lepš 2005), because these indicators might be determined by different ecological processes (Wilsey & Stirling 2007).

When there is a need to effectively assess the diversity patterns at landscape level (see above) the use of single number diversity measures (i.e. number of species) might not be effective due to the different landscape properties (e.g. landscape use history, regional species pools etc.). Use of more complex methods in such cases can bring deeper insight, disentangling these idiosyncratic patterns. The analyses dealing with species-area relationship appeared as a very capable tool. The species-area relationship is considered one of the most robust diversity patterns described in ecology (Huston 1994, Rosenzweig 1995). The relationship is not trivial not only due to fact that larger area can host larger number of individuals and therefore also more species than smaller area but is also affected by plant size, patchiness in plant distribution due to growth constraints or environmental heterogeneity etc. (Schmida & Wilson 1985). Previous studies showed that the shape of the species-area relationship may differ for different communities (Lepš & Štursa 1989, Lepš 2005), considered spatial scale (Crawley & Harral 2001, Fridley et al. 2005, Chiarucci et al. 2006, Drakare et al. 2006), sampling method (Hill et al. 1994, Drakare et al. 2006, Dengler & Oldeland 2010) or the vegetation composition patterns (Martín & Goldenfeld 2006, Tjørve et al. 2008).

The first pioneer studies on species-area relationship can be found in middle of the 19<sup>th</sup> century; the first empirical models emerged in the third decade of the 20<sup>th</sup> century (Arrhenius 1921; Gleason 1922). Numerous models of various complexities from two to

four parameters were then proposed (see review of Tjørve 2003, 2009 and Dengler 2009) and many debates were disputed to find “the best” model providing best accuracy to describe field data. In recent studies the Arrhenius power model (Arrhenius 1921) is advocated as best at most scales it is computed upon (Dengler 2008, Triantis et al. 2012). Sigmoid models, on the other hand, seem to be more appropriate when the spatial range exceeds three orders of magnitude (Triantis et al. 2012). When we use the species-area relationship models to explore the factors determining the diversity patterns, the best fit criteria for selection suitable model might have some constraints. At first, performance of the models is different in relation to patterns and scales studied (Tjørve 2003); and at second, there are models which fitting abilities can be more accurate, but their expression is rather difficult (e.g. they use large number of parameters) which is not trivial to interpret on ecological background. The requirement of simple model which parameters are easily biologically interpretable is of high weight therefore (Connor and McCoy 1979; Lepš 2005). The most used are two models: the Arrhenius power model ( $S = C * A^z$ , often presented as the log transformation:  $\log S = \log C + z * \log A$ , Arrhenius 1921) and the Gleason logarithmic model ( $S = C + z * \log A$ , Gleason 1922). In these two models,  $S$  is species richness,  $A$  is area, and  $C$  and  $z$  are constants. The constant  $C$  ( $\log C$ ) is interpreted as ( $\log$ )species richness per unit area and indicates the realized carrying capacity of the system per unit area (Triantis et al. 2012). The constant  $z$  is the rate at which species richness increases with enlarging area and is indicative of the process establishing species richness and composition patterns (Triantis et al. 2012). The power model is more frequently used than the logarithmic one in modeling species-area relationship of plant assemblages because it usually performs better fit with data. Lepš and Štursa (1989) gave a simple reason for this: the log/log transformation is the best tool for converting a monotonic function without inflection to a straight line. Martín and Goldenfeld (2006) suggested that the power model is robust with respect to the specific details of the distribution of abundance and mechanisms of clustering. Other ecologists argue that the logarithmic function is more useful on small spatial scales (He and Legendre 1996, but see Fridley et al. 2005) or for some groups of organisms (Panitsa et al. 2006). Despite uncertainties, the prevailing approach is to select the model based on best-fit criteria (Connor and McCoy 1979, Lepš and Štursa 1989, Dengler 2009).

It is recommended to use the species-area relationship models for exploring the scale dependent patterns (i.e. productivity-diversity, disturbance-diversity, Sandel & Corbin 2012). The reason is that the extrapolation the results to other scales may be misleading (Lepš & Štursa 1989), because the relationships observed on sampled scale might not be operating when another scale is considered (Schmidha & Wilson 1985). The models have been used so far to describe the patterns connected with species diversity and community productivity (Pastor et al. 1996, Weiher 1999, Chiarucci et al. 2006), environmental characteristics (Désilets & Houle 1996, Weiher 1999), management regimes (de Bello et al. 2007) and succession (Lepš & Štursa 1989, Rejmánek & Rosén 1991).

## **Functional traits**

Plant functional traits are supposed more capable compared to species richness indices, in evaluating the role of various processes in community assembly along ecological gradients (Mudrak et al. 2015) or after land use changes (Lavorel & Garnier 2002). As habitat and species loss is connected with species diversity decline, the decrease of the plant functional traits representation in communities was also recorded (Dıaz et al. 2007). In grasslands, eutrophication and abandonment support the competitive species (tall species, with high specific leaf area). Several recent studies revealed the importance of clonal traits in evaluating effect of eutrophication or abandonment. Species with high lateral spread ability and short persistence of ramet connection are put in advantage under these conditions (Klimešova et al. 2011).

As a huge number of the plant functional traits were introduced into the diversity patterns explorations during the last two decades, a question arisen which functional traits should be selected (Lepš et al. 2006). Westoby (1998) proposed leaf-height-seed plant ecology strategy scheme based on three functional traits (specific leaf area, plant height and seed mass), which mirrors three fundamental processes of plant life: dispersal, establishment and persistence. Lepš et al. (2006) on the other hand concluded that selection of the plant functional traits should be made depending on the ecosystem function interest. As grasslands small scale diversity is very narrowly shaped by competition and the clonal plants are prevailing (Klimeš 1995) in these communities, functional traits pertinent to competitive abilities and clonal growth should be used in the grassland biodiversity patterns analyses.

Another important question is, if the species proportions should be taken in account when evaluating the plant traits distribution in a community. In species rich grasslands, where the communities are composed by several dominants and many subordinates, the analyses based on presence compared to that based on abundance can bring different results (Pakeman et al. 2008, Latzel et al. 2011, Sammul 2011, and also the second paragraph in part “Functional diversity” herein).

## **Functional diversity**

The concept of functional diversity based on the idea that community is not only assembly of species but it may be perceived also as assembly of functional traits appeared as a very promising tool in biodiversity patterns investigation. As the species diversity and functional diversity need not to be correlated (Petchey & Gaston 2006), the functional-trait approach may provide alternative insight when exploring biodiversity patterns. Furthermore, functional diversity may be better tool when assessing ecosystem functioning than by species richness (Mason et al. 2005, Dıaz et al. 2006, Lepš et al. 2006, de Bello et al. 2013).

Functional diversity can be divided into three components: functional richness (the functional traits space - span of functional traits present at community), functional evenness (the evenness of functional traits distribution in functional traits space) and functional divergence/convergence (the relationship between the species richness and the functional traits space size) (Mason et al. 2005). Functional evenness is more affected by functional traits of dominant species and should better reflect the ecosystem processes and short time changes in relation to management (Cingolani et al. 2007, Mokany et al. 2008). On the contrary, functional richness is taking into account also rare species which may possess unique traits affecting ecosystem functioning. Rare species are important components of species richness; they can play a facilitative role in the communities (Boeken & Shachak 1994; Polley et al. 2003). The comparison of the rare species down weighted (functional evenness) and up weighted (functional richness) analyses can therefore show the traits connected with extinction risks. Functional richness and functional divergence are often proposed to be related to community assembly processes (Mason et al. 2010, 2012) or ecosystem functioning (Petchey et al. 2004). Separate analyses of the three components of the functional diversity can reveal more detailed insight in the patterns determining the community structure and dynamics.

### **Objectives of the thesis**

There is large body of studies on the problematic of the diversity patterns of wet meadows and the negative effect of eutrophication and abandonment on the wet meadows diversity. The need of regular wet meadows management is widely accepted among scientists and conservation authorities (Křenová & Lepš 1996). As the maintenance of the species rich grasslands as biodiversity hotspots is considerably cost compared to intensive farming (Kleijn et al. 2009); these activities are conducted with allocation of large assets usually in form of government or international subsidiary programs. As the money sources for management are not infinite we need risk assessment study evaluating priorities in management on the level of whole landscape. We cannot, however, get relevant information by comparing results from individual scientific studies as they are done in different conditions (e.g. climate, management regime, fertilizer composition and dosage), sampling protocols (e.g. different plot areas, species cover / abundance / biomass sampling) and landscape context (e.g. land use history, fragmentation of suitable habitats; Klaus et al. 2013). To overcome above mentioned difficulties, we conducted a project across the broad spectra of the wet meadows communities in selected region of the Železné hory Mts. The wet meadows of the Železné hory can be considered diversity hotspots (Horník & Hrázský 2009; Chytrý et al. 2015) due to their high variability and presence of many endangered species. The project took place on 22 localities which contained all wet meadows vegetation types in present Železné hory Mts. landscape (alliances: *Calthion palustris*, *Caricion canescenti-*



*nigrae*, *Molinion caeruleae*, *Violion caninae*, *Sphagno warnstorffii-Tomentypnion nitensis*, *Sphagno-Caricion canscentis* and *Magno-Caricion gracilis*).

The main objectives of the thesis are: (i) to describe diversity patterns of the wet meadows communities on a regional scale; (ii) to assess changes of vegetation of the wet meadows in dependence on different types of management; (iii) to reveal the differences in the reaction of the communities on the management in relation to their productivity; (iv) to set the priorities for the grassland maintenance at regional scale.

## **Methods**

With respect to the complexity of the biodiversity across the scales and communities, the manipulative experiment with baseline data was done. We selected 22 localities to cover broad spectra of the Železné hory Mts. wet meadows (especially on productivity, nutrient and groundwater depth gradients). On each locality one permanent plot was established containing eight blocks with four combinations of the treatments: mown-fertilized, mown - unfertilized, unmown - fertilized, unmown - unfertilized. The data on vegetation composition were collected in July in 2007 before starting the treatments application (baseline data) and then after every two years (July 2009, 2011). Vegetation composition was sampled as phytosociological relevés at each plot 2 m × 2 m and as presences in 100 small 0.01 × 0.01 m plots arranged in 10 rows and 10 columns in the center in four adjacent blocks (Fig.1). Abiotic conditions and community productivity were measured to evaluate the drivers of both initial species diversity and its change dependent on treatments among the communities. The data analyses were performed using species richness at three area levels (0.01 m<sup>2</sup>, 1 m<sup>2</sup>, 4 m<sup>2</sup>), species evenness, two species-area relationship models and nine plant functional traits.

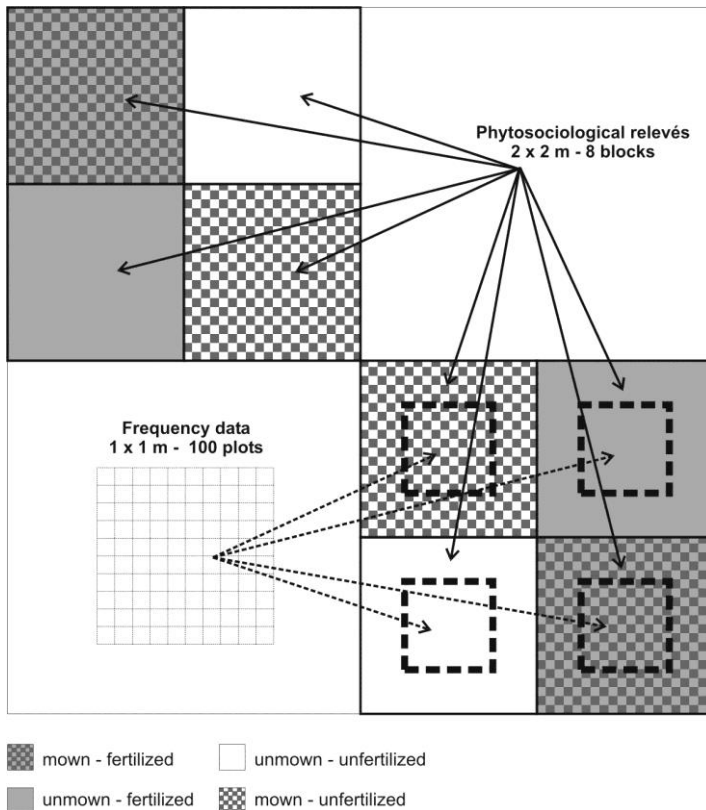


Fig. 1. Permanent plot – sampling scheme

The thesis is composed from three case studies (chapter II, III and IV) and summary.

**Chapter II** describes the diversity of various types of wet meadows communities on main abiotic gradients (soil moisture, nutrient level, soil reaction) and explores determining factors for community composition at different scales

**Chapter III** analyses the short time changes of the vegetation of the wet meadows under different management regimes with respect to functional traits related to productivity, vegetative regeneration and disturbance avoidance

**Chapter IV** compares the effect of different management regimes on functional and taxonomical wet meadow diversity along productivity gradient

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## Chapter II

### **Species-area curves revisited: the effects of model choice on parameter sensitivity to environmental, community, and individual plant characteristics**

Horník, J., Janeček, Š., Klimešová, J., Doležal, J., Janečková, P., Jiráská, Š., & Lanta, V. (2012). *Plant Ecology*, 213(10), 1675-1686



# Species-area curves revisited: the effects of model choice on parameter sensitivity to environmental, community, and individual plant characteristics

## Abstract

Species-area curves are often employed to identify factors affecting biodiversity patterns. The aim of this study was to determine how model choice affects biological interpretation of SAC parameters at a small scale in wet, temperate meadows (Železné hory Mts, Czech Republic). We estimated 88 species-area curves in nested plots on areas ranging from 0.01 to 4 m<sup>2</sup> at 22 localities using four different models (Arrhenius, Gleason, and their log transformations). Relationships were tested between the parameters of the fitted curves (slope and intercept) and a number of environmental and vegetation characteristics (environmental—water table, pH, nutrient availability, organic matter content; community—productivity, evenness; and individual plant—shoot cyclicality, persistence of connection among ramets, multiplication rate, dispersal ability). Species diversity was calculated for 0.01, 1, and 4 m<sup>2</sup>. The corrected Akaike information criterion was used to identify the best model. The models differed in their sensitivity to environmental, community, and individual plant characteristics. The spatial scale that was the most suitable for revealing the factors underlying species diversity was the smallest considered (0.01 m<sup>2</sup>). The most important factors were spatial pattern in community structure (evenness, lateral spread), plant mobility (lateral spread and persistence), and soil properties. Although Gleason model showed better fit to data (both non-log and log transformation) and its intercept was more sensitive to tested biological characteristics, the Arrhenius model was more sensitive when correlating biological characteristics and slope. Choice of model according to best fit criteria restricts possibilities of biological interpretation and deserves further study.

Keywords: Species-area curve, Arrhenius model, Gleason model, Wet meadows, Clonal growth, Akaike information criterion

## Introduction

One of the most important aims in ecology is to understand species diversity and its spatial variation. One important aspect of species diversity is the relationship between the size of a considered area and the number of species it contains. The species-area relationship has been discussed since the mid-19<sup>th</sup> century and was the subject of mathematical formulations in the early 20<sup>th</sup> century (Arrhenius 1921; Gleason 1922). Despite numerous studies, many aspects of the relationship remain unclear and debated. These aspects include how data on the species-area relationship should be collected (Hill et al. 1994; Scheiner 2003), how the data should be mathematically modeled (Tjørve 2003; Dengler 2009), whether and how parameters of species-area relationships can be interpreted biologically (Connor and McCoy 1979; Hill et al. 1994), and whether common patterns of species-area relationships exist across scales and communities (Singh et al. 1996; Fridley et al. 2005). A number of factors have been identified that may affect species-area relationships, such as successional changes (Lepš and Štursa 1989; Rejmánek and Rosén 1992), abiotic conditions (Weiher 1999; Désilets and Houle 2005), disturbance (Lepš and Štursa 1989), productivity (Pastor et al. 1996; Weiher 1999; Chiarucci et al. 2006), or management regimes (de Bello et al. 2007). However, the importance of factors may change with the considered scale (Schmida and Wilson 1985). For example, on the smallest scales, interspecific interactions (i.e., competition) are important (Grime 1973), particularly when plants are fully sessile (van der Maarel and Sykes 1993). With enlarging area, the role of interspecific interactions typically weakens and environmental heterogeneity becomes the main determinant of species richness (Schmida and Wilson 1985).

Spatial variation in species richness is commonly expressed by species-area curves (SAC), which are promising tools for testing potential factors and processes shaping diversity. They are typically estimated by one of two models: the Arrhenius model ( $S = C \times A^z$ , often presented as the log transformation:  $\log S = \log C + z \times \log A$ , Arrhenius 1921) and the Gleason model ( $S = C - z \times \log A$ , Gleason 1922). In these two models,  $S$  is species richness,  $A$  is area, and  $C$  and  $z$  are constants. The constant  $C$  ( $\log C$ ) is interpreted as (log) species richness per unit area and will be called the “intercept” hereafter. The constant  $z$  is interpreted as the rate at which species richness increases with enlarging area and will be called the “slope” hereafter. The Arrhenius and Gleason models are the most commonly used among plant ecologists, largely because they contain a small number of parameters (Connor and McCoy 1979; Lepš 2005) that can be easily and empirically understood. However, when the aim of a study is to identify the biological factors underlying the slope and intercept of SACs, researchers should realize that the different models have different fit to data (e.g., Lepš and Štursa 1989) and that logarithmic transformation changes the relationships between intercepts, slopes, and other factors determining species-area relationships. Although studies concerning plant communities usually consider the intercept of the SAC at the area of 1 m<sup>2</sup> (e.g., Lepš and

Štursa 1989; Chiarucci et al. 2006; de Bello et al. 2007), other sizes relevant to plant communities are equally possible (Rosenzweig 1995) and could help elucidate scale dependency of factors affecting species richness. Consequently, attempts to interpret parameters of SAC biologically require careful consideration of model choice (Tjørve 2003). With respect to the two most commonly used SAC models (Arrhenius and Gleason model), the simple question arises: How does model choice affect biological interpretation of SAC model parameters? To answer this question, we studied species-area relationships in wet meadows in the Železné hory Mts. (Czech Republic). These wet meadows are scattered in the landscape along small streams, in spring areas, and near fish ponds. The species diversity of those marginal habitats is endangered by abandonment, eutrophication, and drainage, and we explored which factors affect the spatial pattern of species richness in these endangered habitats. Using areas ranging from 0.01 to 4 m<sup>2</sup> for determining the intercept, we tested the relationships among SAC parameters (intercepts and slopes as determined by the Arrhenius and Gleason functions and their logarithmic transformations) and environmental characteristics (soil chemical properties and water table depth), community characteristics (productivity and evenness in cover of resident species), and characteristics of individual plants (persistence on a spot and clonal growth). Some characteristics (soil chemical properties, water table depth, and productivity) were tested because they are commonly thought to be relevant to species richness on a plot and could affect function intercepts. Other characteristics (evenness, persistence, and clonal growth) were tested, because they are thought to be relevant to spatial pattern and hence could primarily affect function slopes.

## Methods

### *Study area*

The study area is 340–620 m above sea level in the central part of the Železné hory Mts. (East Bohemia, Czech Republic). The landscape consists of a matrix of forests, arable land, cultural meadows, and urban areas. In this matrix, wet meadows survive as small (and very often abandoned) fragments. Among these wet meadows, there is a broad range in soil moisture, soil fertility, and soil reaction. We collected data in 22 localities of various types of vegetation (alliances *Caricion gracilis*, *Calthion*, *Molinion caeruleae*, *Caricion fuscaee*, *Sphagno recurvi*—*Caricion canescentis* and *Sphagno warnstorffiani*—*Tomentyption*, see Klimešová et al. (2011c) for detailed description; the nomenclature is according to Moravec (1995)).

### *Plant species richness and cover data*

At each locality, we established four 2x 2 m<sup>2</sup> plots in which vegetation was sampled. In every 2 × 2 m<sup>2</sup> “sampling plot,” vegetation was assessed using phytosociological relevés (cover scale: r = rare plant with 1–2 individuals; + = cover less than 1 %; cover greater than 1 % was estimated to the nearest 1 % (up to 20 %) or to the nearest 5 % (over 20 %)). The cover data were used for species-evenness evaluations. For computation of

SAC coefficients, we determined species composition in a nested design over a range of spatial scales from 0.01 to 4 m<sup>2</sup>. The species richness at the largest scale (4 m<sup>2</sup>) was extracted from the phytosociological relevés data. Data for smaller areas (0.01, 0.04, 0.09, 0.16, 0.25, 0.36, 0.49, 0.64, 0.81, and 1 m<sup>2</sup>) were obtained using 1 x 1 m<sup>2</sup> “presence plots,” which were established in the center of each sampling plot and were divided into 100 0.1 × 0.1 m<sup>2</sup> “small plots.” In each small plot, all rooting species were recorded (see Klimešová et al. (2011c) for detailed information). The average species richness was calculated for all possible nested squares that could be placed in the 1 × 1 m<sup>2</sup> area of a presence plot. In total, we sampled data for 88 SACs (four replicates at 22 localities) from 11 spatial scales (0.01, 0.04, 0.09, 0.16, 0.25, 0.36, 0.49, 0.64, 0.81, 1, and 4 m<sup>2</sup>) for every model.

### *SAC models*

In order to test species-area relationships, we used two types of models: the Arrhenius model and the Gleason model. Our statistical analysis also included the logarithmic transformations of both models so that we could evaluate whether the log transformation of the models affects the relationships of the SAC parameters and studied characteristics. Thus, we used four models of SAC:

#### 1) Arrhenius model

$$S = c \times A^z$$

#### 2) Gleason model

$$S = d + y \times \log A$$

#### 3) log Arrhenius model

$$\log S = e + x \times \log A$$

#### 4) log Gleason model

$$\log S = \log (f + w \times \log A)$$

In order to simplify the text, we refer to the constants c, d, e, and f as “intercepts” and the constants w, x, y, and z as “slopes,” although doing so is not correct in a mathematical sense.

### *Environmental characteristics*

The main ecological factors determining the composition and diversity of meadow vegetation in Central Europe are soil water content, nutrient availability, and soil reaction (Ellenberg 1996). We measured these abiotic parameters in the following way.

#### *Groundwater depth*

The depth of the water table was measured using perforated plastic tubes (40 mm in diameter) that were inserted to 90 cm depth and left in the soil for the duration of the sampling period. The measurements were repeated seven times per year during the years 2008–2010 at monthly intervals from April to November. Average values for the five tubes, the seven measurement dates, and 3 years at each locality were used for analyses (the dryer the locality, the greater the value for groundwater depth).

#### *Soil characteristics*

Soil was sampled from the A horizon to 15 cm depth and was analyzed for basic nutrients, soil reaction, and organic matter content. All the characteristics were determined for each sample, and the average value of five samples per locality was used for analyses. Total nitrogen was extracted using the Kjeldahl method (Novozámský et al. 1983), total phosphorus was extracted with perchloric acid (Kopáček and Hejzlar 1995), NO<sub>3</sub> and NH<sub>4</sub> ions were extracted by leaching with KCl (Anonymous 1997), and PO<sub>4</sub> was extracted by leaching with CaCl<sub>2</sub> (Houba 1994). Extracted nutrients were quantified with a FIAstar flow analyzer (Tecator, Sweden). Organic matter content was determined by burning the samples at 550 °C, and the exchangeable soil reaction was measured in a KCl solution according to ISO 10390 (Anonymous 1992).

#### *Community characteristics*

##### *Productivity*

The community productivity was assessed as aboveground biomass of the stand on the two 1 × 1 m<sup>2</sup> presence plots per locality in July 2007 after species composition data were recorded. Aboveground biomass (excluding litter and mosses) was clipped 5 cm above the soil surface, dried for 12 h at 85 °C, and weighed. The average dry mass per locality was used for analyses. Data on plant productivity were log-transformed to improve normality and homoscedasticity.

##### *Evenness*

The description of community composition can be divided into two measures: species richness (i.e., how many species are present in the community) and evenness. Evenness refers to the distribution of the abundance of resident species in a community (Smith and Wilson 1996). Except for some theoretical studies and proposals (Martín and Goldenfeld 2006; Borda-de-Água et al. 2002), there is a lack of studies, especially of experimental

studies exploring the patterns among evenness and the species-area relationship. Many evenness indices have been developed for the study of vegetation but the Shannon-Wiener and Simpson indexes are the most commonly used. These two indexes are species richness dependent (Smith and Wilson 1996). In order to avoid the co-varying of evenness and species richness, we used the converted index of dominance to describe species evenness:

$$E = 1 / (D \times S);$$

where S is total species richness, and D is the index of dominance:

$$D = \sum (n_i/n)^2;$$

where  $n_i$  is cover of  $i$ th species, and  $n$  is total cover. We used cover from the phytosociological relevés for evenness computations.

#### *Plant characteristics*

We selected traits concerning the persistence/mobility of a plant (shoot cyclicality, persistence of connection, and lateral spread) and traits concerning plant aggregation (multiplication rate and lateral spread). Traits were assigned to species according to the CLO-PLA database (Klimešová and Klimeš 2006). The three or four categories for each trait from the database were merged into two broader ones, because some categories were rarely represented (fewer than five times) in the species list and because of multivalent categorization for some species (the CLO-PLA database contains multiple data from various sources). In such cases, we first averaged the values within the records and then averaged the data among the records.

#### *Shoot cyclicality (cyclicality)*

Cyclicality denotes shoot life-span, i.e., the number of growing seasons between shoot sprouting and shoot flowering and death. Two categories were used: 1 year and more than 1 year (aggregation of categories 2 and >2 in CLO-PLA).

#### *Persistence of connection (persistence)*

Persistence of connection describes how long parent and offspring ramets are interconnected. Plants with persistent connection (integrators) prefer less productive habitats than plants with less persistent connections (splitters) (Klimešová et al. 2011a). Two categories were used for persistence: 1 year and more than 1 year (aggregation of categories 2 and >2 in CLO-PLA).

#### *Multiplication rate*

Multiplication rate describes how many new shoots a parental shoot is able to produce in 1 year. This trait is relevant to meadow management, and plants with high multiplication



rates tend to be favored by mowing (Klimešová et al. 2011c). Two categories were used for multiplication rate: 1 shoot or fewer per year (aggregation of categories <1 and 1 in CLO-PLA) and 2 or more shoots per year (aggregation of categories 2–10 and >10 in CLO-PLA).

#### *Lateral spread*

The horizontal spread of a clonal plant is enabled by the growth of rhizomes, stolons, etc. Lateral spread is promoted on more productive habitats (Klimešová et al. 2011a). Two categories were used for lateral spread: less than 1 cm/year and more than 1 cm/year (aggregation of categories 1–25 and >25 in CLOPLA).

For all traits, the mean proportion of the categories in all 88 of the  $2 \times 2 \text{ m}^2$  plots was computed with all species given the same weight (i.e., abundance was ignored). Because all of the traits used only two categories, which were complementary, only one was used in computations (cyclicality >1 year, persistence >1 year, multiplication rate  $\geq 2$  shoots per year, and lateral spread >1 cm/year).

#### *Statistical analyses*

Relationship among studied characteristics In order to avoid co-linearity among explanatory variables, we calculated a correlation matrix and tolerance values for the remaining variables (Quinn and Keough 2002, online resource 1). Since soil characteristics concerning nutrient availability (total nitrogen, total phosphorus,  $\text{NO}_3$ ,  $\text{NH}_4$ ,  $\text{PO}_4$ , and organic matter content) were strongly and positively correlated with each other (tolerance values were <0.25), we created one variable (“soil fertility”) that combined soil nutrient and organic matter contents. Soil fertility was calculated with principal component analysis (PCA) and expressed as the scores on the first ordinal axis. The soil characteristics were centered and standardized by the standard deviation value. The ordination of localities and the three groups of characteristics (environmental, community, and plant) were computed with a, and the characteristics were centered and standardized by the standard deviation value. All multivariate analyses were conducted in the CANOCO software (ter Braak and Šmilauer 2002). The coefficients of SACs were computed using nonlinear regressions. In order to compare the fit of SAC parameters to empirical data, we used the Akaike information criterion. Since sample number was relatively low (SACs were constructed upon 11 points), we used the corrected form (AICc). As shown by Rosenzweig (1995), the intercept is scale dependent; therefore, we constructed SACs upon different scales (the area for which the intercept was calculated: 0.01, 1, and  $4 \text{ m}^2$ ) to test whether the effect of the predicting factors on species richness differs among scales. Similarly, the relationships among the SAC coefficients and predicting factors were analyzed by the lowest AICc criteria. The analyses were computed separately for each group of factors (environmental, community, and individual characteristics). In the first step, we created linear models:  $X \sim Y$ , where X

was the coefficient of the SAC (slope or intercept) or the fit of the SAC (AICc) and Y was all single factors from one group (environmental, community, and individual) and all their combinations. For the group “community”, for example, we created three models:  $X \sim \text{productivity}$ ;  $X \sim \text{evenness}$ ; and  $X \sim \text{productivity} + \text{evenness}$ . In summary, 24 models were compared: four kinds of models (Arrhenius, Gleason, log Arrhenius, and log Gleason)  $\times$  two dependent variables (slope, and intercept)  $\times$  three groups of characteristics (environmental, community, and plant). The locality was assigned as a random factor. All these remaining statistical computations were conducted with the R-statistics software, version 2.13.1 (R Development Core Team, 2009). We used the function “AICcmodavg” package (Mazerolle 2011) to assess the best model from every group of created models.

## Results

### *Fitting models*

In total, 147 species were found across the 88 plots at 22 localities. Species richness ranged from 15 to 51 species at the largest scale (4 m<sup>2</sup>) and from 0.88 to 11.23 species at the smallest scale (0.01 m<sup>2</sup>; mean values from presence plots, see online resource 2). The Gleason model showed better fit than the Arrhenius model in both basic (81 of 88 models) and logarithmic form (77 of 88 models) (Online Resource 2, see also the graphical illustration of fitting of the four SAC models on Fig. 1). Slopes and intercepts of the models were significantly correlated. The correlations were negative in the Arrhenius model but positive in the Gleason model. The strength of correlations varied along the spatial scale for which the models were computed. The Arrhenius model and the log Arrhenius model showed the strongest correlations between SAC coefficients when they were computed for the smallest area (intercept at 0.01 m<sup>2</sup>). On the other hand, correlations between SAC coefficients of the Gleason and log Gleason models were the strongest when they were computed over the largest area (intercept at 4 m<sup>2</sup>) (Table 1).

### *Correlation among predicting factors*

Interrelationships of the environmental, community, and individual plant characteristics are shown in Fig. 2. Soil fertility was negatively correlated with water table depth. Evenness was negatively correlated with productivity. Persistence and cyclicity were negatively correlated with lateral spread. Productivity was negatively correlated with soil fertility.

### *SAC slopes—predicting factors*

For community characteristics, correlations between slopes and evenness were negative for all models except the log Gleason model. For individual characteristics, correlations between slopes and persistence and lateral spread were positive for the Arrhenius and log Arrhenius models (Table 2).

### *SAC intercepts—predicting factors*

Correlations between intercepts and characteristics were significant only when they were computed upon the smallest area (intercept at 0.01 m<sup>2</sup>), i.e., correlations were never significant at the scale of 1 or 4 m<sup>2</sup>. For environmental characteristics, correlations between intercepts were not significant for any factor (Table 3). For community characteristics, correlations between intercepts and evenness were positive for all models except the Arrhenius model. For individual characteristics, correlations between intercepts and persistence of the connection among ramets were negative for all models except the log Arrhenius model (Table 3). We tested whether relationship between productivity and intercepts was rather non-linear; however, a quadratic model was not significant (data not shown). Despite this, relationship between species richness at a small scale (per 0.01 m<sup>2</sup>) and productivity (average per 1 m<sup>2</sup>) was nearly hump shaped.

### **Discussion**

The sensitivity of the SAC model parameters to environmental, community, and individual plant factors depended on the model (Arrhenius, Gleason, log Arrhenius, or log Gleason) and on scale, i.e., on the plot size for which an intercept was calculated (0.01, 1, or 4 m<sup>2</sup>). The slope of Arrhenius and log Arrhenius models, the intercept of Gleason and log Gleason models, and the smallest spatial scale were the most sensitive SAC model parameters for the examination of environmental, community, and individual plant characteristics. Both logarithmic and non-logarithmic versions of the models enabled consistent biological interpretation of the models; this was not affected by the model fit to the data.

The slopes of the curves were affected both by characteristics that are generally thought to be relevant to species richness (soil properties) and by small-scale spatial distribution of plant individuals and their mobility (evenness, lateral spread, and persistence). These results suggest that, in our study system, a fine-grained spatial pattern of species composition was typical for high diversity plots, whereas an aggregated pattern (one with a few dominants and scattered subordinate species) was typical for low diversity plots. These patterns were associated with soil properties but not with productivity. The low importance of soil fertility in this relationship is surprising. A possible explanation is that nutrients and organic matter are conserved by the high water table in our study area, which limits nutrient availability for plants (Verhoeven et al.

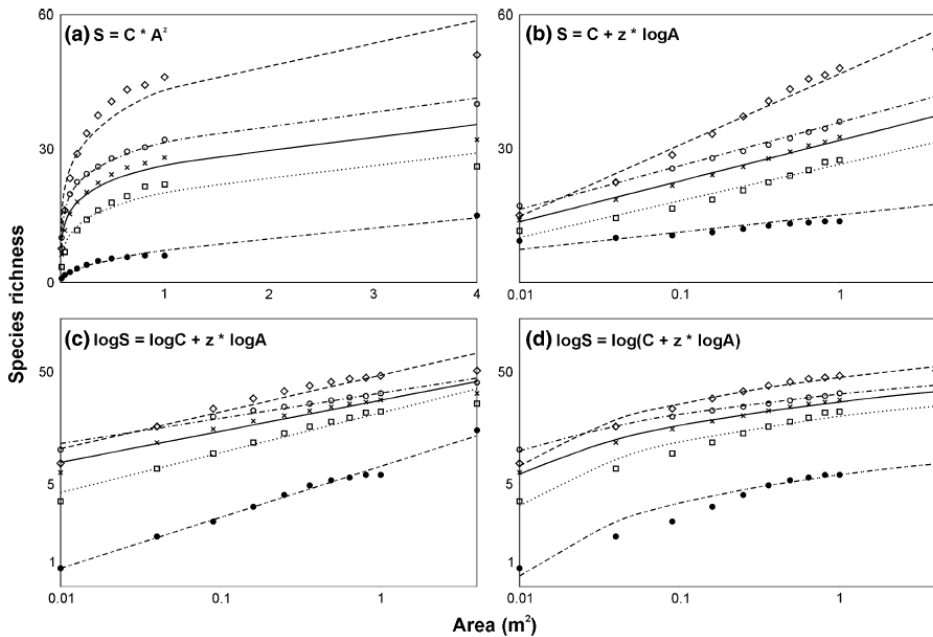


Fig. 1 Examples of SAC curves (five out of 88) fitted by four models (a Arrhenius, b Gleason, c log Arrhenius, and d log Gleason). Symbols are observed values. The five curves were selected to cover broad spectrum of vegetation types; diamonds *Caricion fuscaea*, empty circles species rich *Calthion*, crosses *Molinion caeruleae*, squares *Caricion gracilis*, full circles species poor *Calthion*

Table 1. Correlations between slopes and intercepts of four tested models

Model	Scale (area for which the intercept was calculated)					
	0.01 m <sup>2</sup>		1 m <sup>2</sup>		4 m <sup>2</sup>	
	Pearson <i>r</i>	<i>p</i>	Pearson <i>r</i>	<i>p</i>	Pearson <i>r</i>	<i>p</i>
Arrhenius	-0.648	<0.0001	-0.390	0.0002	-0.253	0.0173
Gleason	0.350	0.0008	0.925	<0.0001	0.950	<0.0001
log Arrhenius	-0.691	<0.0001	-0.205	0.0549	-0.019	0.8638
log Gleason	0.618	<0.0001	0.972	<0.0001	0.981	<0.0001

1993). This argumentation is supported by the negative correlation between soil fertility and water depth in our dataset (Fig. 2). In contrast, none of the three environmental characteristics that are generally thought to be relevant to species richness on a plot (soil chemical properties, water table depth, productivity Ellenberg (1996)) affected SAC intercepts. Surprisingly, this basic parameter of species diversity was mainly determined by characteristics relevant to small-scale spatial pattern and mobility of species in a community (evenness and persistence). The lack of an obvious relationship between productivity and intercepts on SAC models at any examined scale might indicate that the relationship between productivity and species richness is not linear but “hump-shaped,”

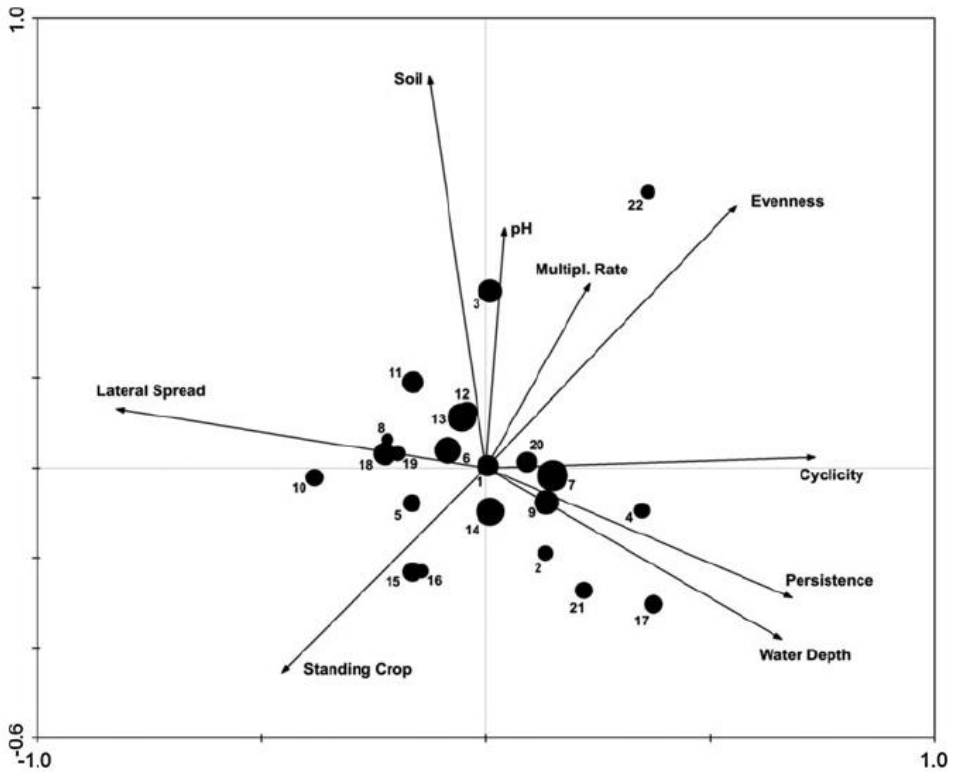


Fig. 2 Relationship among environmental characteristics (soil, pH, and water depth), community characteristics (evenness and productivity), and individual plant characteristics (cyclicality, persistence, multiplication rate, and lateral spread) for the studied data-set as visualized by a PCA ordination diagram. Six environmental characteristics were combined into the characteristic soil (organic matter, P total, N total, PO<sub>4</sub>, NO<sub>3</sub>, and NH<sub>4</sub>). Points 1–22 represent centroids of four sampling plots per locality, and their diameter corresponds to mean species richness per 2 x 2 m<sup>2</sup> plot

Table 2. The correlations between slopes of Arrhenius, log Arrhenius, Gleason, and log Gleason models and three groups of characteristics (environmental, community, and individual plant).

Model	Environmental			Community			Individual		
	<i>r</i>	Factor	<i>p</i> value	<i>r</i>	Factor	<i>p</i> value	<i>r</i>	Factor	<i>p</i> value
Arrhenius	-0.02	Soil	0.0072	-0.17	Evenness	0.0001	0.38	Persistence	<0.0001
							0.41	Lateral spread	0.0001
Gleason	-	-	-	-2.3165	Evenness	0.0164	-	-	-
log Arrhenius	-0.02	Soil	0.0152	-0.19	Evenness	<0.0001	0.30	Persistence	0.0020
							0.34	Lateral spread	0.0010
log Gleason	-	-	-	-	-	-	-	-	-

- Not significant

as has often been reported (Al-Mufti et al. 1977 and others). Depicting the relationship between species number at the smallest scale (0.01 m<sup>2</sup>) and plot productivity partly supported this idea (Fig. 3) (although polynomial regression of intercepts and productivity at any of examined scales was not significant, data not shown). Moreover, the hump-shaped scatter of plots in the biplot indicates that productivity and species richness are clearly related to the lateral spread and persistence of connection among ramets (Fig. 3). The ascending arm of the hump-shaped diversity-productivity relationship correlates with increasing lateral spread and decreasing persistence of connection among ramets, while the descending arm correlates again with increasing lateral spread but not with persistence, which remains constant. The hump-shaped diversity-productivity relationship reported from herbaceous communities by numerous authors (Al-Mufti et al. 1977; Pastor et al. 1996) was hypothesized to be caused by plant clonality (Laanisto et al. 2008). Researchers have also proposed that the descending arm of the hump-shaped productivity-diversity relationship in grasslands can be explained by the competitive superiority of clonal plants (due to their foraging behavior) in heterogeneous environments (Eilts et al. 2011). A problem with this explanation is that most species in grasslands grow clonally (Klimeš et al. 1997), and so clonality would not result in a competitive advantage. Clonality, however, might explain various spatial patterns that result in the hump-shaped diversity-productivity relationship, as discussed in the next paragraph.

Table 3. Correlations between intercepts of Arrhenius, log Arrhenius, Gleason, and log Gleason models (at a scale of 0.01 m<sup>2</sup>; correlations were not significant at scales of 1 or 4 m<sup>2</sup>) and three groups of characteristics (environmental, community, and individual plant)

Model	Environmental			Community			Individual		
	<i>r</i>	Factor	<i>p</i> value	<i>r</i>	Factor	<i>p</i> value	<i>r</i>	Factor	<i>p</i> value
Arrhenius	–			–			–11.02	Persistence	0.0309
Gleason	–			7.35	Evenness	0.0001	–14.11	Persistence	0.0049
log Arrhenius	–			4.26	Evenness	0.0035	–		
log Gleason	–			3.91	Evenness	0.0011	–6.65	Persistence	0.0381

– Not significant

According to our study, the hump-shaped diversity-productivity relationship might be explained by the spatial mosaic caused by different combinations of clonal traits (like lateral spread and persistence). While high diversity plots in our dataset were characterized by even abundance and by plant species with intermediate lateral spread and low persistence of rhizomes, low diversity plots were characterized by an aggregation of dominants. This aggregated or clumped spatial pattern resulted in low diversity in small plots at both the low and high end of the productivity gradient. The trends mentioned above are illustrated in Fig. 3: localities located in the bottom left of the biplot are species poor and unproductive and are dominated by tussock graminoids

(e.g., *Deschampsia cespitosa*, *Nardus stricta*, *Festuca filiformis*, or *Carex davalliana*) with low lateral spread and high persistence of connection among ramets; localities in the middle of the biplot have the highest species diversity and intermediate productivity and are characterized by an even distribution of abundances, intermediate lateral spread, and short-lived connection among ramets; and the less species rich and productive localities in the right of the biplot are dominated by tall herbs with long rhizomes (e.g.,

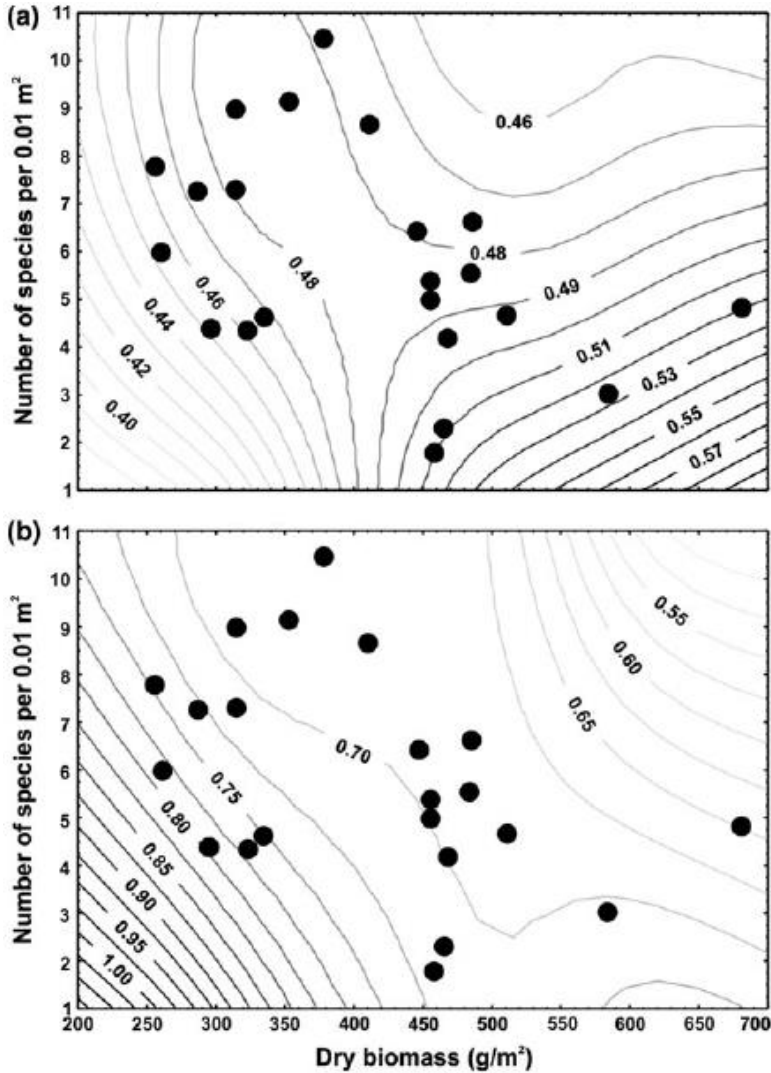


Fig. 3 Relationship between productivity (average per 1 m<sup>2</sup>) and species richness at a small scale (per 0.01 m<sup>2</sup>) for 22 meadows in the Železné Hory Mts. The upper graph shows how the relationship is affected by average lateral spread for community weighted by species abundance, and the bottom graph shows how the relationship is affected by persistence among ramets for community weighted by species abundance

*Filipendula ulmaria*, *Scirpus sylvaticus*, and *Carex acuta*). Damgaard et al. (2012) found a similar relationship between plant aggregation and plant productivity/species richness in acidic grasslands, and proposed that this relationship indicates that species diversity is threatened.

Previous research has established that increasing nutrient availability is likely to have neutral effects on species-area relationships because an increase in nutrients causes an increase in shoot size but a decrease in shoot number (Oksanen 1996; Stevens and Carson 1999). Since the lateral spread of rhizomatous plants correlates with their shoot size (Klimešová et al. 2011b) and clonal rhizomatous plants are stronger competitors than non-clonal plants, clonality also contributes to the random processes responsible for species richness decline in productive plots (Suding et al. 2005, Eilts et al. 2011). Our results indicate that the spatial pattern generated by plant clonality affects species richness of herbaceous communities at small scales. The relevance of clonality to species-area relationships in other herb-dominated ecosystems remains to be tested.

## Conclusions

The aim of this study was to determine how model choice affects biological interpretation of SAC parameters. Based on analysis of a data-set from 22 wet meadows, we showed that:

(1) The slope of Arrhenius and log Arrhenius models and the intercept of Gleason and log Gleason models were the most sensitive SAC model parameters for the examination of environmental, community, and individual plant characteristics.

(2) The spatial scale that revealed which biological factors explained species diversity in our study system was the smallest considered (0.01 m<sup>2</sup>).

(3) The factors that most affected species richness (and hence SAC model parameters) at the small scale were the spatial pattern of community structure and soil properties. Persistence of connection among ramets and lateral spread were correlated with the parameters of species richness. Thus, as a side-result we provided the first data in support of the idea that clonal traits affect the species-area relationship by creating small-scale mosaics of species in a community.

(4) Although we found no contradictory results, the models differed in factors they were correlated with and the models with best fit were not the most sensitive to tested biological factors. We, therefore, advocate against use of the best fit criteria as the problem deserves deeper study.

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## **Chapter III**

### **Effect of the method of assessing and weighting abundance on the interpretation of the relationship between plant clonal traits and meadow management.**

Klimešová, J., Janeček, Š., Horník, J., & Doležal, J. (2011). *Preslia*, 83(3), 437-453



## Effect of the method of assessing and weighting abundance on the interpretation of the relationship between plant clonal traits and meadow management

### **Abstract**

The role of clonal traits in a plant's response to changes in management of semi-natural grasslands is poorly known and the few studies examining their importance have yielded contradictory results. For a better understanding of the role of plant functional traits in determining competitive ability and clonal growth in response to early changes in management, we mowed and applied fertilizer to 22 wet meadows in the Železné hory Mts, Czech Republic. We used two methods of assessing abundance (plant cover and species frequency) to determine whether changes in frequency induced by changes in management are better predicted by clonal traits while changes in cover are mainly determined by competitive traits such as plant height. We evaluated (i) the response of individual species to changes in management and (ii) the response of the whole community, with and without taking abundance of individual plants into account, in order to separate the effect of local extinction and immigration from changes in abundance. The plant functional traits tested were generally found to be important soon after the changes in the management of the semi-natural grasslands occurred: competitively superior resident species (possessing tall erosulate, monocyclic shoots) that are able to spread far and multiply clonally (having a high clonal index) were favoured by applying fertilizer and/or suppressed by mowing. Some other traits supposed to be important in the response to changes in management did not change (persistence of connection between ramets). Results for the two methods of assessing abundance differed; however, neither was better at detecting the response of particular types of traits (i.e. relevant to clonal growth and competitive ability). The initial response of the whole community, with and without taking abundance of individual plants into account, was consistent indicating that species that went extinct possessed the same traits as those that decreased in abundance. The clonal index proved to be a useful characteristic of meadow plants. Our results further imply that (i) the method used to assess abundance significantly affects the output of analyses of the response of functional traits, and (ii) a comparison of analyses based on weighting abundance and unweighted means resulted in a deeper insight into the changes in the spectra of functional traits that occurred after changes in meadow management.

Keywords: abandonment, clonal traits, clonal index, fertilization, mowing, plant height, seminatural grasslands, SLA

## Introduction

The most serious losses in plant species diversity in temperate Europe are connected with changes in land use, namely the application of fertilizer and abandonment of semi-natural grasslands, and their fragmentation and conversion into arable land (Stöcklin et al. 2000, Isselstein et al. 2005, Rudmann-Maurer et al. 2008). Besides changes in species diversity and species composition, changes in the spectra of functional traits are also recorded (Maurer et al. 2003, Kahmen & Poschlod 2004, 2008, Díaz et al. 2007, Garnier et al. 2007, Römermann et al. 2008, Pakeman et al. 2009, Pakeman & Marriott 2010); following both abandonment and the application of fertilizer, tall competitive plants with erosulate shoots and high specific leaf area (SLA) replace smaller plants with rosette shoots and low SLA. The clonal traits, which are important for local spread and plant persistence, have so far only rarely been assessed in semi-natural grasslands subjected to changes in management. A comparative analysis of the clonal growth organs of plant species on red lists vs those in entire floras indicate that plants with root tubers are especially prone to extinction (Klimeš & Klimešová 2000). On the other hand, there is much controversy about how other clonal traits change with abandonment. Some studies record that the meadow species that spread after the cessation of management are typically those with a high lateral spread and high rate of multiplication (Kahmen et al. 2002, Pakeman & Marriott 2010), while others report opposite trends (Kahmen & Poschlod 2008, Sammul 2011). Studies on clonal growth in relation to environmental gradients indicate that plants attaining dominance after the application of fertilizer or abandonment are characterized by the ability to spread laterally over great distances and split up connections among ramets (Klimešová et al. 2011). However, detection of a general pattern might be difficult as results of analyses are affected by the way the traits are scaled at the community level, i.e. whether plant abundance is taken into account and which measurement of plant abundance is used (Guo 2003, Sammul 2011).

Plant communities are usually dominated by a few species and the other species are less abundant. Therefore species-based and abundance-weighted analyses can produce different results (Pakeman et al. 2008), as is also demonstrated for clonal traits (Latzel et al. 2011, Sammul 2011). Many authors (e.g. Grime 1998, Cingolani et al. 2007, Mokany et al. 2008) argue that dominant species better reflect ecosystem processes than subordinate species. The rare species are, however, important for species richness and might be characterized by unique functional traits that substantially affect ecosystem functioning (Grime 1998) and the functional richness of a community (Thompson et al. 2010). Because rare plants are more prone to random extinction when habitats change, their inclusion in analyses may affect the results, and in the case that the rare species have unique traits will result in the loss of part of the functional diversity, but the reason for their extinction is not necessarily their unique traits but their rarity (Grime 1998). These random effects diminish with time and do not affect the results of studies that focus on the consequences of changes in management for plant traits in a

community long after the first application of different treatments (e.g. Kahmen & Poschold 2008, Pakeman & Marriott 2010). However, they might be important when assessing the initial response of plants in a community with the aim of detecting the key traits that play a role at the beginning of the process as a whole.

To understand the role of functional traits in the divergent developments that follow the establishment of different management treatments, we focused on the early course of events in the responses of meadow species and whole plant communities. We expected shifts in species abundance rather than substantial changes in species composition. In this case, weighting the data by species abundance or not have implications as a community mean calculated from presence/absence data reflects changes in species composition and is affected by local extinction of rare species, whereas a community mean weighted by species abundance includes effects of changes in abundance.

To study the response of plants with different clonal traits to management, we performed a manipulative experiment in 22 wet meadows spread across a landscape mosaic in the Železné hory Mts, Czech Republic. Different management treatments combining mowing and application of fertilizer were applied for three years in a fully factorial design in each meadow. Besides the seldom studied focal clonal traits we tested whether the clonal index (defined by Johansson et al. 2011) as a proxy of the degree of clonal growth responds to changes in management. We also included in the analysis the more often studied traits relevant to competitive ability and carbon economy.

There are several methods for assessing species abundance, e.g. plant cover, plant frequency, shoot density and biomass (Kent & Coker 1992, Dierschke 1994, Bråkenheim & Liu 1995, Mucina et al. 2000, Röttgermann et al. 2000, Brathen & Hagberg 2004). While in some methods the abundance of a plant species having the same number of shoots is affected by shoot size (plant cover, biomass), in others it is affected by shoot number (plant density and frequency in small plots). The relationship between plant functional traits and meadow management might therefore be affected by the method used to assess plant abundance (e.g. Chiarucci et al. 1999, Pavlů et al. 2009, Sammuli 2011). In our study two assessments of abundance, cover and frequency, were used. We may expect that estimates of cover are more sensitive to changes in management because it records the increasing/decreasing size of individual shoots together with changes in their number, whereas frequency assessment records only changes in shoot number. It is moreover expected that changes in plant cover are explained better by traits connected with plant dominance and carbon economy, whereas changes in plant frequency will be explained better by traits connected with clonal growth.

We presumed that (i) competitive resident species with tall erosulate and monocyclic shoots would be able to spread far, multiply clonally and have annual shoots, and that their large SLA and splitting rhizomes would be favoured by the application of fertilizer and/or suppressed by mowing; (ii) assessments of abundance using plant frequency would be more sensitive for detecting the response of plants that employ different clonal traits, while cover will be more sensitive for detecting the

response of plants that employ different competitive traits; and (iii) the results at the community level would be affected by the method of weighting abundance and thus the same results from particular analyses would mean that the extinct species and species with decreasing abundance did not differ in traits.

## **Methods**

### *Study area*

The study area was located at an elevation of 340 to 550 m a.s.l. in the Železné hory Mts, eastern Bohemia, Czech Republic. The landscape consists of a mosaic of forests, arable land, intensively managed meadows and urban areas. Semi-natural, species-rich wet meadows occupy less than 5% of the area (Fig. 1), but host more than 70% of endangered Klimešová et al.: Plant clonal traits and meadow management 439 plant species (Horník & Hrázský 2009). For the experiment we selected 22 wet meadows (Electronic Appendix 1) distributed over an area of  $9 \times 21.5$  km, covering a range of soil moisture, fertility, soil reaction values and types of management.

### *Experiment*

Blocks of experimental plots  $4.5 \times 4.5$  m in size were established in spring 2007 at each of the 22 meadows. Each block consisted of 4 plots ( $2 \times 2$  m) representing four treatments in full factorial design: application of fertilizer and mowing, application of fertilizer and abandonment, no application of fertilizer and mowing, no application of fertilizer and abandonment. The buffering zone between the plots was 0.5 m wide. Plant cover was assessed in the plots, using the following scale: r = rare plant with 1–3 individuals, + = cover less than 1%; cover higher than 1% was visually estimated with an accuracy of 1% (up to 20%) and 5% (over 20%), respectively. The semi-quantitative values + and r were transformed for analyses as: r = 0.001% and + = 0.5%. In total the plant cover in 88 plots was assessed during the season. Plant frequency was assessed for plant shoots rooting in  $10 \times 10$  cm quadrates in the central  $1 \times 1$  m quadrate of a plot, altogether there were 88 assessments per season (2007, 2009). Baseline data on vegetation composition were recorded in the first half of July 2007. Mowing was done after vegetation assessment and then in the following years at the same time of year. Fertilizer was applied at a dosage of 20 g mineral NPK (10% N, 10% P<sub>2</sub>O<sub>5</sub>, 10% K<sub>2</sub>O) per m<sup>2</sup> at the end of July 2007 and 50 g/m<sup>2</sup> in the second half of April 2008 and 2009. The dosage represents the upper amount recommended by the producer for extensive grasslands (30–50 g/m<sup>2</sup>). Data on the response to the short period of management were collected in the first half of July 2009. Abundance was assessed by two or three observers who worked together and consulted over identification and adjusted cover



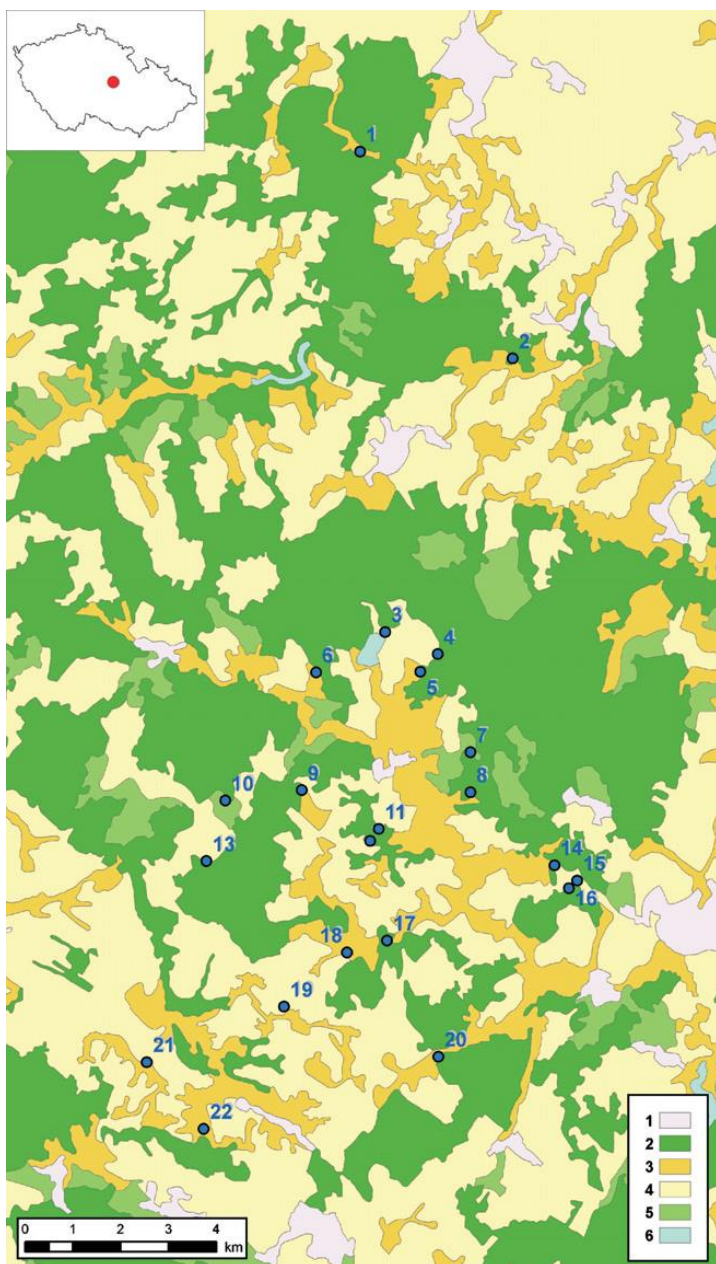


Fig. 1. – Land cover map of the Železné hory Mts with their location in the Czech Republic. Corine land cover categories: 1 – Artificial surfaces (including Discontinuous urban buildings, Industrial or commercial units, Mineral extraction sites, Sport and leisure facilities); 2 – Forests (including Coniferous forest, Mixed forests, Transitional woodland-shrub); 3 – Land principally used for agriculture, with significant areas of natural vegetation; 4 – Nonirrigated arable land; 5 – Pastures; 6 –Water bodies. Blue dots (1–22) denote the localities studied; for their characteristics see Table 1

estimates in order to avoid identification or estimation biases (for species list, see Electronic Appendix 2).

### *Plant functional traits*

We selected eight functional traits indicating (i) competitive ability (shoot height, shoot cyclicity, shoot architecture), (ii) clonal growth (multiplication rate, lateral spread, clonal index) and (3) carbon economy (SLA, persistence of ramet connection) (for details, see Table 1).

### *Statistical analysis*

Both univariate and multivariate analyses were performed to reflect the BACI (Before-After/Control-Impact) design of the experiment. In this design, the main interest is not the effect of the treatment itself but its effect on changes that occur after treatment was applied.

Table 1. – Plant functional traits used in the analyses.

Function	Trait	Attributes	Source
Competitive ability	shoot height	cm	Kubát et al. 2002
	shoot cyclicity	1; 2; > 2 years	CLO-PLA database <sup>1</sup>
	shoot architecture	rosulate; semirosette; erosulate	CLO-PLA database <sup>1</sup>
Clonal growth	multiplication rate	< 1; 1; 2–10; >10 shoots per mother shoot per year	CLO-PLA database <sup>1</sup>
	lateral spread	< 0.01; 0.01–0.25; > 0.25 m per year	CLO-PLA database <sup>1</sup>
	clonal index	sum of ordinal values of multiplication rate and lateral spread	CLO-PLA database <sup>1</sup> according to Johansson et al. 2011
Carbon economy	specific leaf area	gram per m <sup>2</sup>	LEDA traitbase <sup>2</sup>
	persistence of ramet connection	1/2;>2 years	CLO-PLA database <sup>1</sup>

<sup>1</sup>Klimešová & Klimeš 2006, <sup>2</sup>Kleyer et al. 2008.

### *Effect of the application of fertilizer/mowing on the composition of the vegetation*

To test the effect of treatment on changes in the composition of the vegetation (in terms of plant frequency or plant cover) we used redundancy analysis (RDA) with the Monte Carlo permutation test in the Canoco 4.5 program (ter Braak & Šmilauer 2002). Our analyses were analogous to those described by Lepš & Šmilauer (2003, p. 215–235). RDA represents the constrained form of principal component analysis (PCA), in which the canonical axis is defined (constrained) by linear combinations of explanatory variables.

In our analyses, following the BACI design of our experiment, we were interested in the effect of Treatment (mowing or application of fertilizer) × Year interaction. As we used only one explanatory variable in each of the analyses (the interaction), the explanatory variable was fully associated with the first ordination axis (see Table 1). We used as covariates (i) the interaction between year and treatment that was not included in

the explanatory interaction term to eliminate the vegetation changes caused by this factor and (ii) the plot identifiers (Plot ID) coded as 88 dummy variables, to eliminate between-plot variation. Because the Plot IDs were used as covariates the main effect (application of fertilizer and mowing) does not explain any variability and we finally did not include it as a covariate. In the sample-species matrix, data were centered by species and non-standardised by samples.

Data on percentage cover were  $\log(y+1)$  transformed as recommended by Lepš & Šmilauer (2003, p. 13–15). Using the Monte Carlo permutation test a split-plot permutation design was employed: assessments of abundance made in different years in the same plot were considered as split-plots, whereas individual plots represented the whole plots. Permutations at both split-plot and whole-plot levels were performed 499 times. Species scores on the first (canonical) axis, the one determined by our single explanatory variable, represented the responses of the species to individual treatments and were used in the following analysis as the plant characteristics that were to be associated with particular plant traits. For more details on the RDA analysis in CANOCO see ter Braak & Šmilauer (2002) or Lepš & Šmilauer (2003), in R software Borcard et al. (2011).

#### *Effect of the application of fertilizer/mowing on plant traits: response of individual species*

In these analyses we aimed to explain plant responses to treatments (responses were determined by RDA scores from previous analyses) in terms of particular plant traits. These analyses were performed for 93 species of plants, which were present in more than eight plots. Rare species were excluded as recommended by Lepš & Šmilauer (2003) as they may not be responding, because they are missing from most of the plots both before and after the treatment.

Individual plant traits (except for shoot architecture) were considered quantitative variables. The quantitative traits were calculated considering semi-quantitative categorization in the CLO-PLA database and the number of records for each category. If plant traits were classified into more than one semi-quantitative category in the CLO-PLA database (Klimešová & Klimeš 2006), we calculated the weighted mean of the trait. In this case, weighting by number of records in the category was used, e.g. when four records of lateral spread were in category 2 (0.01–0.25 m/year) and seven in category 3 (> 0.25 m/year), then the used value in the analyses was  $((4 \times 2) + (7 \times 3)) / (4 + 7) = 2.64$ . To analyze the effect of plant traits on plant response to management we used backward stepwise selection in general regression models (STATISTICA for Windows; Statsoft 2010a, b). To avoid co-linearity among explanatory variables (plant traits) we (i) excluded the dichotomous variable “erosulate” where the erosulate plant species were coded, because this variable was fully collinear with two other dichotomous variables (rosette and semi-rosette), and (ii) calculated a correlation matrix and tolerance values for the remaining variables (Electronic Appendix 3). Tolerance for a variable is defined

as 1 minus the  $r^2$  of this variable with all other independent variables in the regression and it is recommended that variables with a tolerance value lower than 0.1 are excluded (Quinn & Keough 2007). As the lowest tolerance value detected was 0.29, we left all explanatory variables in the model. Plant height was log-transformed to decrease the effect of the tallest species *Phragmites australis*.

*Effect of the application of fertilizer/mowing on plant traits: frequency versus cover data*

To test if individual plant traits cause different responses to management from the frequency vs plant cover point of view we calculated general linear models with split-plot design. Species represented the whole plots and the type of its response to treatment (RDA scores calculated from frequency or cover data) was treated as a within-plot factor. The interactions between individual traits and within-plot factors were tested.

*Effect of the application of fertilizer/mowing on plant traits: response of the community*

We calculated community means and community-weighted means considering both frequency and cover data for individual plant traits for each plot before the 2007 and after the 2009 treatment. In these calculations all plant species (including the rare ones) were considered. The community mean was calculated as mean trait value of all species in the plot, whereas the community weighted mean was calculated by weighting the trait value by plant

abundance (simply  $\bar{x} = \frac{\sum_{i=1}^n w_i t_i}{\sum_{i=1}^n w_i}$ , where  $w_i$  is the cover or frequency and  $t_i$  is the trait value of species  $i$ ). The

effects of treatments on changes in the community means and community weighted means were analysed by split-plot designed ANOVA, where time represented the within-plot factor. Locality identification was used as a random factor in the analyses. Effect of the application of fertilizer/mowing on the composition of the vegetation The response of the plant species to mowing and the application of fertilizer differed significantly, but only accounted for a low percentage of total variability (Table 2). Most of the variability (more than 87% in all analyses) was determined by co-variables. The responses of species to changes in management were similar when both ways of assessing abundance were used, as is revealed by the correlation between RDA scores for frequency and RDA scores for plant cover ( $n = 97$ ; application of fertilizer,  $r = 0.397$ ,  $P < 0.01$ ; mowing  $r = 0.663$ ,  $P < 0.01$ ).

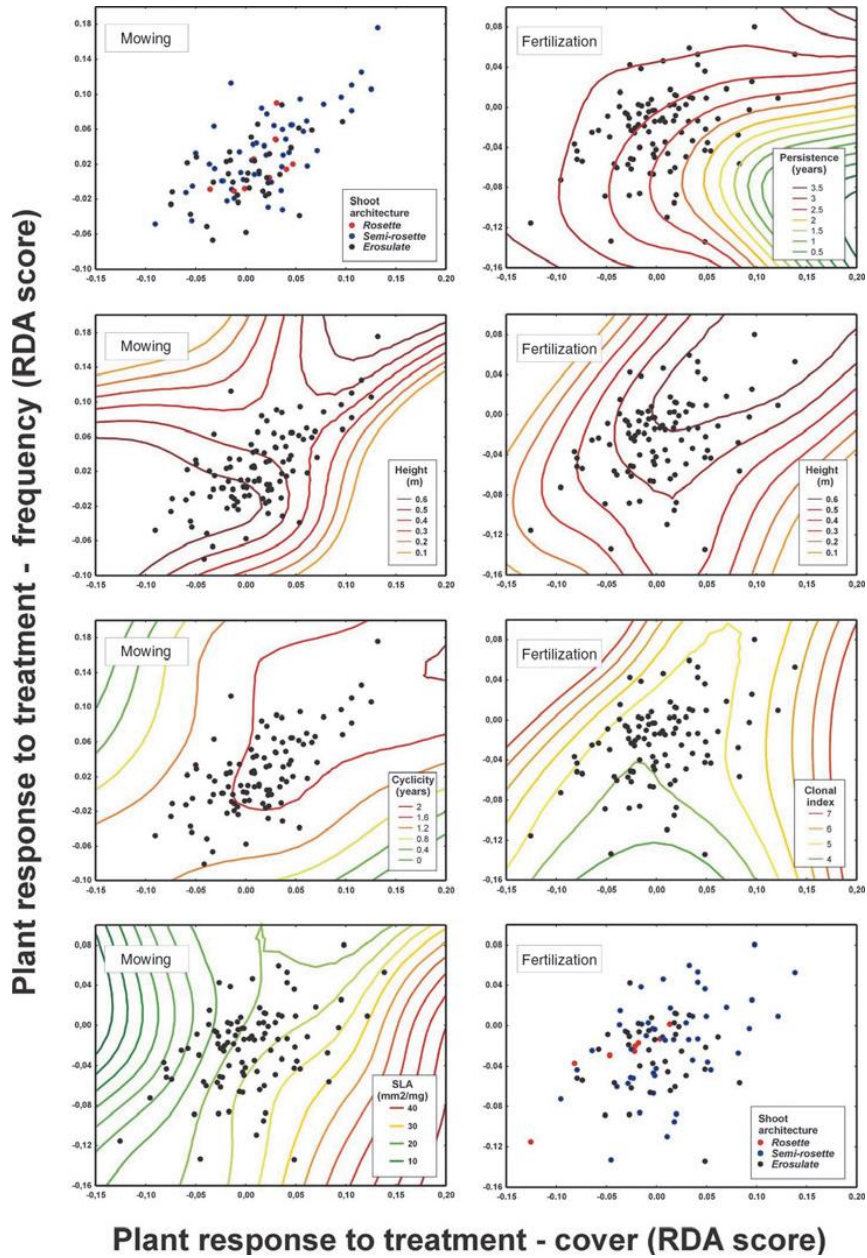


Fig. 2. – Regressions of the responses (RDA score) of individual species to management (mowing or application of fertilizer), based on plant abundance assessed in terms of cover (x-axis) and frequency (y-axis). Particular curve colours represent different continuous trait values, whereby plants of different categorical traits (shoot architecture) are colour-coded. Only traits that were significantly affected by the treatment (Table 4) and/or relationships that differed significantly when the two methods used to assess abundance were used (Table 5), are shown

## Results

### *Plant traits: response of individual species*

The correlations with traits relevant to competitive ability were more often significant than those with clonal traits. Shoot cyclicity correlated positively with the response of plant cover to mowing; plant height correlated negatively with plant cover and response in plant frequency to mowing but positively with cover and response in the frequency of plants to the application of fertilizer; SLA correlated positively with the response of plant cover to the application of fertilizer. Plants with a distinct shoot architecture responded differently to mowing in terms of frequency and to application of fertilizer in terms of cover (Table 3). The three clonal traits, lateral spread, multiplication rate and persistence of ramet connection correlated neither with species responses based on frequencies nor with those based on relative cover after changes in management (Table 3). Clonal index showed only one significant (positive) correlation, i.e. the response of plant frequency to the application of fertilizer (Table 3).

### *Effect of the application of fertilizer/mowing on plant traits: frequency vs cover*

In evaluating the effects of plant traits on the different responses of plants to treatment based on either frequency or cover, three out of nine traits tested – persistence of ramet connection, SLA and shoot architecture (rosette plants) – had a significant effect (Table 4, Fig. 2). These traits were, however, significant only when responses to the application of fertilizer were tested (Table 4). Plants that produced ramets that remained connected to the mother plant for a short period exhibited a more positive response to the application of fertilizer in terms of cover than frequency, whereas plants with a low SLA showed a more positive response in terms of frequency than cover. Rosette plants showed a more positive response in terms of frequency than cover (Fig. 2).

### *Plant traits: response of community*

Considering frequency, the application of fertilizer led to an increase in clonal index, multiplication rate and plant height both in terms of changes in abundance (weighted mean) and species composition (unweighted mean). Some of these trends were also obvious when cover was used (Table 5). Mowing, on the other hand, led to increased cyclicity, but lower lateral spread only when weighted means were used. A decreased representation of erosulate shoots was found irrespective of weighting. Multiplication rate and the representation of semi-rosette shoots positively responded to both types of management in both weighted and unweighted analyses (Table 5). Only one functional trait, persistence of ramet connection, did not show any response to change in management at the community level.

Table 2. – Effect of application of fertilizer and mowing on the composition of the vegetation of 22 wet meadows. Abundance expressed either as plant cover or plant frequency. F – fertilizer applied, M – mowing, Yr – Year number (2007: 0, 2009: 1), PlotID – identifier of each plot, %exp. var. – percentage of species variability explained by the first (constrained) axis, r – species-treatment correlation on the first axis, P – significance.

Method of abundance assessment	Explanatory variables	Covariables	% exp. var.	r (1st axis)	F-ratio	P-value
Plant cover	Application of fertilizer*Yr	Yr, PlotID, M*Yr	0.2	0.635	1.633	0.018
	Mowing*Yr	Yr, PlotID, F*Yr	0.2	0.630	1.737	0.004
Plant frequency	Application of fertilizer*Yr	Yr, PlotID, M*Yr	0.3	0.623	2.708	0.002
	Mowing*Yr	Yr, PlotID, F*Yr	0.4	0.624	4.024	0.002

Table 3. – Effect of plant traits on plant response to treatments analysed by using general linear models with backward stepwise selection. Only traits with a significant response ( $P < 0.05$ ) are shown.  $\beta$  – correlation coefficient.

Mowing				Application of fertilizer			
Cover		Frequency		Cover		Frequency	
Traits	$\beta$	Traits	$\beta$	Traits	$\beta$	Traits	$\beta$
Cyclicality	0.2327	Semi-rosette	0.3497	Height (log)	0.2800	Height (log)	0.2564
Height (log)	-0.2948	Height (log)	-0.2442	SLA	0.3482	Clonal index	0.2307
				Semi-rosette	0.2455		

Table 4. – Effects of plant traits on differences in plant responses to treatments based on cover and frequency data. General linear models with split-plot design were used. Plant species represented the entire plots and plant response types, (i.e. responses calculated using frequency or cover data), were treated as within-plot factors. Interactions between plant traits and within factors were tested. F-values are shown, significant effects are in bold: \*  $0.05 > P > 0.01$ , \*\*  $P < 0.01$ , ns – non-significant; effect df = 1; error d.f. = 93 (except for SLA analyses, where error d.f. = 89 as we do not have SLA values for all plants).

Response to	Clonal index	Cyclicality	Persistence of connection	Multiplication rate	Lateral spread	Plant height	SLA	Rosette	Semirosette	Erosulate
Mowing	0.45 <sup>ns</sup>	0.05 <sup>ns</sup>	0.70 <sup>ns</sup>	0.14 <sup>ns</sup>	2.14 <sup>ns</sup>	0.81 <sup>ns</sup>	0.33 <sup>ns</sup>	0.28 <sup>ns</sup>	2.25 <sup>ns</sup>	1.47 <sup>ns</sup>
Fertilizer applied	0.84 <sup>ns</sup>	1.59 <sup>ns</sup>	<b>16.48**</b>	0.05 <sup>ns</sup>	0.51 <sup>ns</sup>	0.08 <sup>ns</sup>	<b>12.84**</b>	4.21*	0.72 <sup>ns</sup>	0.12 <sup>ns</sup>

### Summary of results

All traits important at the species level were important in the analysis at the community level when data were weighted by plant abundance. Also the analyses at the community level, which treated all species equally and reflected changes in species composition in communities after changes in management with one exception, were confirmed by the analyses that took plant abundance into account (Table 6). However, all three methods produced the same result only for three traits: clonal index, plant height responding

positively to the application of fertilizer and semirosette shoots responding positively to mowing. While the response in terms of plant height to the application of fertilizer was consistent using both methods of assessing abundance, the response of two other traits were consistent only when frequency was used (Table 6). The traits are moreover the same for rare and common species, whereas traits which responded to changes in species composition (community mean) – rosette and erosulate shoots, multiplication rate – were probably typical of rare species.

The analysis using community-weighted means produced the highest number of significant responses as it combined two effects: changes in abundance and changes in species composition (Table 6).

Table 5. – Effect of treatments on changes in individual community traits analysed by repeated-measures ANOVA with time as a within plot factor. F-values are shown, significant effects are in bold: \* 0.05 > P > 0.01, \*\* P < 0.01, n.s. – non-significant; effect d.f. = 1; error d.f. = 21.

Explanatory variable (data used) Dependent variable	Clonal index	Cyclicality	Persistence of connection	Multiplication rate	Lateral spread	Plant height	SLA	Rosette	Semi-rosette	Erosulate
Mowing × Time (cover)										
Community mean	0.40 <sup>ns</sup>	2.74 <sup>ns</sup>	0.17 <sup>ns</sup>	2.76 <sup>ns</sup>	3.48 <sup>ns</sup>	1.19 <sup>ns</sup>	0.05 <sup>ns</sup>	<b>4.34*</b>	7.17*	3.68 <sup>ns</sup>
Community-weighted mean	0.07 <sup>ns</sup>	20.12 <sup>**</sup>	0.05 <sup>ns</sup>	20.12 <sup>**</sup>	<b>5.87*</b>	12.28 <sup>**</sup>	1.25 <sup>ns</sup>	2.50 <sup>ns</sup>	<b>21.34<sup>**</sup></b>	14.07 <sup>**</sup>
Mowing × Time (frequency)										
Community mean	1.97 <sup>ns</sup>	0.40 <sup>ns</sup>	1.13 <sup>ns</sup>	0.12 <sup>ns</sup>	2.78 <sup>ns</sup>	2.61 <sup>ns</sup>	3.34 <sup>ns</sup>	0.91 <sup>ns</sup>	<b>10.48*</b>	6.60*
Community-weighted mean	0.41 <sup>ns</sup>	<b>6.07*</b>	2.02 <sup>ns</sup>	0.01 <sup>ns</sup>	<b>8.97<sup>**</sup></b>	14.39 <sup>**</sup>	5.87*	1.89 <sup>ns</sup>	<b>22.11<sup>**</sup></b>	11.44 <sup>**</sup>
Application of fertilizer × Time (cover)										
Community mean	3.74 <sup>ns</sup>	1.02 <sup>ns</sup>	0.05 <sup>ns</sup>	1.02 <sup>ns</sup>	3.84 <sup>ns</sup>	<b>9.04<sup>**</sup></b>	1.24 <sup>ns</sup>	0.64 <sup>ns</sup>	0.21 <sup>ns</sup>	0.71 <sup>ns</sup>
Community-weighted mean	<b>4.42<sup>**</sup></b>	0.10 <sup>ns</sup>	0.10 <sup>ns</sup>	0.10 <sup>ns</sup>	0.24 <sup>ns</sup>	<b>4.54*</b>	10.56 <sup>**</sup>	9.88 <sup>**</sup>	4.45*	0.28 <sup>ns</sup>
Application of fertilizer × Time (frequency)										
Community mean	<b>4.67*</b>	0.07 <sup>ns</sup>	0.54 <sup>ns</sup>	<b>4.73*</b>	2.98 <sup>ns</sup>	<b>4.35<sup>**</sup></b>	0.06 <sup>ns</sup>	0.18 <sup>ns</sup>	1.91 <sup>ns</sup>	1.68 <sup>ns</sup>
Community-weighted mean	<b>25.29<sup>**</sup></b>	0.63 <sup>ns</sup>	3.29 <sup>ns</sup>	<b>25.41<sup>**</sup></b>	0.53 <sup>ns</sup>	<b>21.35<sup>**</sup></b>	0.04 <sup>ns</sup>	<b>9.63<sup>**</sup></b>	5.14*	1.43 <sup>ns</sup>

## Discussion

Analysis of the results of a short-term experiment in which 22 wet meadows in central Europe were managed by mowing or applying fertilizer revealed that: (i) as expected, applying fertilizer favoured whereas mowing suppressed dominant, competitive species with tall erosulate and monocyclic shoots that can spread and multiply clonally over long distances (have a high clonal index); (ii) although the results were affected by method of assessing abundance there was no clear relationship between abundance and two groups of traits; responses of plant cover to management were not better predicted by traits related to competitive ability, and that of plant frequency to management were not better predicted by clonal traits; (iii) trends at the species level in several cases occurred also at the community level as immigration and extinction had already occurred in the communities three years after the change in management, and plants that became extinct locally (and hence were rare) had (at least partly) the same traits as plants that decreased in abundance.



Table 6. – Summary of results showing the effects of plant traits on their responses to treatment (species) and effects of treatments on changes in community mean and community-weighted mean trait values. R – rosette; S – semi-rosette; E – erosulate. Note that the factor erosulate was not tested in the analyses of effects of traits on plant responses to treatment (see Statistical analyses for details).

Treatment	Method	Clonal index	Cyclicality	Persistence	Multiplication rate	Lateral spread	Plant height	SLA	R	S	E
Mowing (cover)	species		↑				↓				
	community mean								↓	↑	
	community-weighted mean		↑		↑	↓	↓			↑	↓
Mowing (frequency)	species						↓			↑	
	community mean									↑	↓
	community-weighted mean		↑			↓	↓	↑		↑	↓
Application of fertilizer (cover)	species						↑	↑		↑	
	community mean						↑				
	community-weighted mean	↑					↑	↑	↓	↑	
Application of fertilizer (frequency)	species	↑					↑				
	community mean	↑			↑		↑				
	community-weighted mean	↑			↑		↑		↓	↑	

### Plant traits

The trait that characterized the changes in species composition in the mowing treatment was shoot architecture; ratio of plants with semirosette shoots increased at the expense of plants with either rosette or erosulate shoots (Table 6). This result accords well with the finding that plants with semirosette shoots are at an advantage in grassland managed by mowing, because their shoot ontogeny is synchronized with the management (Klimešová et al. 2008). As shoot architecture correlates with shoot cyclicality (see Electronic Appendix 3) and responded to the mowing treatment we can conclude that our analyses confirm the idea that abandonment favours plants with erosulate shoots and a short lifespan (low cyclicality). Similar trends, although weaker, occurred in the response to the application of fertilizer, which did not affect plants with erosulate shoots and low cyclicality but favoured those with semirosette shoots, which increased in importance.

Other clonal traits rarely responded to mowing: multiplication rate increased and lateral spread decreased. These two opposite trends resulted probably from a lack of response in the clonal index, which is based on the two traits. On the other hand, the importance of the clonal index increased in the treatment in which fertilizer was applied, in which multiplication rate increased but lateral spread was unaffected. The clonal index proposed by Johannson et al. (2011) was the only clonal trait responding at all three levels analysed (plant species, community mean and community-weighted mean, see below) and proved to be a useful and better parameter of the degree of clonality than the simple division into clonal and non-clonal species in those communities where clonal plants prevail. Traits associated with carbon economy, persistence of connections

between shoots and SLA, responded very weakly. On the other hand, plant height, the most often used trait in studies determining the response of functional traits to management (e.g. Díaz et al. 2001, 2007, Veski et al. 2004), responded consistently: low growing plants were favoured by mowing and tall plants were favoured by the application of fertilizer, which accords with the expectations (see also Klimešová et al. 2010).

#### *Methods of assessing abundance*

The method used to assess abundance affected the results of this experiment (see also Pavlů et al. 2009). Although our results differed from those obtained using individual methods of assessing abundance, they were not contradictory as was, for example, found for seed size (Chiarucci et al. 1999, Guo 2003). Contrary to our expectations, the two methods used to assess abundance (frequency and cover) did not affect the sensitivity of the analysis in terms of trait types (clonal, competitive and carbon economy). It is difficult, however, to combine different methods of assessing abundance in one analysis (see Garnier et al. 2007, Sammul 2011).

We compared results obtained using the two methods of assessing abundance considering only their principal differences as proposed by Chiarucci et al. (1999), i.e. effect of shoot size on cover data and number of shoots on frequency data, without taking into account the accuracy of the description of the vegetation obtained using these methods. Studies that evaluate the quality of data collected using the different method for assessing abundance (e.g. Lepš & Hadincová 1992, Klimeš 2003, Vittoz et al. 2010) indicate that the data are constrained by misidentifications, difficulties with estimating cover of species with similar and inconspicuous growth forms, overestimating large and conspicuous species, different skills of the observers participating in the monitoring, etc. We aimed to limit these methodological difficulties as much as possible; although our field study would not have been possible without the participation of numerous observers, minimally two or three trained observers worked together, compared their estimates and consulted over plant identifications. Although estimating cover is the most inaccurate technique for repeated sampling, the results were not in contradiction with those obtained by assessing frequency. However, it gives results that are the least consistent for all three analyses (see below).

#### *Species vs community level, weighting by abundance*

By combining the three analyses of the dataset: the species, the community mean and the community-weighted mean enabled us to separate the effects of changing plant cover (the species level), immigration and extinction (the community mean). Only three traits were found to consistently respond at all tree levels: semirosette shoots increased with mowing, clonal index and plant height increased after the application of fertilizer. The method combining both effects (community-weighted mean) logically was the most sensitive measure of trait changes that occurred in our study and with one exception

confirmed trends revealed by the two other methods. Although it would be convenient if the community weighted mean was the most powerful tool for such analyses, we advocate that the selection of the method should be determined by the research question being addressed (see Pakeman et al. 2008).

Preceding studies using abundance weighting together with community means for trait parameters combined with both the methods for separating the role of environmental filtering (whether the species can immigrate into the community) from performance of species in a community (whether the species can spread in the community by seeds or clonal growth) (Cingolani et al. 2007, de Bello et al. 2007, Pakeman et al. 2008, Latzel et al. 2011, Sammul 2011). Here we show that in short-term experiments the two methods can separate the effect of changes in species composition (immigration and extinction) from changes in the abundance of resident species. Although fluctuations in species composition shortly after the change in management might be partly caused by random processes (see Introduction), the traits found to respond only to changes in species composition (rosette and erosulate shoots, multiplication rate) can be considered important for meadow species. This implies that early responses in species composition to changes in management are (at least partly) caused by filtering of traits based on the type of management.

## **Conclusion**

Clonal index as a proxy of the degree of clonal growth is a better measure of clonality than the more often used division into clonal and non-clonal species. Our results further imply that (i) the method of assessing abundance significantly affects the analysis of the response of functional traits to changes in management at the community level, and (ii) comparison of analyses based on abundance weighting and unweighted means results in a deeper insight into the changes in the spectra of functional traits that occur after a community is disturbed.

See <http://www.preslia.cz> for Electronic Appendix 1–3.

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## **Chapter IV**

### **Effects of land-use changes on plant functional and taxonomic diversity along a productivity gradient in wet meadows.**

Janeček, Š., Bello, F., Horník, J., Bartoš, M., Černý, T., Doležal, J., Dvorský, M., Fajmon, K., Janečková, P., Jiráská, Š., Mudrák O. & Klimešová, J. (2013). *Journal of Vegetation Science*, 24(5), 898-909

Effects of land-use changes on plant functional and taxonomic diversity along a productivity gradient in wet meadows.

### **Abstract**

**Questions:** To what extent do changes in management (abandonment and fertilization) affect plant functional and taxonomic diversity in wet meadow communities? To what extent do the changes in functional and taxonomic diversity depend on site productivity?

**Location:** Železné hory Mts., Czech Republic.

**Methods:** Experimental plots were established on 21 wet meadows differing in productivity and species composition. In each meadow, in 2007, four  $1 \times 1$  m plots were established, representing a full factorial design with abandonment and fertilization as the factors. In each plot, the number of species present was recorded in 100 subplots ( $0.1 \times 0.1$  m) in the years 2007, 2009 and 2011. Different indicators of functional diversity (functional richness, functional evenness, and Rao's quadratic entropy) were calculated using five functional traits (SLA, LDMC, seed mass, plant height and clonality). Both abundance-weighted and non-weighted diversity indices were calculated. Randomization tests (conducted with PERMANOVA) were used to assess the effect of site productivity and management on both  $\alpha$ - and  $\beta$ -diversity components.

**Results:** Meadows along the productivity gradient differed in functional and taxonomic diversity. Both abandonment and fertilization decreased taxonomic diversity. Whereas fertilization decreased functional richness and Rao's quadratic entropy, abandonment decreased functional evenness. The changes in both taxonomic and functional diversity caused by abandonment and fertilization occurred faster in more productive meadows.

**Conclusions:** The increased dominance of tall species with abandonment and fertilization, followed by the loss of species and the decrease in various indicators of functional diversity, suggest that increased competition for light resulted in increased trait convergence among co-existing species. In addition, many processes occurring after abandonment and fertilization depend on meadow productivity. Results suggest that abundance- and non-abundance-weighted diversity indices give complementary insights on community structure. These results imply that changes are needed in current meadow management and conservation.

**Keywords:** Abandonment; Biomass; Clonal index; Fertilization; Leaf dry matter content; Management; Plant height; Plant traits; Seedmass; Specific leaf area



## **Introduction**

Biodiversity is a multifaceted concept that can be measured with a variety of indicators (Izsák & Papp 2000; Ricotta 2002, 2005). Traditionally, most research on biodiversity has focused on taxonomic diversity, measured as species richness, species evenness or by various indices that combine richness and evenness. Although many ecological theories concerning the mechanisms underlying community structure focus on taxonomic diversity and its changes due to environmental and land-use modification, the mechanisms by which communities respond to these changes are expected to depend on the functional traits of the species composing these communities (Lavorel & Garnier 2002). Environmental filtering, disturbance tolerance, competitive interactions or facilitation, for example, are processes that select for species with specific functional traits related to competitive ability, resource acquisition and storage, reproduction, dispersal, persistence, etc. (Violle et al. 2007; Spasojevic & Suding 2012). This sorting of species based on their traits often results in non-random distribution of traits in a community. Consequently, researchers assessing the effect of environmental changes on community structure are increasingly considering both taxonomic diversity and functional diversity (Mayfield et al. 2010). Like taxonomic diversity, functional diversity can be expressed using several indices (Villéger et al. 2008); among these, functional richness, functional evenness and functional divergence indices should cover the main dimensions of functional diversity (Villéger et al. 2008).

At a small spatial scale, the most taxonomically diverse plant communities on Earth are European hay meadows (Wilson et al. 2012), even though species richness in these meadows has been reduced by modifications in agricultural practices during the last decades (Linusson et al. 1998; Jensen & Schrautzer 1999; Helm et al. 2006; Halda et al. 2008). Many experimental studies have reported that the most important factors affecting meadow species diversity are eutrophication and abandonment (Kull & Zobel 1991; Willems et al. 1993; Lepš 1999; Klimeš et al. 2000; Krahulec et al. 2001; Pavlů et al. 2007; Ceulemans et al. 2011). Although the negative effect of land-use changes on species richness is well documented, the changes in functional diversity may not simply follow the decrease in taxonomic diversity because functional diversity and taxonomic diversity can be both mathematically and biologically independent (Díaz & Cabido 2001; Villéger et al. 2008; Sasaki et al. 2009; Mayfield et al. 2010). It is becoming increasingly evident that characterizing the relationship between taxonomic and functional diversity can provide insight into how different disturbances or management strategies affect community assembly and ecosystem functioning (Sasaki et al. 2009; Mayfield et al. 2010).

Changes in both taxonomic and functional diversity due to land-use changes can be region-specific (Díaz et al. 2007; Mayfield et al. 2010) and can differ along environmental gradients (Díaz et al. 1998; de Bello et al. 2006, 2012; Bernhardt-Römermann et al. 2011; Lavorel et al. 2011; Pakeman 2004, 2011). Productivity has

been pointed out as one of the most important gradients determining plant biodiversity response to management (Milchunas & Lauenroth 1993; Venterink et al. 2002). Theoretical predictions are, however, to a certain extent contradictory. On the one hand, changes in taxonomic diversity are expected to be more pronounced on less productive, nutrient-limited sites than on productive ones because of the increasing competition that occurs after nutrient addition to less productive sites (Tilman 1987). On the other hand, changes in taxonomic diversity are expected to be pronounced on more productive sites because plant growth and hence population dynamics are faster with high productivity (Huston 1979). Like theoretical predictions, results of empirical studies have not been uniform (e.g. Milchunas & Lauenroth 1993; Gough et al. 2000) perhaps because both theoretical and empirical studies have not considered functional diversity along with taxonomic diversity. We expect that the assessment of functional diversity in combination with taxonomic diversity will provide important insights into how the communities respond to land-use changes. This approach should include the comparison of the different spatial components of diversity ( $\alpha$ -,  $\beta$ - and  $\gamma$ -diversities, i.e. within-community, between-community and total diversity; de Bello et al. 2010). In particular,  $\beta$ -diversity within a site (i.e. the turnover among experimental plots with different management established on one meadow) should verify the expectations of a greater vs. lesser response to land-use changes within more productive sites.  $\beta$ -diversity among sites (i.e. turnover across meadows) will, in contrast, test for the effect of environmental filtering on species and functional trait turnover along the productivity gradient.

In this study, we therefore asked: to what extent do the changes in taxonomic or functional diversity (both within and between plots) in response to abandonment and eutrophication depend on site productivity? This question, which has seldom been the topic of experimentation, has both theoretical and practical importance. From a practical perspective, the results can help indicate whether limited conservation resources should be directed toward more productive or less productive meadows. In this study, we evaluated the effect of abandonment and fertilization on taxonomic and functional diversity on 21 semi-natural wet meadows along a productivity gradient.

## **Methods**

### *Experimental setup*

The study area is located in the Železné hory Mts. (eastern Bohemia, Czech Republic). The landscape is composed of forest fragments, arable fields, villages, ponds and different semi-natural meadows. Meadows represent regional biodiversity hotspots and are often protected as nature reserves. We selected 21 of these wet meadows, differing in species composition and productivity (Appendix S1). As a surrogate for meadow productivity, we used standing crop at the peak of the growing season (July). The standing crop, as measured at the beginning of the experiment (see below), ranged from 255 to 680 g m<sup>-2</sup> of dry biomass. On each of the 21 meadows, four 1 × 1 m plots

were established early in the spring of 2007 (84 in total). In July 2007, plant species occurrences in 100 subplots ( $10 \times 10$  cm) in each of the 84 plots were recorded as baseline data. We then assigned treatments to each of the four plots per meadow: the plots were mown, mown–fertilized, abandoned or abandoned–fertilized. Mowing was done in July of every year. Fertilizer was applied at 20 g of mineral NPK (10% N, 10% P<sub>2</sub>O<sub>5</sub>, 10% K<sub>2</sub>O) per m<sup>2</sup> at the end of July 2007 and at 50 g m<sup>-2</sup> in the second half of April in subsequent years. These doses represent the maximum amount recommended for extensive grasslands (30–50 g m<sup>-2</sup>). Species composition in each of the  $10 \times 10$  cm subplots in each plot were recorded again after 2 yrs (2009) and 4 yrs (2011). The standing crop biomass was clipped from two  $1 \times 1$  m plots on each meadow in 2007 after the species were recorded (these two plots were subsequently assigned to mown and mown–fertilized treatments). The biomass was dried for 12 h at 85 °C, and weighed. The biomass per m<sup>2</sup> was then calculated as the mean value from these two plots.

#### *Plant functional traits*

We selected five functional traits related to key plant functions. Three of them, specific leaf area (SLA), height and seed mass, were suggested in Westoby (1998) as traits that express fundamental differences in ecological behavior of plant species. SLA was calculated as the leaf area of the fresh leaf divided by its dry mass (in m<sup>2</sup> kg<sup>-1</sup>). SLA is usually positively correlated with potential relative growth rate and negatively correlated with investment into leaf protection (Schierenbeck et al. 1994; Westoby 1998). Plant height is closely related to competitive ability and represents the trade-off between benefits resulting from access to light and costs of stem construction and maintenance (Falster & Westoby 2003). Moreover, these costs can be higher when the regular mowing removes a large proportion of above-ground biomass (Klimešová et al. 2010). Seed mass is related to the ability to survive hazards that occur at the start of seedling growth (Westoby et al. 1996). In addition to these three traits, we used leaf dry matter content (LDMC), which represents the ratio of leaf dry mass to fresh mass (in g kg<sup>-1</sup>), and the clonal index. Although LDMC is often correlated with SLA, it does not always have the same biological functions (Cornelissen et al. 2003). The clonal index reflects the degree of clonal reproduction and space occupancy and is important in meadow communities where most species are perennial (Johansson et al. 2011; Klimešová et al. 2011). Data on SLA, LDMC and seed mass were taken from the LEDA Traitbase (Kleyer et al. 2008), and the plant height was taken from Kubát et al. (2002). Clonal index was calculated as the sum of ordinal values of multiplication rate and lateral spread reported in the CLO-PLA database (Klimešová & Klimeš 2006). For more details on clonal index calculation, see Johansson et al. (2011).

#### *Alpha-diversity*

We used three indices of functional diversity (FD) and four parameters of taxonomic diversity (TD). FD was described by functional richness (FRich; Mason et al. 2005;

Villéger et al. 2008), functional evenness (FEve; Mason et al. 2005; Villéger et al. 2008) and Rao's quadratic entropy expressing functional divergence (Rao index; Rao 1982). FRich is defined as the functional trait space that is occupied by the community, and for one trait it represents the range of trait values in a community. FEve represents the evenness in both the abundance and trait values of species in a community (Villéger et al. 2008). The Rao index represents the extent of functional dissimilarity between species pairs (Rao 1982):

$$\text{Rao} = \sum_{i=1}^N \sum_{j=1}^N d_{ij} p_i p_j \quad (1)$$

where  $d_{ij}$  is the dissimilarity between species  $i$  and  $j$ , and  $p_i$  and  $p_j$  is the relative abundance of species  $i$  and  $j$ , respectively. The relative abundance of the particular species was calculated as the number of  $10 \times 10$  cm subplots where the species was present over the sum of these presences for all species in the  $1 \times 1$  m plot. For a more detailed description of functional indices, see Appendix S2. Rao index and functional evenness were computed both with and without consideration of differences in species abundance (the calculations without abundance effect assume that all species have equal abundances). This was done to assess how species occurrence alone vs. species composition including species abundance affected FD. All functional diversity indices were calculated using the five functional traits mentioned above. Data on SLA, LDMC, plant height and seed mass were log transformed to decrease the effect of extreme values. Subsequently, multivariate species trait dissimilarities were combined and standardized using the Gower approach to compute FD (Gower 1971; Podani 1999; Pavoine et al. 2009; de Bello et al. 2012).

To characterize TD, we used species evenness, species richness, the Simpson reciprocal diversity index and the total number of presences in a community (i.e. the sum of richness of all 100  $10 \times 10$  cm subplots). To describe the community evenness independently of species richness, we used the converted index of dominance (Williams 1964):  $E = 1/(D*S)$ , where  $S$  is the species richness and  $D$  is the index of dominance.  $D = \sum(n_i/N)^2$ , where  $n_i$  is the number of species presences of the  $i$ th species in 100 subplots, and  $N$  is the total sum of number of all species presences in the community. The Simpson reciprocal diversity index was expressed as  $1/D$ , where  $D$  is the index of dominance and corresponds to the correction proposed by Jost (2007) and de Bello et al. (2010) to decompose diversity into  $\alpha$ -,  $\beta$ - and  $\gamma$ -diversities (see next section).

### *Beta-diversity*

The taxonomic and functional  $\beta$ -diversity (i.e. taxonomic and functional dissimilarity between individual pairs of plots) was computed according to de Bello et al. (2010) (see also Jost 2007; Ricotta & Szeidl 2009). The  $\beta$ -diversity expresses a dissimilarity, or

‘turnover’, between each pair of plots based on either taxonomic or functional characteristics. We followed four steps. First, we calculated the Rao and reciprocal Simpson index for each plot in each year ( $\alpha$ -diversity) for summarizing FD and TD, respectively. Note that the Rao index is the generalization of the Simpson index of diversity (Lepš et al. 2006), and if dissimilarity  $d_{ij}$  in eq. 1 is 1, then Rao = 1 – Simpson dominance. Therefore, taxonomic and functional diversity can be compared within the same mathematical framework. Second, the  $\alpha$ -diversity for taxonomic diversity was calculated for each plot as the Simpson reciprocal diversity index (1/D) and similarly for functional diversity as  $1/(1 - \text{Rao})$ . Third, the  $\gamma$ -diversity was determined for each pair of plots:  $p_i$  and  $p_j$  in eq. 1 represent the mean abundance of species  $j$  and  $i$  in both plots. Fourth,  $\beta$ -diversity between each pair of plots was subsequently calculated as  $\beta = ((\gamma - \text{mean } \alpha) * 100) / \gamma$ . R software (R Foundation for Statistical Computing, Vienna, AT) and the function ‘Rao’ (de Bello et al. 2010) were used for these calculations.

### *Statistical analyses*

We used different models based on randomization tests and the program PERMANOVA+ for PRIMER (Anderson et al. 2008) to assess management and site productivity. We constructed three models for each diversity index (Table 1). Model 1 was used to test the effect of abandonment and fertilization on changes in diversity indices over time. In this model, meadow identification was used as a random factor, abandonment and fertilization were used as fixed factors, and year was used as a continuous fixed predictor. Model 2 determined whether the changes between the first and third year of the experiment (i.e. whether changes after 2 yrs of experimental manipulation) depended on site productivity of individual meadows. Changes were expressed as the diversity index or plant trait value in the third year divided by the value for the same parameter in first year of the experiment. Model 3 was similar to model 2 but compared the first and fifth year, i.e. it determined whether changes after 4 yrs of experimental manipulation depended on site productivity of individual meadows. In models 2 and 3, fertilization and abandonment were used as fixed factors and productivity was the continuous fixed effect variable. The same approach was used for both  $\alpha$ -diversity indices and for  $\beta$ -diversity (i.e. functional and taxonomic dissimilarity between plots). To test whether dissimilarity between plots within particular meadows (i.e. dissimilarity between mown, mown–fertilized, abandoned and abandoned–fertilized plots) depended on meadow productivity, we calculated for each meadow, year and dissimilarity index the mean distance to the common centroid (or ‘meadow heterogeneity’) using the PERMDISP utility in the program PERMANOVA+ for PRIMER. Dependence of these mean distances on productivity was then analyzed with linear regression models in which meadow heterogeneity (either taxonomic or functional) was the dependent variable and standing biomass was the predictor.

## Results

### *Taxonomic diversity*

The meadows differed in all parameters of taxonomic diversity (Table 1). Three taxonomic indices, i.e. species richness, the Simpson index and total number of species presences, decreased over time in response to both meadow abandonment and fertilization (Fig. 1a, b, c, Table 1; interactions were significant for Year\*Treatment) whereas species evenness did not show any significant trend (Table 1). The response of taxonomic diversity indices to changes in management, mainly fertilization, depended on meadow productivity particularly over the first 2 yrs (Table 1, Model 2 and 3). These trends, however, disappeared when changes after 4 yrs were considered in that the response became more similar across meadows (Table 1, Model 3). The changes after 2 yrs were most obvious for the Simpson diversity index. The Simpson index decreased rapidly mainly in abandoned–fertilized plots on more productive meadows (Fig. 2). These trends were also similar for the other three taxonomic indices (Table 1, Appendix S3).

### *Functional diversity*

With regard to the effects of treatments and productivity on individual traits used for calculations of functional diversity indices, effects on plant height were the most consistent. The mean plant height, both weighted and not weighted by species abundance, increased after abandonment and fertilization (Appendix S3). The responses of other traits to treatments differed depending on treatment (fertilization vs. abandonment) and whether community means or community weighted means were considered (for details, see Appendix S3).

Except for functional evenness, when species abundance was taken into account, the meadows differed in all examined parameters of functional diversity (Table 1). The effects of treatments were much more variable on functional diversity than on taxonomic diversity.

With regard to changes that were not affected by species abundance, abandonment decreased functional evenness (Fig. 1e, Table 1), and after 2 yrs of experimental manipulations functional evenness decreased more after abandonment on meadows with high productivity than low productivity (Fig. 2, Table 1). Moreover, fertilization reduced functional richness (Fig. 1d), while the effects of fertilization along the productivity gradient on the Rao index were only marginally significant. The marginal effect on the Rao index was manifest mainly by an increase in the Rao index in abandoned–fertilized plots after 2 yrs of experimental manipulation on meadows with high biomass production (Table 1, Appendix S4). The single and only marginally significant effect on changes in diversity along the productivity gradient after 4 yrs of the experimental

Table 1. The effect of fertilization, abandonment and productivity on individual parameters of functional (FD) and taxonomic (TD) diversity as indicated by permutation P-values.

	E	Richness	Sim.	NSP	FRich	FEve	FEve-Ab	Rao	Rao-Ab
<i>Model 1</i>									
Meadow	0.000	0.000	0.000	0.000	0.000	0.000	<i>n.s.</i>	0.000	0.000
Year (Y)	0.030	0.000	0.000	0.000	0.000	0.001	<i>n.s.</i>	0.003	0.001
Abandonment (A)	<i>0.076</i>	0.000	0.004	0.000	0.022	0.001	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Fertilization (F)	<i>n.s.</i>	0.001	0.000	0.000	0.011	<i>n.s.</i>	<i>n.s.</i>	0.033	0.006
Y*A	<i>n.s.</i>	0.000 <sup>(+)</sup>	0.001 <sup>(+)</sup>	0.000 <sup>(+)</sup>	<i>n.s.</i>	0.012 <sup>(+)</sup>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Y*F	<i>n.s.</i>	0.000 <sup>(+)</sup>	0.000 <sup>(+)</sup>	0.001 <sup>(+)</sup>	0.017 <sup>(+)</sup>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	0.008 <sup>(+)</sup>
A*F	<i>n.s.</i>	<i>0.060</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Y*A*F	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
<i>Model 2 (3rd/1st year)</i>									
Biomass (B)	<i>0.056<sup>(+)</sup></i>	<i>0.056<sup>(+)</sup></i>	0.000 <sup>(+)</sup>	<i>0.080<sup>(+)</sup></i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.097<sup>(+)</sup></i>
Abandonment (A)	<i>n.s.</i>	<i>n.s.</i>	0.001 <sup>(+)</sup>	0.000 <sup>(+)</sup>	<i>n.s.</i>	<i>0.079<sup>(+)</sup></i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Fertilization (F)	<i>n.s.</i>	<i>n.s.</i>	0.001 <sup>(+)</sup>	0.000 <sup>(+)</sup>	<i>n.s.</i>	0.010 <sup>(+)</sup>	<i>n.s.</i>	<i>n.s.</i>	0.001 <sup>(+)</sup>
B*A	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	0.023	<i>n.s.</i>	<i>n.s.</i>	<i>0.061</i>
B*F	<i>0.091</i>	<i>0.091</i>	0.010	<i>0.074</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.0590</i>	<i>0.079</i>	<i>n.s.</i>
AxF	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
B*A*F	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.079</i>	<i>n.s.</i>
<i>Model 3 (5th/1st year)</i>									
Biomass (B)	0.027 <sup>(+)</sup>	0.006 <sup>(+)</sup>	0.000 <sup>(+)</sup>	0.008 <sup>(+)</sup>	<i>n.s.</i>	0.020 <sup>(+)</sup>	<i>n.s.</i>	<i>n.s.</i>	0.023 <sup>(+)</sup>
Abandonment (A)	<i>n.s.</i>	0.001 <sup>(+)</sup>	0.001 <sup>(+)</sup>	0.000 <sup>(+)</sup>	<i>n.s.</i>	0.011 <sup>(+)</sup>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Fertilization (F)	<i>n.s.</i>	0.001 <sup>(+)</sup>	0.000 <sup>(+)</sup>	0.000 <sup>(+)</sup>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	0.004 <sup>(+)</sup>
B*A	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.057</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
B*F	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
AxF	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
B*A*F	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Superscript signs (-) or (+) indicate a negative or positive effect of explanatory variables. E – Taxonomic evenness, Sim– Simpson reciprocal diversity index, NSP – Total number of species present, FRich – Functional richness, FEve –Functional evenness, Rao – Rao's quadratic entropy, -Ab – Functional index considering the effect of abundance. Values with  $P < 0.05$  and marginally significant  $P$ -values  $0.1 > P > 0.05$  are displayed in bold and in bold italics, respectively. Model 1: Effect of fertilization and abandonment on FD and TD changes in the course of the experiment. The explanatory variable year was considered as a continuous variable. Because interactions with year are of the greatest interest, they are the only ones accompanied with signs (-) or (+). Model 2: Effect of fertilization, abandonment and biomass productivity on ratios between values of FD or TD parameters in the third vs. first year of the experiment (i.e. 2 yrs after experimental manipulations began). Model 3: Effect of fertilization, abandonment and biomass productivity on ratios between values of FD or TD parameters in the fifth vs. first year of the experiment (i.e. 4 yrs after experimental manipulations began). For interaction effects of models 2 and 3, see Fig. 3 and Appendix S4.

manipulation was the effect of abandonment on functional evenness (Model 3 in Table 1). This effect was related mainly to a decrease in functional evenness in abandoned–fertilized plots (Table 1, Appendix S4).

When calculation of functional diversity indices included the effect of plant abundance, fertilization negatively affected the Rao index (Table 1, Fig. 1f). The effect of fertilization on functional evenness was marginally significant when calculations included plant abundance; functional evenness decreased more in the second year after experimental manipulation due to fertilization in meadows with high rather than with low productivity (Table 1, Appendix S4). Taxonomic and functional dissimilarity among plots Treatments generally increased both taxonomic and functional dissimilarity between plots within a meadow, especially when plant abundance was considered. When plant abundance was not considered, fertilization and abandonment (the latter was only

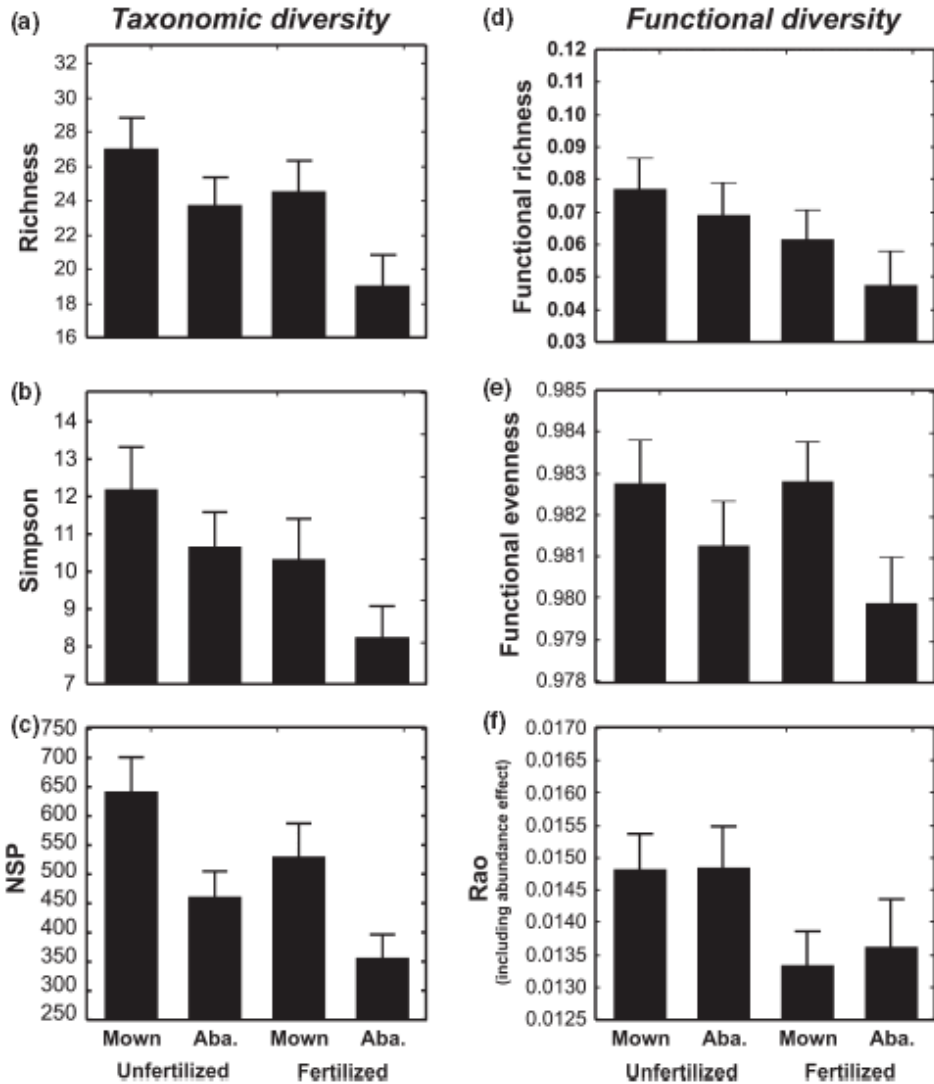


Fig. 1. Functional and taxonomic diversity in the last year of the experiment (2011). Taxonomic diversity expressed as (a) species richness, (b) Simpson diversity index, (c) NSP. Functional diversity expressed as (d) functional richness, (e) Functional evenness and (f) Rao index. Aba – abandoned. NSP – total number of species present. Mean  $\pm$  SE

marginally significant) affected only functional dissimilarity (Rao index; Table 2, Whole model). The two models, which were tested separately for the third and fifth year of the experiment, showed basically the same pattern (with the exception of the Rao index when abundance was not considered). Interestingly, the extent of taxonomic and functional responses to treatments (as indicated by the distance to the meadow centroid) on individual meadows was positively related to meadow productivity in both 2009 and



2011 (Fig. 3). The one exception was taxonomic diversity when the effect of abundance was included in 2011 (Fig. 3, Table 3).

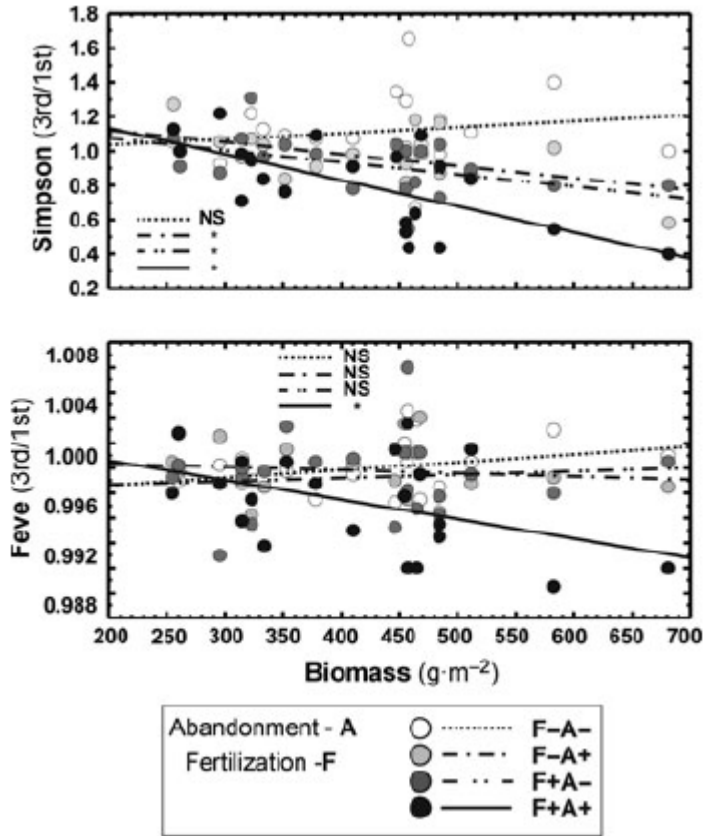


Fig. 2. Dependence of changes in Simpson diversity and functional evenness on meadow productivity after 2 yrs of experimental manipulation. The statistical significances of regression lines for individual treatments are presented: n.s. –not significant, \* $P < 0.05$ .

## Discussion

Our study revealed that abandonment and eutrophication (due to fertilization) reduce both the taxonomic and functional components of plant diversity in meadows. The response to abandonment and eutrophication, however, often depended on meadow productivity. The more productive meadows lost taxonomic as well as functional diversity more quickly than less productive meadows. These findings are important for understanding the ecological processes in semi-natural meadows and suggest that conservation efforts should be targeted not only on species-rich, low-productivity meadows but also on the more productive meadows that can more rapidly show changes in plant diversity.

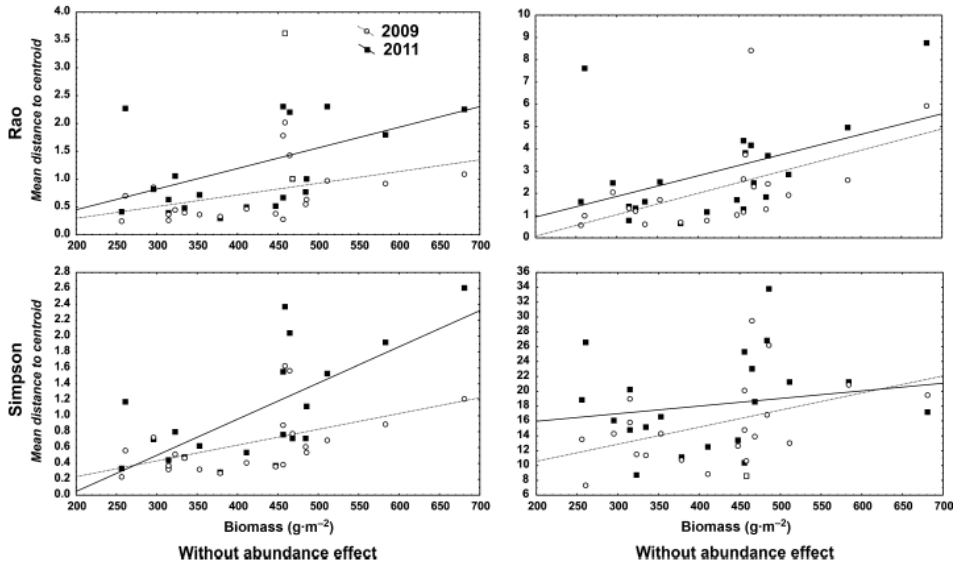


Fig. 3. Dissimilarity of plots on individual meadows as affected by meadow productivity. Points are related to individual meadows in individual years and represent the mean distance of four plots (i.e. mown, mown-fertilized, abandoned, and abandoned-fertilized) to their common centroid in functional or taxonomic space.

### *Taxonomic diversity*

We detected a general negative impact of abandonment and fertilization on taxonomic diversity in that most diversity indices decreased with both abandonment and fertilization. These changes were most obvious in the abandoned-fertilized treatment. Our results therefore correspond with those of similar studies (e.g. Kull & Zobel 1991; Willems et al. 1993; Lepš 1999; Honsová et al. 2007; Pavlů et al. 2007; Ceulemans et al. 2011) and highlight the threats caused by recent land-use changes (Isselstein et al. 2005; Henle et al. 2008). We found, however, that changes in taxonomic diversity differed along the productivity gradient, i.e. more productive meadows lost taxonomic diversity more quickly than less productive meadows 2 yrs after start of the experiment. Such a relationship is consistent with the idea that eutrophication accelerates loss of species diversity (Grime 1973; Keddy et al. 1997; Hautier et al. 2009; Li et al. 2011), but shows that these effects are productivity-dependent. Nevertheless, this trend disappeared when we considered changes 5 yrs after land-use changes were introduced. This indicates that degradation of meadows of both low and high productivity could level off over longer periods, while short-term effects are more intense in productive meadows.

### *Functional diversity*

The effects of abandonment and fertilization were more variable on functional diversity than on taxonomic diversity. This agrees with the absence of a simple linear relationship between functional and taxonomic diversity (see also Díaz & Cabido 2001; Sasaki et al.

Table 2. Effect of abandonment and fertilization on functional and taxonomic dissimilarity (b-diversity) as indicated by permutation P-values. In all significant tests, abandonment and fertilization increased b-diversity.

	Simpson	Simpson-Ab	Rao	Rao-Ab
<i>Whole model</i>				
Meadow	0.000	0.000	0.000	0.000
Year (Y)	0.015	0.000	0.000	0.000
Abandonment (A)	0.048	0.000	<i>n.s.</i>	0.006
Fertilization (F)	<i>n.s.</i>	0.000	<i>n.s.</i>	0.074
Y*A	<i>n.s.</i>	0.000	0.055	0.003
Y*F	<i>n.s.</i>	0.001	0.035	0.006
A*F	<i>n.s.</i>	0.004	<i>n.s.</i>	<i>n.s.</i>
Y*A*F	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	
<i>Model 2009</i>				
Meadow	0.000	0.000	0.000	0.000
Fertilization (F)	<i>n.s.</i>	0.000	<i>n.s.</i>	0.066
Abandonment (A)	<i>n.s.</i>	0.000	<i>n.s.</i>	0.014
AxF	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
<i>Model 2011</i>				
Meadow	0.000	0.000	0.000	0.000
Fertilization (F)	<i>n.s.</i>	0.000	0.075	0.011
Abandonment (A)	<i>n.s.</i>	0.000	<i>n.s.</i>	0.001
AxF	<i>n.s.</i>	0.069	<i>n.s.</i>	<i>n.s.</i>

-Ab – the effect of plant abundance was included. The whole model tests dissimilarity across all years (including the first year – 2007 – when baseline data were collected). Model 2009 and Model 2011 test dissimilarity in the third and fifth year of the experiment, respectively.

2009). In contrast to de Bello et al. (2006), who found a positive effect of a treatment (grazing) on species richness and a negative effect on functional diversity in the moistest locations, we found the same negative effect of treatments on both taxonomic and functional diversity. The different responses of species vs. plant traits were demonstrated by Pakeman (2004). He compared data from ten published experiments on grasslands and demonstrated that responses of traits to grazing are more often modulated by

Table 3. Dependence of dissimilarity between four plots on meadow productivity.

Year	Simpson		Simpson-Ab		Rao		Rao-Ab	
	r	P	r	P	r	P	r	P
2007	0.2368	<i>n.s.</i>	0.3864	<i>n.s.</i>	0.2778	<i>n.s.</i>	0.1470	<i>n.s.</i>
2009	0.5424	0.0111	0.4517	0.0398	0.4528	0.0393	0.5487	0.0100
2011	0.6913	0.0005	0.1688	<i>n.s.</i>	0.4382	0.0469	0.4669	0.0329

Dissimilarity was measured as the mean distance of the four plots in the meadow to the centroid. Mean distances were calculated with PERMDISP. -Ab – the effect of plant abundance was included.

productivity than responses of plant species. Díaz et al. (2007) showed in their meta-analysis that effect of defoliation (grazing) on individual traits can differ according to different climate and herbivory history, and Mason et al. (2011) demonstrated that mowing increase abundance-weighted niche overlap (approximated as overlap of quantitative functional traits) between species in a wet meadow in South Bohemia. What is the reason for context-specific effects of changes in grassland management? One reason could be different assemblage rules in individual plant communities (e.g. Keddy 1992). Due to environmental filters, the plants in a community represent only a certain range of functional traits that are available in the species pool, and these ranges differ among communities (Díaz & Cabido 2001). Consequently, decreases in species richness due to abandonment of traditional management (e.g. grazing or mowing) or increasing nutrient level can have different effects on species diversity in comparison with functional diversity. In our study, fertilization decreased the volume of functional space (i.e. functional richness), indicating that species with unique trait combinations were excluded from the community and that resident species became more similar (as indicated by the Rao index). These conclusions are in agreement with the recent study of Gerhold et al. (this issue), who report that fertilization of mesophytic grasslands in Estonia leads to exclusion of functionally dissimilar species. The increased dominance of tall species with abandonment and fertilization in this study (as supported by changes in the community trait mean; Appendix S3), followed by the loss of species and the decrease in various facets of functional diversity, suggest that increased competition for light increased trait convergence between co-existing species. Our result that functional diversity decreases with fertilization is also in accordance with Bowman et al. (1995), who showed that some growth forms having similar traits (e. g. dominant graminoids) are able to increase nitrogen acquisition after fertilization and invest more in photosynthesis than other growth forms. In contrast to eutrophication, defoliation (mowing or grazing) is expected to have negative effects on these growth forms as they can be more affected by loss of the above-ground biomass investment (Klimešová et al. 2010); assemblage-level thinning resulting from increases in individual plant size could enhance this effect (Oksanen 1996; Stevens & Carson 1999). These processes were especially obvious on more productive sites. The influence of meadow productivity on treatment effects was similar for taxonomic as well as functional diversity and fell over time.

Furthermore, we have shown that the observed changes in  $\alpha$ - and  $\beta$ -diversities are often dependent on whether or not we consider abundance of individual species. When abundance is not considered, the diversity indices are affected only by the changes in occurrence, whereas with abundance weighting, the results are also affected by changes in relative abundances of individual species. Ecologists and conservationists should keep in the mind that abundance-weighted and non-weighted matrices represent complementary rather than alternative points of views on processes in the plant communities (Pakeman et al. 2008; Klimešová et al. 2011).

### *Implications for management*

The taxonomic and functional dissimilarities among plots within individual meadows increased with productivity. This finding confirms the classical hypotheses of Milchunas & Lauenroth (1993) that dissimilarity between meadow communities under different management increases with site productivity. This hypothesis, however, has largely not been verified empirically, and to the best of our knowledge, the current study is the first to test the hypothesis in terms of functional diversity (but see Carmona et al. 2012). Moreover, we have shown that this productivity-dependent response to management is particularly evident in the short term, but tends to weaken with time. In other words, the changes caused by eutrophication and/or abandonment in meadows occur faster on more productive meadows than on less productive meadows, and are most evident shortly after the meadow fertilization or abandonment; this finding has important implications for conservation. We suggest that the appropriate frequency of monitoring to observe trajectories in diversity changes could differ for different types of meadow and priorities in management should be set with respect to meadow productivity. Because budgets for meadow management are often limited, the conservation authorities usually allocate all resources into regular management of a small number of the most diverse and less productive meadows. These meadows are then mown every year, whereas other less diverse and more productive meadows hosting fewer rare plants are mostly abandoned. Our results suggest, however, that extensive management of diverse meadows with low productivity and intensive management of less diverse meadows with high productivity could prevent their rapid degradation.

### **Conclusions**

This study documented that abandonment and fertilization reduce both plant functional and taxonomic diversity of wet meadows along a productivity gradient. The changes were faster and more pronounced on more productive meadows, where exclusion of functionally dissimilar species resulted in convergence. Based on these findings, we suggest that conservation management plans should involve not only species-rich, nutrient-poor meadows but also those that are relatively species-poor and nutrient-rich.

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### **Supporting Information**

Additional supporting information may be found in the online version of this article:

Appendix S1. Description of 21 studied meadows in the Železné hory Mts.

Appendix S2. Functional indices used in the manuscript.

Appendix S3. Effect of abandonment, fertilization and productivity on individual plant traits that were used for calculations of diversity indices.

Appendix S4. Influence of meadow productivity on functional diversity.



## Summary of results

The main objectives of the thesis was: (i) to describe diversity patterns of the wet meadows communities on the regional scale (the Železné hory Mts.); (ii) to assess the changes of the vegetation of the wet meadows in dependence on different type of management; (iii) to reveal the differences in the reaction of the communities on the management in relation to their productivity; (iv) to set the priorities for the grassland maintenance at regional scale.

The results show the importance of the multiple-scale approach for revealing the most important factors affecting species diversity and functional diversity changes. Species-area relationship appeared as a most sensitive measure in describing initial diversity patterns; best-fit criterion is quite not appropriate for decision among the models. Combination of analyses based on both abundance weighting and unweighted means can better untangle the processes underlying the changes of wet meadows species/functional diversity shortly after changed management regimes. Plant functional traits related to clonal growth appeared as a very effective tool in accounting of wet meadows diversity patterns and played important role in shaping species – area relationship. The analyses furthermore acknowledged the contribution of plant functional traits concerning clonal growth, competition strategies and carbon economy in explorations in depicting the patterns of responses of the wet meadow vegetation to management changes, described by.

Answers on main objectives of the thesis, based on the results of three proposed studies, are:

(i) The diversity of Železné hory Mts. wet meadows was very variable and its structure was foremost determined by processes on smallest scales. Species area relationship models appeared as a very sensitive measure, revealing negative effect of soil properties on the rate of the species richness increase with area. No significant relationship productivity-richness and productivity-species area relationship was found and showed that this problematic is more complex and that for example clonal growth patterns can play important role (Chapter I).

(ii) Both abandonment and eutrophication appeared serious threats for the diversity of wet meadows across broad soil moisture and productivity gradients (Chapter II, Chapter III). The significant feedbacks of various plant traits yet two years after the change of the meadow management demonstrate that wet meadows communities are very dynamic in reaction on land use change. Positive relationship of the traits concerning the competitive abilities both with abandonment and fertilization clearly disclosed that the species richness decrease is primarily induced by increasing competition asymmetry among the species (Chapter II).

(iii) The negative effect of eutrophication and abandonment on both species and functional diversity were amplified on more productive sites (Chapter III). Regular management should be therefore kept preferentially on more productive habitats, which are more threatened by absence of appropriate management.

(iv) Above mentioned findings have important implication for conservation management practices. Low productive habitats were more affected by land use changes during last century, their area decreased more rapidly and most of the specialists on oligotrophic conditions have become endangered consequently. Therefore, these habitats should be the major objects of conservation effort; and indeed most of the natural reserves were designated to protect these habitats. These localities are usually regularly mown but according to our findings they are less endangered than more productive sites; although they are less species rich still provide suitable habitats in landscape mosaic and host site specialist and when abandoned degrade more quickly than oligotrophic sites. Restoration of these habitats and they regular yearly mowing, is crucial for their conservation.

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