Three decades of vegetation changes in a submontane grassland after the cessation of intensive fertilization

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Mesic semi-natural grasslands have usually been and mostly still are, too intensively managed in central Europe, which includes over application of fertilizer. Consequently, restoration of the structure of this vegetation and its species richness is desirable. We investigated three decades of spontaneous recovery of a submontane grassland (western part of the Czech Republic) after the cessation of two decades of applying 320 kg/ha of nitrogen fertilizer per year. The number of plant species in plots that were previously heavily treated with fertilizer approached the numbers in the controls within five years and in total species composition in approximately two decades. The number of species in both types of plots continued to increase until the end of the second decade and then more or less stabilized. Typical grassland species were mostly responsible for the increase. In the third decade, the parallel trajectories in both types of plots were substantially altered, probably due to a change in climate, which is recorded in the local meteorological data, but the type of vegetation in the grassland remained the same. Effective and low-cost spontaneous recovery of species richness in mesic grasslands in central Europe previously subject to yearly applications of fertilizer may work provided the landscape has not been too much altered by human activity and target species are still present in the surroundings. Under these conditions, no active restoration measures are needed to reduce soil nutrients and sowing seeds of target species is unnecessary.

Keywords: climate fluctuations, fertilization, mesic grassland, nitrogen, restoration, species richness, vegetation

Introduction

European semi-natural grasslands are valuable secondary habitats (Leuschner & Ellenberg 2017), often with high biodiversity (Wilson et al. 2012, Feurdean et al. 2014). However, in the second half of the 20th century, many were subjected either to land-use intensification, typically accompanied by the application of fertilizer, or were abandoned in less productive marginal areas, which usually led to succession towards woodland (Bakker 1989, Dengler et al. 2014). In the past few decades, the intensity of agricultural use has generally decreased in at least some parts of Europe, particularly in terms of a reduction in the amounts of fertilizer used (Waldén & Lindborg 2016). Consequently, the effects of

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a reduction or cessation in the use of fertilizer should be of interest to both ecologists and agriculturalists.

There are many studies on the association between the decline in species richness with intensive application of fertilizers, mostly as nitrogen or in combination with phosphorus (Willems et al. 1993, Crawley et al. 2005, Silvertown et al. 2006, Honsová et al. 2007). An abundance of nutrients usually leads to an increase in biomass and, consequently, intensive competition for light and the elimination of less competitive species (Harpole & Tilman 2007, Kirkham et al. 2014). There are very few studies on changes in community parameters after fertilizers ceased to be used, especially long-term studies (Olff & Bakker 1991, Spiegelberger et al. 2006, Hejcman et al. 2007a, b, Schmidt 2007, Semelová et al. 2008, Heinsoo et al. 2020). An increase in richness of target species after the cessation of the application of fertilizers is a good indicator of successful recovery or restoration of degraded grasslands towards semi-natural vegetation (Mountford et al. 1996, Bakker & van Diggelen 2006, Grootjans et al. 2006). The few studies of the spontaneous recovery of plant diversity after the cessation of the application of fertilizers in secondary grasslands in Europe report both fast (Mountford et al. 1996, Schmidt 2007) and slow recovery (Walker et al. 2004, Spiegelberger et al. 2006, Semelová et al. 2008). Walker et al. (2004) conclude that the reassembly of plant communities resembling those in semi-natural grasslands has generally been slow, while Semelová et al. (2008) discuss possible irreversible changes in species composition.

The influence of changing climate on ecosystems is frequently discussed (Vermaat et al. 2017), however, its effect on their species composition based on quantitative data is rarely demonstrated, even for grasslands (Dong et al. 2020). One-time extreme fluctuations in weather conditions are reported to change species composition only temporarily (Osbornová et al. 1990).

We studied changes in the vegetation in a mesic grassland after a two-decade experimental application of fertilizer ceased and the grassland was allowed to recover for nearly three decades. The results for experimental plots that received frequent applications of fertilizer, compared with that of controls in which no fertilizer was applied, were presented and the trajectory of the changes in vegetation up to 2006 was described in a previous paper (Královec et al. 2009). Here, we report the changes in vegetation that occurred over a longer period of time of more than another decade. We asked: (i) What is the long-term trajectory in the changes in vegetation that occurred after the cessation of the application of fertilizer? (ii) Are there any recent changes in trends?

**Methods**

**Study site**

The study site is located in the western part of the Czech Republic (49°58'37.2N, 12°45'14.4E), at elevation of 750 m. The climate is moderately cold and wet, with a mean annual temperature of 6.4 °C and about 700 mm of precipitation per year (measured by a meteorological station at the locality). Soil is classified as acidic cambisol. In 1966, the entire previously extensively managed (mown and grazed) grassland was ploughed and seeded with a standard commercial mixture used for agricultural reclamation (for more details about soil conditions and seed mixture, see Královec et al. 2009).
Experimental plots of 2.5 × 6.0 m were established in the central part of the reclaimed grassland in 1968. A randomized block design was used with the following types of plots: controls – no fertilizer applied; treated with applications of 80, 160 (the most common at that time), 240 and 320 kg/ha of nitrogen (N) in the form of ammonium nitrate per year. Long-term records are available only for controls (4 replicates) and plots that were previously treated with the highest dose of fertilizer (8 replicates). The fertilizer was either applied in a single dose (4 replicates) or in three separate amounts during the growing season (4 replicates). The differences in application did not appear to have a significant effect (see Fryček et al. 1992), therefore, the variants are not distinguished here. Except for the controls, all of the plots were treated with constant doses of phosphorus (32 kg/ha P as superphosphate) and potassium (100 kg/ha K as potassium chloride). The application of fertilizer ceased in 1989. Since that time, the sward was only cut two times a year (June, September). After 1989, the intensity of management of the whole surrounding landscape decreased.

Data collection and processing

Visual estimates of the percentage-cover of all vascular species of plants were made in a 4 × 2 m subplot within each experimental plot to avoid edge effects. Data was collected in the first half of June 1990, 1994, 1996, 1997, 1999, 2003, 2006, 2009, 2012, 2015 and 2018, before the first mowing of the season. Vegetation data was processed using the unconstrained ordination method (Detrended Correspondence Analysis, DCA) in Canoco 5 software (ter Braak & Šmilauer 2012). Variability within the data was 2.8 SD units. Cover data were logarithmically transformed to downweigh dominant species (Šmilauer & Lepš 2014). Centroids of the samples were plotted. Time since the cessation of the application of fertilizer, the number of species per plot, the number of grassland species per plot (classified as diagnostic and constant species, based on Chytrý 2007) and the mean Ellenberg indicator values for moisture and nutrients (Chytrý et al. 2018) were used as passive variables in the ordination diagrams. To explore the differences in mean species number between the controls and the plots that were previously treated with fertilizer each year, the F-test (testing the homogeneity of variances) was used, followed by a two-way t-test (Šmilauer & Lepš 2014). Univariate analyses were performed in R software (R Core Team 2017).

Daily temperature and precipitation were measured at the locality over the entire period of the study (by J. Královec).

Results

After the cessation of fertilizer application, it was approximately two decades before the vegetation of the plots that were previously treated with fertilizer approached that of the control plots (Fig. 1A). However, after the first decade of more or less constant composition, directional changes were recorded in the controls at the end of the second and beginning of the third decade. In the last decade, both variants (plots previously treated with fertilizer and controls) followed more or less the same parallel trajectories. The greatest recent changes happened in both variants between 2012 and 2015. We attribute this mainly to the extremely warm and dry season in 2015 (see below). Isolines in Fig. 1A
indicate a continuous increase in species number in the plots previously treated with fertilizer until the beginning of the third decade after which it stabilized. Most species in the experimental plots are typical of mesic grassland sites (Fig. 1B). Passively projected community characteristics indicate similar increases in grassland species and total species richness over time, which indicates that the increase in the total number of species was mainly due to an increase in the number of grassland species. The arrows corresponding to the Ellenberg indicator values indicate a decrease in nutrients with time and a reduction in moisture in the third decade of the study. The last two samples collected are located on the ordination diagram in opposite positions to the arrow corresponding to wetter site conditions. It is likely that this is due to the warm and dry seasons in the second half of the third decade (see Fig. 2), especially the extremely dry conditions in 2015, which pushed the species composition in a different direction. In 2015, the lowest precipitation in the growing season in 30 years was recorded (range of 260–629 mm). Also, the precipitation in the following three growing seasons was below the average of 412 mm. At the same time, the years 2014–2018 were much warmer than the 29-year average (8.5 vs 7.3 °C), and also the growing seasons (April–September) were nearly one degree Celsius warmer than the average (14.3 vs 13.4 °C). The mean annual temperature significantly increased during the whole observation period and the decrease in precipitation was insignificant (Fig. 2).

The mean number of species in the plots previously treated with fertilizer reached the initial species number in the controls within ~5 years, but the species number increased even in the controls. The differences in the mean number of species in the plots previously treated with fertilizer and the controls were significant during the first two decades (except 1999) and non-significant in the third decade (Fig. 3). This suggests that after two decades the vegetation in those previously treated with fertilizer and control plots was more or less equal in terms of species richness.
Fig. 2. – Trends in mean precipitation and temperature during the growing seasons. The mean temperature in the growing season (dashed line, black circles) significantly increased during the observation period (linear regression: F = 12.6, P < 0.001) whereas the trend in mean precipitation (full line, empty squares) was not significant.

Fig. 3. – Comparison of mean species richness (per 4 × 2 m) in the controls and the plots previously treated with fertilizer (320 kg N/ha) in the period from 1990 to 2018. The results of a two-way t-test are shown above the boxes: significant differences (P < 0.05) are indicated by asterisk, n.s. – not significant.
Discussion

Spontaneous recovery after the cessation of applying large amounts of fertilizer, measured in terms of total species composition and species richness of grassland vegetation, appears to be effective, as we previously concluded after 16 years of succession in the same system (Královec et al. 2009). Since the end of the second decade, both the controls and the plots previously heavily treated with fertilizer were nearly equal in terms of their species composition and in the third decade the difference in the numbers of species in the plots were not significant. In the last decade, the vegetation in the plots previously treated with fertilizer and control plots changed in favour of species that prefer drier conditions, such as *Leontodon hispidus*, *Luzula campestris* and *Galium verum*; this trend was associated with a decrease in site moisture. We are aware that the shift was only indirectly indicated by the Ellenberg indicator values, but it was evident. Between 2012 and the next sample recorded in the extremely dry year 2015, both the controls and the plots previously treated with fertilizer exhibited by far the greatest change in species composition during the entire study, but in a different direction than before. This trend continued due to low precipitation and higher temperatures, which exacerbated the lack of precipitation, and is most likely the cause of the shift. It appears that the change in climate resulted in the system following another trajectory. On the other hand, the grassland studied did not change substantially in terms of physiognomy and probably functioning, as the type of vegetation is still that of submontane meadows typical of central Europe at this particular altitude (Chytrý 2009).

Various worldwide studies have investigated responses of grasslands to interannual fluctuations in climate (Craine et al. 2013), usually in terms of phenology (Wu et al. 2012, Cui et al. 2017), productivity (Knapp et al. 2017, Zhao et al. 2018), the effect of disturbance such as grazing in an area subject to a fluctuating climate (Dong et al. 2020), and species composition (Bodner & Robles 2017, Serafini et al. 2019). Generally, grasslands exhibit no or negligible (Sternberg et al. 2017) to great (Sebastià et al. 2008) changes in various ecosystem characteristics. Sometimes, the changes are irreversible or at least long-lasting, when a certain threshold is exceeded (De Boeck et al. 2018). Still, most grasslands are highly resistant (Grime et al. 2008) or resilient (Anjos & de Toledo 2018, Abbas et al. 2019) to fluctuations in climate. Synergic effects of warming and drought are reported (De Boeck et al. 2016). Although the grassland studied was probably induced to follow a different trajectory by recent changes in climate, we cannot exclude a potential return if there is a series of wet and colder years in the future. But this seems to be less and less probable under the current change in climate (Xu et al. 2018).

The recovery from previous applications of large amounts of fertilizer recorded in this study was faster than in most other studies on similarly treated grasslands (e.g. Grootjans et al. 2006, Spiegelberger et al. 2006, Heinsoo et al. 2020). Slow (Grootjans et al. 2006) or even no recovery (Oomes et al. 1996, after 10 years) are also reported, especially at productive sites with a largely depleted local species pool. Semelová et al. (2008) report differences in species composition of plots treated with fertilizer and control plots that were not treated as long as 62 years after the application of fertilizer stopped in unproductive subalpine meadows. Fast recovery is reported by Schmidt (2007), who describes a species-poor *Alopecurus pratensis* meadow developing into a species-rich meadow five years after the cessation of applying fertilizer. A relatively fast recovery is also reported
by Mountford et al. (1996), but in this case the fertilizer was only applied in four years.
Species replacement (reported by e.g. Olff & Bakker 1991) has not yet occurred at our
study site, but there were quantitative changes in the cover of resident species and the
establishment of new species. Especially species typical of semi-natural grasslands
(Molinio-Arrhenatheretea and Festuco-Brometea classes of the continental vegetation
system; Chytrý & Tichý 2003) increased in number and cover, which is desirable from an
ecological restoration point of view (Allison & Murphy 2017). A similar fast recovery of
bacterial soil communities is recorded for our site, but a period of 20 years following the
cessation of the application of fertilizer was not enough for fungal soil communities to
recover (Čuhel et al. 2019). The recovery after the cessation of the application of fertilizer
is discussed more extensively by Královec et al. (2009). Various grasslands obviously
differ greatly in the way the plant communities recover after the application of fertilizer
cesses, making any generalization very tentative.

The propagule input from both the controls and the broader surroundings into the plots
previously treated with fertilizer is likely to have resulted in the increases both in the
numbers of species and typical grassland species (Královec et al. 2009). Since the land-
scape where the experimental plots were located was less intensively managed after
1989, species typical of more natural grasslands have increased in abundance. Such
changes in the local species pool (Zobel et al. 1998) are thus assumed to have contributed
to the increase in species richness recorded in our experimental plots and that they reflect
changes in metapopulation dynamics of individual species (Silvertown et al. 2006).

It will be interesting to know whether the different trajectory, which started in the past
decade, will continue or the composition of the vegetation will change yet again and what
the role of future fluctuations in climate will be (Gibson & Newman 2019).

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Souhrn
Mezické polopřirozené trávníky ve střední Evropě byly, a leckde stále jsou, intenzivně obhospodařované, včet-
ně přílišného hnojení, které vedlo k jejich degradaci. Proto je všeobecně žádoucí obnova jejich druhového slo-
žení a diverzity. Po dobu téměř tří desetiletí jsme studovali spontánní obnovu polopřirozené podhorské louky,
která byla předtím každoročně hnojena množstvím dusíku v přepočtu 320 kg/ha po dobu dvaceti let. Změny
v druhovém složení jsme porovnávali s nehnojenými kontrolami. Během prvních dvou desetiletí se druhové
složení na obou typech ploch téměř vyrovnalo. Počet druhů na hnojených plochách se vyrovnal kontrolám
v době počátku sledování již po ~5 letech, avšak jak na kontrolách, tak na hnojených plochách stoupal i nadále
a stabilizoval se po ~20 letech, kdy rozdíly již nebyly průkazné. V poslední dekádě sledování se paralelní
trajektorie obou typů ploch zásadně změnily, pravděpodobně v důsledku climatických výkyvů – konkrétně
extrémně suchého roku 2015 a suchých a teplých let následujících. Spontánní, a tudíž levná obnova dříve
přehnojovaných mezických luk může v podmínkách střední Evropy fungovat, pokud okolní krajina není příliš
pozměněna člověkem a cílové druhy se v okolí stále vyskytují. Za těchto okolností nejsou potřebná dražší
obnovná opatření, jako je redukce živin v půdě (např. odstranění svrchní vrstvy půdy) nebo vysévání cílo-
vých druhů.
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