The Grass Garden in the Giant Mts. (Czech Republic): Residual effect of long-term fertilization after 62 years

Věra Semelová a, Michal Hejcman b,*, Vilém Pavlů c, Stanislav Vacek a, Vilém Podrážský a

a Department of Silviculture, Czech University of Life Sciences, Kamýcká 1176, CZ-165 21 Prague 6, Suchdol, Czech Republic
b Department of Ecology and Environment, Czech University of Life Sciences, Kamýcká 1176, CZ-165 21 Prague 6, Suchdol, Czech Republic
c Department of Plant Ecology and Weed Science, Crop Research Institute, Rolnická 6, CZ-460 01 Liberec, Czech Republic

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Abstract

The aim of the study was to investigate how plant species’ composition, soil parameters and nutrient concentrations in plant biomass differ between fertilized and control plots 62 years after the last fertilizer application on a sub-alpine grassland.

A piece of land called the Grass Garden (GG), fertilized with wood ash and manure for at least 200 years, was rediscovered in the Giant Mts. (Krkonoše, Karkonosze) in 2006. The last fertilization was applied in 1944. The central part of GG (treatment A), the edge of GG (treatment B) and never-fertilized control plots outside of GG (treatment C) were distinguished.

Sixty-two years after the last fertilization Nardus stricta was dominant in treatment C and Deschampsia cespitosa and Avenella flexuosa in treatments A and B. The predominance of these grasses was first described in 1786 and repeatedly during the 19th and 20th centuries and indicated the long-term stability of plant species’ composition in the sub-alpine grassland. In the case of GG, long-term fertilization has had a long-term “stable after-effect” upon differences in plant species’ composition.

Ca concentration in the soil was more than two times higher in treatments A and B than in the control, indicating that it was very difficult to deplete applied Ca even on extreme podzol soils and under the climatic conditions of the sub-alpine vegetation belt.

In above-ground plant biomass, Mg and P concentrations and N:P ratio were still significantly affected by treatment.

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Keywords: Ca; Mg and P concentration; Plant species’ composition; Long-term experiment; Mountain grassland; Resilience

1. Introduction

The Park Grass Experiment (PGE), established in Rothamsted in 1856, was described by Silvertown et al. (2006) as the oldest still running fertilizer and ecological experiment in the world.

In 2006 the Grass Garden (GG) composed of a piece of land fertilized with organic fertilizers and a never fertilized control was identified in the Giant Mts. (Krkonoše, Karkonosze, and Riesengebirge in Czech, Polish, and German, respectively). The highly contrasting plant species’ composition between fertilized and control plots was first described in 1786 (Haenke et al., 1791). The oldest written record concerning GG was made in 1778, but GG was probably established together with Meadow Chalet in the second half of the 16th century (Lokvenc, 1978).

GG has been permanently marked on maps and has been delimited by boundary stones in the field. Therefore the border between plots with different fertilizer regimes has been stable for at least the last 250 years and was accurate to a few dm. Further GG was in permanent use by owners of the Meadow Chalet, who regularly fertilized the grassland year after year. Fertilized and the never-fertilized control grassland was cut or sometimes grazed by livestock (Lokvenc, 1978).

Given these specific features, the GG was denoted probably as the oldest still running grassland fertilizer experiment in existence.
A comparison of data collected at the same place under different terms enables the investigation of the stability of plant communities. GG enabled research into the stability of grassland communities even after more than 200 years, probably making it the longest stability test in plant ecology worldwide. Furthermore, perturbation by fertilizer application can affect a wide range of ecosystems functioning for many years (Willems and van Nieuwstadt, 1996; Pettersson and Hogbom, 2004; Comerford et al., 2002; Sammul et al., 2003; Marriott et al., 2005; Niinemets and Kull, 2005; Koo’s and Németh, 2006; Heyel and Day, 2006; Remesˇ and Podrážský, 2006; Sardi et al., 2006; Vacek et al., 2006). Spiegelberger et al. (2006) and Hejcman et al. (2007) revealed that short-term perturbation by fertilizer application could cause long-term changes in grassland functioning. GG provided researchers with answers on grassland resilience (the ability of a system to return to normal after a disturbance or stress period (Lepš et al., 1982)) after a long-term perturbation by fertilization. To date, resilience of grasslands after such a long period of fertilization has not been described in literature.

Therefore, the aim of this paper was to answer the following question: how do (1) chemical soil properties, (2) plant species composition and (3) concentrations of nutrients in plant biomass differ between fertilized and control plots more than 60 years after the last fertilizer application?

2. Materials and methods

The Grass Garden is located on the top of the east part of the Giant Mts. in the borderland between the Czech Republic and Poland. The study site lies above the upper tree limit at altitudes ranging from 1415 to 1430 m above sea level. The mean annual temperature was 2 °C and the mean annual precipitation was 1380 mm (Vrbatova Bouda Meteorological Station). In the study area, podzols have developed on medium grain porphyric granite with the following attributes: the mean thickness of litter (L), fermentation (F), and humus (H) layers was 5 cm and the mineral Ah horizon was 3 cm thick and dark with 40–50% organic matter and characterized by pH (H2O) 3.9–4.2.

The area of GG was approximately 5 ha large and was fertilized by manure and wood ash for a period longer than 200 years. The GG and surrounding grassland had been cut in never fertilized grassland (treatment C), a transition zone (B) on the edge of GG, and the central part of GG (A). B treatment was probably fertilized by lower nutrient doses than A treatment. It was possible to suppose that the plant species’ composition was the same before the start of fertilization because of the same environmental and soil conditions of all treatments. All data were collected in 10 horizontal triplets at the end of July 2006. To eliminate pseudoreplication, the distance between triplets was made as long as possible and the distance between treatments as short as possible. Each triplet consisted of treatments A, B, and C. Plots of 5 m × 5 m were used for the collection of all data: soil samples, relevés (vegetation samples based on cover estimates), biomass samples, and compressed sward height. Soil cores were taken from four places within each plot after relevés were made and biomass samples clipped. Upper litter layers were removed and then the humus Ah horizon was sampled. The four samples from each plot were then mixed, oven-dried at 105 °C, and sieved (<2 mm). All analyses were performed in an accredited national laboratory according to the Mehlich III method to predict plant-available Ca, K, Mg and P (Mehlich, 1984). To measure pH (H2O), 5 g of soil was mixed with 25 ml of distilled water.

The cover of each vascular species was estimated directly in percentages. Nomenclature of vascular plants followed Kubát et al. (2002). Biomass samples were taken thereafter and the sward was clipped to a target height of 3 cm. Biomass samples were dried and analyzed for nutrients and mineral concentrations. Crude protein (CP), and crude fibre (CF) were determined using the Weende analyses method (AOAC, 1984). Ca, Mg, Na, P, and K concentrations were determined by means of colorimetry, photometry, and atomic absorption spectrometry in an accredited national laboratory.

Compressed sward height was measured using the rising plate meter method originally developed to simply estimate sward height on pastures (see Correll et al., 2003).

2.1. Data analysis

Redundancy analysis (RDA) in the CANOCO 4.5 program (ter Braak and Šmilauer, 2002) was used to evaluate multivariate plant species data. Redundancy analysis is a direct gradient analysis based upon the assumption of linear response. This was used because data sets were relatively homogeneous and several environmental variables (treatments) were categorical. Monte Carlo permutation tests were performed completely at random within each triplet. In all analyses 999 permutations were used.

One-way ANOVA in Statistica 5.0 (StatSoft, 1995) followed by the Tukey HSD post hoc test were used to analyze univariate data.

3. Results

Mean Ca concentration was 1.59, 1.28, and 0.57% in treatments A, B, and C, respectively. The lowest Ca
Soil parameters

Soil pH (H₂O) was in the range 3.9–4.2 without any significant differences. Differences in the remaining soil characteristics were not significant, although the lowest concentration of P and Mg was detected in the control and vice versa in the case of total N and K concentrations. Soil pH (H₂O) was in the range 3.9–4.2 without any significant effect of treatment.

Fertilizer regime had a decisive effect upon plant species composition. Treatment was the significant predictor of sward structure. This alone explained 17.7% variability of plant cover data (analysis a3 in Table 3). Soil parameters explained 89.1% of variability in plant cover data (analysis a1 in Table 3). The explanatory power of all measured soil parameters was substantially lower than the treatments (analysis a2 in Table 3). Soil parameters explained collectively only 58.2% of variability in plant cover data. Ca concentration was the most powerful and significant predictor of sward structure. This alone explained 17.7% variability of plant cover data (analysis a3 in Table 3).

Concentrations are given in percentages. Values with the same letter are not significantly different. ‘±’ indicates S.D.

Soil parameters

<table>
<thead>
<tr>
<th>Tested variable</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Treatment C</th>
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<tbody>
<tr>
<td>Ca concentration</td>
<td>1.59 ± 0.48</td>
<td>1.28 ± 0.50</td>
<td>0.58 ± 0.04</td>
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<tr>
<td>Mg concentration</td>
<td>0.29 ± 0.05</td>
<td>0.26 ± 0.06</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>K concentration</td>
<td>0.30 ± 0.11</td>
<td>0.28 ± 0.03</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>N concentration (total)</td>
<td>0.20 ± 0.12</td>
<td>0.22 ± 0.04</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>pH/H₂O</td>
<td>1.38 ± 0.50</td>
<td>1.65 ± 0.66</td>
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Biomass characteristics

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mean Mg concentration was 0.13, 0.11, and 0.08%, and the mean P concentration was 0.34, 0.28, and 0.15% in treatments A, B, and C, respectively (Tables 1 and 2). The highest concentrations of crude protein and Ca were in treatment A and the highest concentration of crude fibre in the control. However, the differences were not significant. The effect of treatment on the N/P ratio in biomass was significant. The N/P ratio was 6.3, 7.0 and 11.5 in treatments A, B, and C, respectively.

The mean compressed sward height was 16, 12 and 11 cm in treatments A, B, and C, respectively, and the differences were significant (Tables 1 and 2).

4. Discussion

A sharply contrasting floristic composition of fertilized and control treatments was first described in 1786 (Haenke, 1791); in GG the dominant grasses D. cespitosa and A. flexuosa sharply contrasted with the dominant N. stricta in the control. The same “pattern” of dominant species was again described at the beginning of the 19th century (Hoser, 1804), in 1960s (Štursa and Rejmánek, unpublished data; Štursová, 1974), and 2006. The same dominant species in fertilized and control plots for a period of at least 220 years indicate a relatively high stability of sub-alpine grassland communities created by different fertilizing regimes. Long-term stability of alpine grasslands was consistent with results obtained from the experiment conducted by Dr. Lüdy in the Alps (Spiegelberger et al., 2006) and with long-term results by Zhou et al. (2006) from the Tibetan Plateau. The long-term stability of alpine grassland, composed of only several species, contradicts the conclusions of Tilman et al. (2006) who held that temporal stability of an ecosystem increases with species diversity.

In GG, the most interesting finding was the relatively high stability of plant species composition even 62 years after the last fertilization. N. stricta was still almost missing in the fertilized plots and, conversely, D. cespitosa in the control. This was in contrast with the results of Spiegelberger et al. (2006) and Hejcman et al. (2007) revealing an asymptotical approach of fertilized treatments of the control plots. In contrast to both studies discussed above, in which perturbation by fertilization was only short-term, long-term fertilization in the case of GG had a long-term “stable after-effect” on differences in plant species composition generated by fertilization. Results from the above studies and GG indicate that in the case of sub-alpine grassland, resilience of a plant community after short-term perturbation by fertilization can be achieved after many years. However, a change of plant community after long-term perturbation can take more than several decades or may be irreversible. To the author’s knowledge, such a long stable after-effect of fertilization on plant species’ composition has not been documented in literature to date. In alpine grassland, the dominance of D. cespitosa in plots which had a history of considerable manure usage was also mentioned by Bovolenta et al. (2002), but further details were not published. Although management of GG was terminated in 1944, regular grazing of fertilized plots by red deer and hares was recorded by hunters in many vegetation seasons preceding data collection. A. flexuosa was preferred and was selected from tall vegetation dominated by less grazed D. cespitosa; N. stricta was completely omitted in the control. The amount of grazed biomass was estimated at 10–20% of available forage in GG. A similar observation was recorded in Dr. Štursova’s experiment (Hejcman et al., 2007) located in close vicinity to GG: plots fertilized by phosphorus 40 years ago were selectively grazed by red deer, causing total disappearance of available above-ground biomass in 2006 (Hejcman, unpublished data). Selective grazing of fertilized plots can be explained by their higher forage quality due to increased concentrations of Ca, Mg, P, crude protein and lower concentrations of crude fibre in the biomass. This explanation was consistent with results by Schutz et al. (2006) and Jewell et al. (2007) from the Alps. Selective grazing evidently contributed to the transport of nutrients from previously fertilized plots, as most defecation occurred outside of GG. Despite this fact, nutrient depletion, with the exception of K, has not been achieved so far, as evidenced by higher concentrations of Ca, Mg, and P in the soil. Additionally, significantly higher concentrations of P and Mg, and significant differences in N/P ratio in plant biomass from the fertilized plots compared to the control in 2006 were recorded. The significant long-term after-effect of

### Table 3

Results of RDA analyses of plant species composition data

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Explanatory variables</th>
<th>Covariables</th>
<th>% ax1(all)</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>Treatments (A, B, C)</td>
<td>–</td>
<td>86.7 (89.1)</td>
<td>104.4 (65.07)</td>
<td>0.001 (0.001)</td>
</tr>
<tr>
<td>a2</td>
<td>Ca, K, Mg, N, P, pH</td>
<td>–</td>
<td>51.8 (58.2)</td>
<td>21.46 (4.65)</td>
<td>0.001 (0.001)</td>
</tr>
<tr>
<td>a3</td>
<td>Ca</td>
<td>K, Mg, N, P, pH</td>
<td>17.7</td>
<td>4.3</td>
<td>0.027</td>
</tr>
<tr>
<td>a4</td>
<td>Mg</td>
<td>Ca, K, N, P, pH</td>
<td>6.3</td>
<td>1.34</td>
<td>0.246</td>
</tr>
<tr>
<td>a5</td>
<td>P</td>
<td>Ca, K, Mg, N, pH</td>
<td>7.5</td>
<td>1.62</td>
<td>0.188</td>
</tr>
<tr>
<td>a6</td>
<td>K</td>
<td>Ca, Mg, N, P, pH</td>
<td>3.7</td>
<td>0.77</td>
<td>0.431</td>
</tr>
<tr>
<td>a7</td>
<td>N (total)</td>
<td>Ca, Mg, P, pH</td>
<td>3</td>
<td>0.62</td>
<td>0.527</td>
</tr>
<tr>
<td>a8</td>
<td>pH (H2O)</td>
<td>Ca, K, Mg, N, P</td>
<td>2.1</td>
<td>0.44</td>
<td>0.625</td>
</tr>
</tbody>
</table>

Analysis, analysis code; % ax1 (all), species variability explained by canonical axis 1 or by all axes (measure of explanatory power of the environmental variables); F-ratio, F statistics for the test of the particular analysis; P-value, corresponding probability value obtained by the Monte Carlo permutation test. 999 permutations were used in all analyses. Significant results are in bold. Results in parentheses are for all constrained axes.
fertilization on the concentration of nutrients in the above-ground biomass was consistent with results by Hegg et al. (1992) and Hejman et al. (2007). The higher Ca concentration in the soil was at least partly consistent with results by Spiegelberger et al. (2006) who recorded an increased Ca concentration 70 years after the last liming. In GG, Ca concentration in the fertilized plots was almost three times higher than in the control 62 years after the last wood ash and manure application. This result indicated that it was very difficult to deplete applied Ca even in extreme podzol soils and the climatic conditions of sub-alpine vegetation zone. The revealed N/P ratio indicated a much higher P efficiency in the *N. stricta* dominated sward in the control than in the fertilized treatments. This concurred with the results of Gusewell et al. (2005). Furthermore, the current differences in sward structure probably reflected much larger differences in soil nutrients’ concentration in the past. All dominant grasses were recognized as long-term living species, which can remain at the same place for many years (Grime et al., 1988). The establishment of *N. stricta* is dependent on germinable caryopses (Hejman et al., 2005), but the conditions for its germination and seedling survival in dense swards dominated by *D. cespitosa* and *A. flexuosa* were probably unfavourable. Furthermore, *N. stricta* is a shade-intolerant species and so was probably not able to persist under the tall canopy of *D. cespitosa*.

*A. alpinum* quickly responded positively to fertilization even in other long-term fertilizer experiments in the sub-alpine zone of the Giant Mts. (see Hejman et al., 2007 and citations therein). *A. alpinum* was identified by Filipová and Krahulec (2006) as an S strategist, but its flexible response to fertilization was a trait rather common in R strategists.

At the time of *S. dioica* flowering, the red colour of GG sharply contrasted with the yellowish surrounding dominated by *N. stricta*. This remarkable contrast was first described in 1786 and repeatedly during the 19th and 20th centuries, as was the appearance of *B. major* (Štursova, 1974, Lokvenc, personal communication), a species dominating especially in the eastern part of GG. According to Pecháčkova and Krahulec (1995) the dominance of *B. major* in long-term abandoned grasslands was due to its ability to accumulate N in underground storage organs and to use stored N for quick growth in early spring. *Ranunculus acris* was not recorded in the relevés collected in 2006, although a high abundance of this species was reported in the western part of GG in 1960s (Štursova, 1974; Štursa and Lokvenc, personal communication) as well as in historical records (Haenke et al., 1791; Hoser, 1804). During the last 40 years there was a retreat of this species, probably for reasons of terminated management and unfavourable conditions for seedling emergence under the dense canopy. In the control, a higher abundance of *C. bigelowii* indicated its relatively low competitive ability to grow in tall vegetation. According to Fabiszewski and Wojtun (2001), *C. bigelowii* substantially expanded in *N. stricta* dominated swards due to eutrophication during the last 35 years. Results from GG did not support this finding as the expansion had probably only a local character.

Results from GG and other discussed studies show that resilience of plant community after short-term perturbation by fertilization can be achieved in the case of sub-alpine grassland after many years, but changes in sub-alpine grassland functioning after long-term perturbation take more than several decades or may even be irreversible.

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