Science of the Total Environment 808 (2022) 152081

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The Admont Grassland Experiment: 70 years of fertilizer application and its effects on soil and vegetation properties in an alluvial meadow managed under a three-cut regime

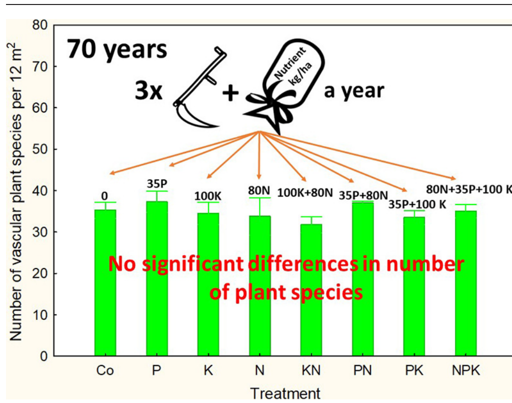
Lenka Pavlů [a], Erich M. Poetsch [b], Vilém V. Pavlů [a,c,*], Jan Titěra [a,c], Michal Hejcman [a,c], Jan Gaisler [a], Alan Hopkins [d]

[a] Department of Weeds and Vegetation of Agroecosystems, Grassland Research Station Liberec, Crop Research Institute, Rolnická 6, CZ 460 01 Liberec, Czechia
[b] Federal Research and Education Centre Raumberg-Gumpenstein, 8952 Irdning, Austria
[c] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 1176, CZ 165 21 Prague 6-Suchdol, Czechia
[d] GES Consulting, Exeter EX6 7JJ, UK

HIGHLIGHTS

- Fertilizer application is a key driver of changes in soil and vegetation in grassland.
- Different N, P, K fertilization rates with 3 cuts per year applied since 1946.
- Long-term application of different fertilisers affected soil pH.
- Dry matter biomass yield in the unfertilized treatment was co-limited by N and P.
- 70 years of fertilization affected species composition but not the species richness.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 November 2020
Received in revised form 26 November 2021
Accepted 26 November 2021
Available online 1 December 2021

Editor: Charlotta Poschenrieder

Keywords:
Biomass
Cutting
Herbage
Meadow
Nutrients
Species richness

ABSTRACT

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

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

* Corresponding author at: Department of Weeds and Vegetation of Agroecosystems, Grassland Research Station Liberec, Crop Research Institute, Rolnická 6, CZ 460 01 Liberec, Czechia.
E-mail addresses: pavlu@vurv.cz grass@volny.cz pavluv@fzp.czu.cz (V.V. Pavlů).

http://dx.doi.org/10.1016/j.scitotenv.2021.152081

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

1. Introduction

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

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

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

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

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

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

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

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

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

2. Material and methods

2.1. Study site

The long-term field fertilization experiment was established in 1946 in Admont, province of Styria (Austria), (47°34′52″N and 14°27′04″E; 635 m a.s.l.). The long-term average annual precipitation at this site is 1227 mm, and long-term mean annual air temperature is 6.8 °C. The mean temperature and average precipitation for the years 2015, 2016 and for the period 2007–2016 are presented in Table 1. The parent material at the experimental site is associated with the nearby Enns-river. According to the WRB-system the soil is classified as Gleyic Fluvic Dystric Cambisol with no supplementary qualifiers (IUSS Working Group WRB, 2015). In 1946 the soil characteristics were: pH/$CaCl_2$ = 6.05, P = 23 mg kg^{-1} (extracted by calcium acetate lactate - CAL), K = 323 mg kg^{-1} (extracted by CAL). The indicated the P concentration was classified as very low, whereas the K concentration was classified as high (BMLFUW, 2017). The experimental field had previously been used for cereal experiments in the 1930s and later reverted to an unsown grassland vegetation. In spring 1946 the field was ploughed and resown (24th May) with a grass-clover mixture with the following species and seeding rates: *Arrhenatherum elatius* 10.0 kg ha^{-1}; *Trisetum flavescens* 4.0 kg ha^{-1}; *Festuca pratensis* 20.0 kg ha^{-1}; *Poa pratensis* 4.5 kg ha^{-1}; *Phleum pratense* 4.5 kg ha^{-1}; *Agrostis stolonifera* 1.0 kg ha^{-1}; *Trifolium repens* 3.0 kg ha^{-1}; *Lotus corniculatus* 1.5 kg ha^{-1}. The present dominant species are *Agrostis capillaris*, *Anthoxanthum odoratum*, *Trisetum flavescens*, *Leontodon hispidus*, *Plantago lanceolata* and *Trifolium pratense*.

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

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

2.2. Design of the experiment

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

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

2.3. Species richness and plant species composition

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

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

2.4. Compressed sward height and dry matter biomass yield

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

2.5. Soil chemical properties

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

2.6. Data analysis

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

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

Redundancy analysis (RDA) in the CANOCO 5 program (ter Braak and Šmilauer, 2012) was used to evaluate multivariate vegetation, and soil and herbage chemical properties data. All plant cover, soil and herbage chemical properties data in RDA were logarithmically transformed [$y = \log(y + 1)$]. For all analyses 999 permutations were performed, with blocks used as covariables to restrict permutations into blocks. To visualize the results of the RDA analysis a standard bi-plot ordination diagram was used.

3. Results

3.1. Soil chemical properties

A significant effect of different management treatments was observed for pH/H_2O, pH/$CaCl_2$, N_{tot} and measured concentrations of plant-available P, K, Ca and Mg (Fig. 1). The mean value of pH/H_2O was lowest in the NK treatment (pH 4.58) and highest in the P (5.61) and NP (5.53) treatments (Fig. 1a). There were similar trends for pH/$CaCl_2$ and values were lower than the pH 6.05 obtained in 1946 at the start of the experiment (Fig. 1b). The highest mean values of N_{tot} were found in the NK (3.9 g kg^{-1}) and in the NPK (3.8 g kg^{-1}) treatments, and the lowest value was in the K treatment (3.2 g kg^{-1}) (Fig. 1c). The highest mean values for plant available P were in the P (125.4 mg kg^{-1}) and PK (114.4 mg kg^{-1}) treatments and the lowest values were in the NK (23.1 mg kg^{-1}), N (28.4 mg kg^{-1}), K (32.6 mg kg^{-1}) and Control (43.6 mg kg^{-1}) treatments (Fig. 1d). The highest mean value for plant available K was revealed in the K treatment (238.8 mg kg^{-1}), and the lowest value was in the NP (56.6 mg kg^{-1}) treatment (Fig. 1e). The highest mean plant available Ca concentrations were in the P (2148 mg kg^{-1}) and NP (2099 mg kg^{-1}) treatments, and the lowest values were in K (309 mg kg^{-1}) and NK (501 mg kg^{-1}) treatments (Fig. 1f). Further, the highest mean concentration of plant available Mg was found in the Control (134.9 mg kg^{-1}) treatment and the lowest concentration was in the NP (51.7 mg kg^{-1}) treatment (Fig. 1g). No significant effect of fertilization on the concentration of C_{org} was detected (Fig. 1h).

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

3.2. Plant species richness

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

3.3. Plant species composition

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

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

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

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

The proportion of the sward contributed by *A. capillaris* varied from 36.3% in the N-only treatment to 1.8% in the NPK treatment. Apart from the NPK treatment there was a tendency for *A. capillaris* to be the dominant species in other N-application treatments (N, NK and NP) (Fig. 3a). *Anthoxanthum odoratum* was also an abundant short-grass species but it showed no significant response to fertilization (Table S1). *Trisetum flavescens* was present in relatively high proportions in treatments with P fertilization: for NPK, PK, NP and P its mean cover values were 30.0, 17.8, 16.3 and 12.0%, respectively. The lowest cover values for *T. flavescens* were in treatment K (0.4%) and Control (0.6%). No effect of treatment on cover was found for *F. rubra* agg. (Fig. 3a). The cover of

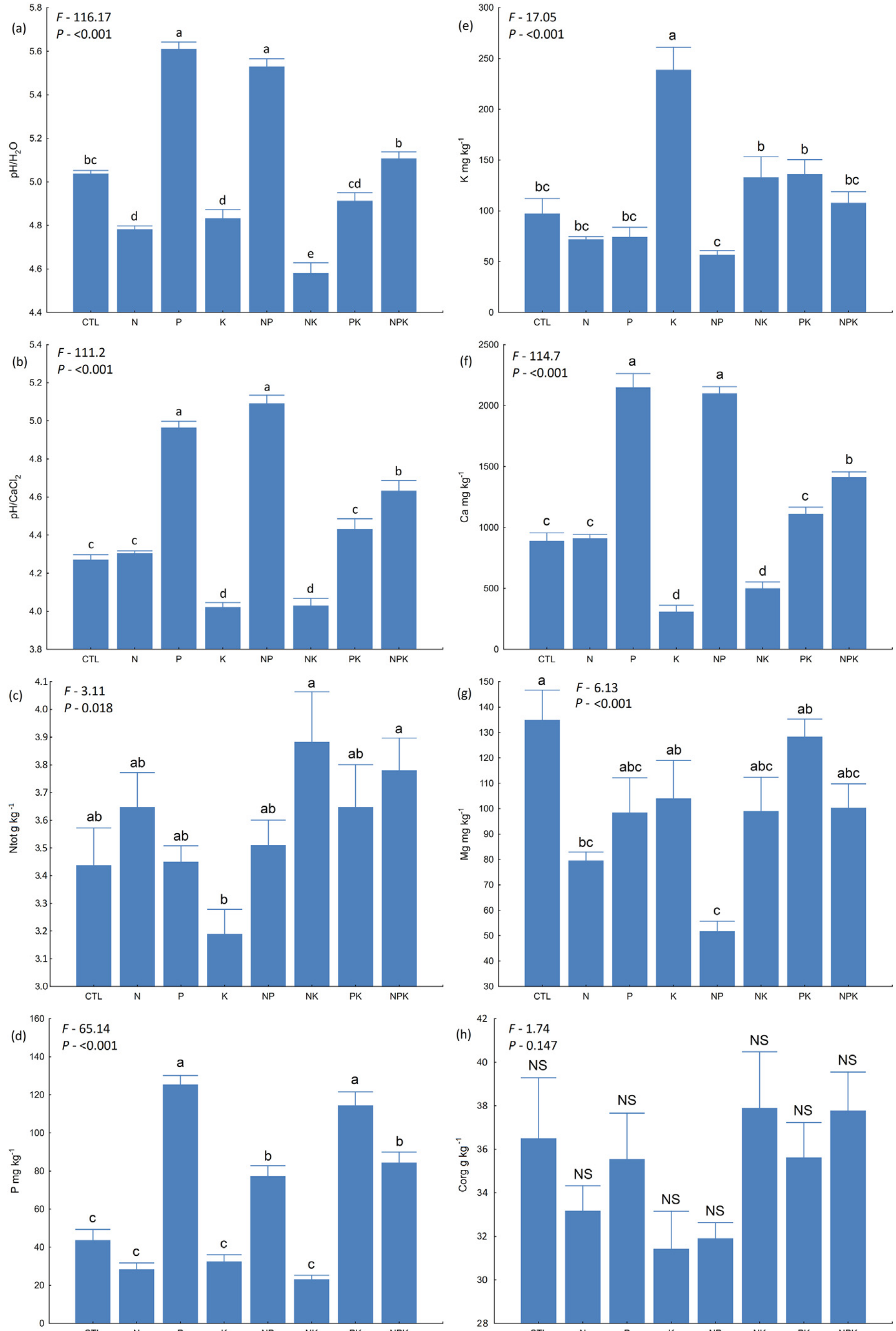


Fig. 1. Effect of fertilizer treatment on soil chemical properties: a) pH/H_2O, b) pH/$CaCl_2$, c) N_{tot}, d) P, e) K, f) Ca, g) Mg, h) C_{org}. Treatment abbreviations: CTL – non-fertilized control, N – N fertilization, P – P fertilization, K – K fertilization, NK – NK fertilization, NP – NP fertilization,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

L. campestris was highest in treatments N (9.3%), Control (6.3%) and K (5.5%) but was almost absent from the NPK treatment (0.1%) (Fig. 3a). Although *L. hispidus* was among the most abundant species recorded in the experiment, there were no effects of fertilization in relation to its cover (Fig. 3b). Among other forb species, *Achillea millefolium* had a significantly higher cover in the NK treatment (17.0%) than in other treatments (Fig. 3b). The cover of *Taraxacum officinale* agg. was also affected by treatment: it was highest in the NPK (5.1%) treatment and lowest in the K (0.2%) and Control (0.2%) treatments (Fig. 3b). *Alchemilla* spp. and *P. lanceolata* were not affected by fertilization (Table S1).

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

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

Variables	pH/H_2O	P	K	Ca	Mg
TNPS	0.39*	0.19	−0.15	0.29	−0.15
NPS ≤ 0.1	0.01	−0.20	0.11	−0.15	−0.17
DMB Yield 1st cut	0.22	0.35*	−0.39*	0.46**	−0.33
Total DMB yield	0.24	0.44*	−0.31	0.46**	−0.27
Graminoids					
Agrostis capillaris	−0.32	−0.46**	−0.26	−0.18	−0.44*
Anthoxanthum odoratum	−0.08	−0.21	−0.27	0.01	−0.31
Festuca rubra agg.	0.20	−0.18	−0.38*	0.23	−0.42*
Luzula campestris	−0.28	−0.39*	−0.14	−0.28	−0.17
Poa pratensis	0.63***	0.34	−0.53**	0.66***	−0.40*
Trisetum flavescens	0.41*	0.58***	−0.20	0.51**	−0.09
Forbs					
Achillea millefolium	−0.60***	−0.46**	0.35	−0.54**	0.08
Lathyrus pratensis	0.02	0.44*	0.01	0.11	0.24
Leontodon hispidus	−0.06	0.30	0.40*	−0.03	0.56***
Plantago lanceolata	0.47**	0.31	−0.28	0.54**	−0.23
Taraxacum officinale agg.	0.28	0.22	−0.24	0.33	−0.24
Trifolium pratense	0.11	0.40*	0.34	0.02	0.48**
Trifolium repens	−0.19	0.34	0.23	−0.15	0.35

F. rubra agg. in the ordination diagram was between the second and the third group. The third group was created predominantly by short graminoids (*A. capillaris*, *L. campestris* and *Carex* species), *Ajuga reptans* and *Hypochaeris radicata*, whereas *A. millefolium*, *Leucanthemum vulgaris* and mosses were connected with the fourth group.

3.4. Dry matter biomass yield and compressed sward height

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

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

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

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

4. Discussion

4.1. Soil chemical properties

In the P-only fertilization treatment the P was supplied as basic slag from 1946 to 1997 and from 1998 as superphosphate. Both of these

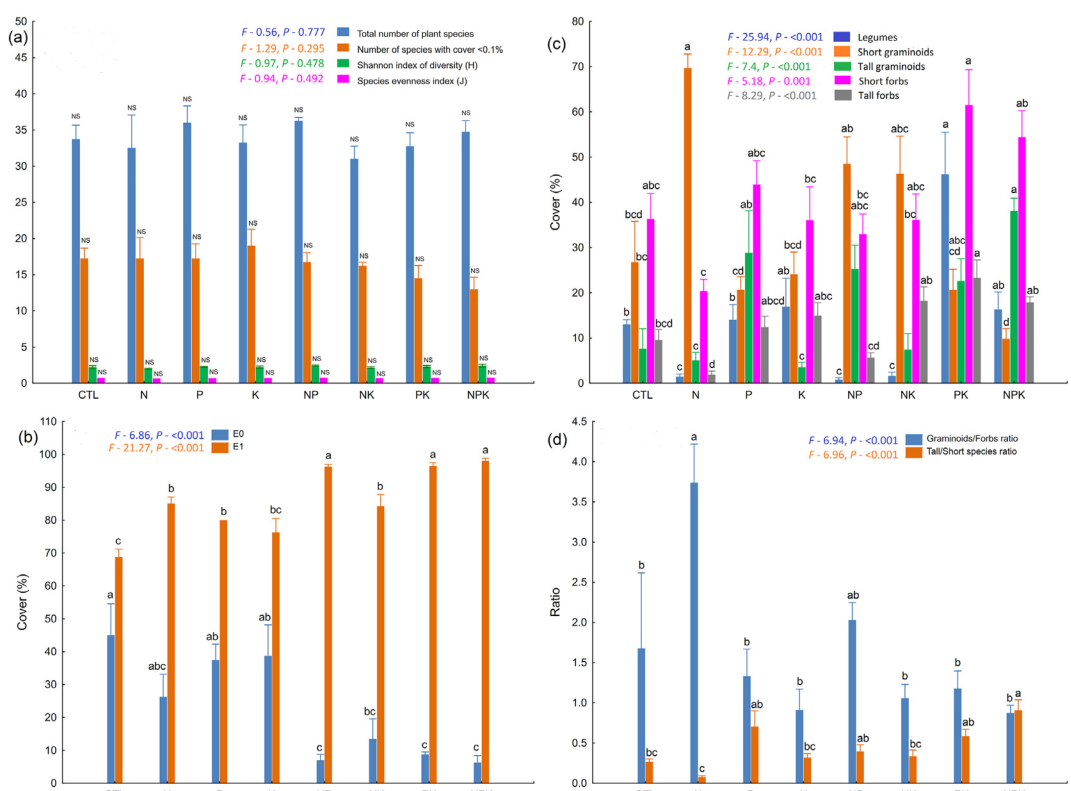


Fig. 2. Effect of fertilizer treatment on: a) species richness, b) cover (%) of E_0 (mosses) and E_1 (cover of vascular plant species), c) cover (%) of functional groups, d) ratios of graminoids/forbs and tall/short species. Treatment abbreviations are given in Fig. 1. In cases of significant differences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

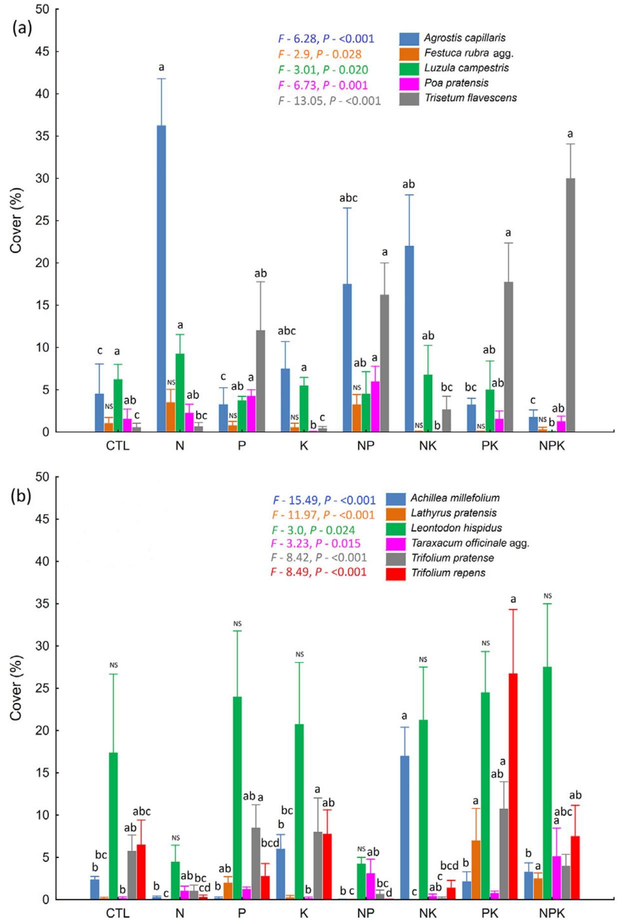


Fig. 3. Effect of fertilizer treatment on cover (%) of the most abundant vascular plant species: a) graminoids, b) forbs. Treatment abbreviations are given in Fig. 1. In cases of significant differences obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction, the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

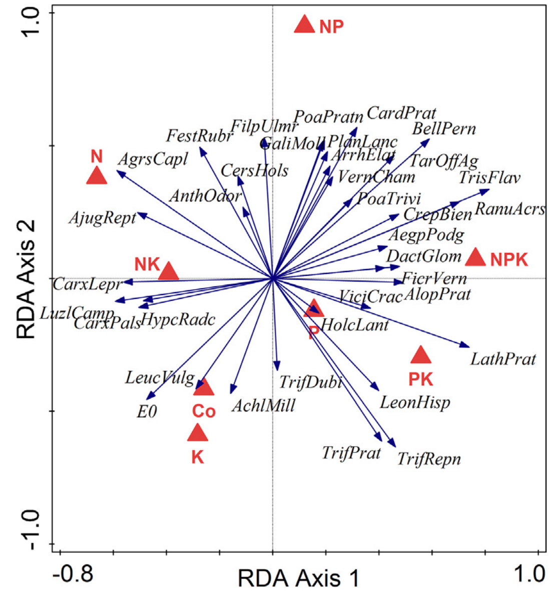


Fig. 4. Ordination diagram presenting the results of the RDA analysis showing changes in plant species composition, with treatments used as predictors. Treatment abbreviations are given in Fig. 1. Species abbreviations: *AchlMill* = *Achillea millefolium*, *AegpPodg* = *Aegopodium podagraria*, *AnthOdor*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

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

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

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

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

Soil P and K concentrations in the control treatment remained sufficient to meet fertilizer recommendations for grassland, including those of the Czech Agriculture Department recommendations for grassland management (Anonymous, 2009). This was despite the long-term biomass removal without any additional P and K inputs. In the case of P, this element is very stable in soils (Janssens et al., 1998) and the quantities removed in herbage biomass are typically small (2–4 kg P/kg DM) (Hopkins et al., 1994; Hrevušová et al., 2014; Marrs, 1993; Pavlů et al., 2013). In the case of K, the mineral-rich alluvial clay soils on this site are able to release considerable amount of K (Schellberg et al., 1999; Hejcman et al., 2010) and thus maintain a sufficient K level in the soil despite the high amount of K removed with harvested herbage (Alfaro et al., 2003).

4.2. Plant species richness

Many previous studies have shown that long-term fertilizer applications lead to reduced plant species richness in grasslands (e.g. Silvertown, 1980; Schellberg et al., 1999; Hejcman et al., 2007; Kidd et al., 2017; Titěra et al., 2020). However, among the treatments examined in the Admont experiment we did not detect any significant effects of fertilization on species

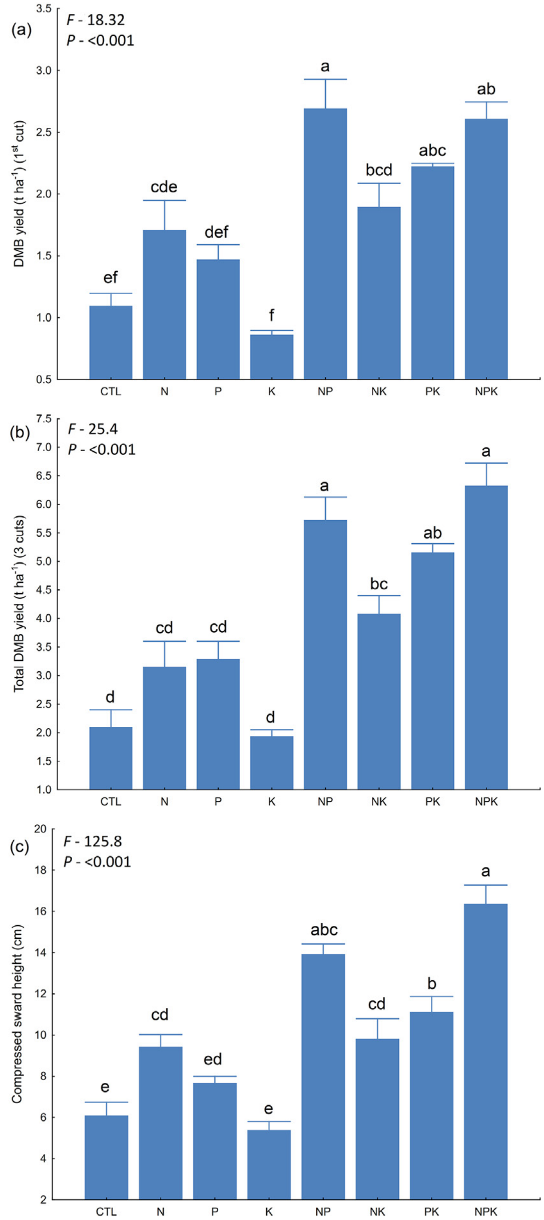


Fig. 5. Effect of fertilizer treatment on a) dry matter biomass (DMB) yield before the 1st cut, b) total DMB yield, c) compressed sward height. Treatment abbreviations are given in Fig. 1. In cases of significant differences (P < 0.05) obtained by linear mixed-effects modelling, the post hoc comparison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

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

Variable	E_1	E_0	TNPS	NPS $\leq$ 0.1	CSH
E_0	−0.84***				
TNPS	0.01	0.017			
NPS $\leq$ 0.1	−0.38*	0.38*	0.59***		
CSH	0.84***	−0.73***	0.1	−0.37*	
DMB Yield ^{1st} cut	0.85***	−0.72***	0.07	−0.33	0.90***

richness. It is also unlikely that weather conditions before the vegetation data collection (autumn 2015 and spring 2016) would have influenced species richness and botanical composition.

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

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

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

4.3. Plant species composition

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

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

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

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

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

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

4.4. DMB yield and compressed sward height

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

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

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

5. Conclusion

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

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

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

CRediT authorship contribution statement

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

Declaration of competing interest

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

Acknowledgements

The long-term experiment is maintained by AREC Raumberg-Gumpenstein. Botanical survey and paper preparation was supported by the Ministry of Agriculture of the Czech Republic, Project No. RO0418 and by the Internal Grant Agency - Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Project No. 20184239. Useful comments from anonymous reviewers are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152081.

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