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RESEARCH ARTICLE

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Response of grassland vegetation composition to different fertilizer treatments recorded over ten years following 64 years of fertilizer applications in the Rengen Grassland Experiment

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Abstract

Questions: Fertilizer application is a key driver affecting the diversity and conservation value grassland vegetation. Using a long-term fertilization experiment in mountain grassland, we addressed the following questions: (a) what is the effect of long-term fertilizer applications on species richness and plant species composition, and (b) is there any detectable trend in plant species composition during ten years of continuous observation?

Location: The Rengen Grassland Experiment, Eifel Mountains, Germany (established in 1941).

Methods: Five treatments including different fertilizers applied annually and one unfertilized control were analysed: Ca (718 kg Ca ha^{-1}); CaN (752 Ca and 100 N kg ha^{-1}); CaNP (752 Ca, 100 N and 35 P kg ha^{-1}); CaNP-KCl (752 Ca, 100 N, 35 P and 133 K kg ha^{-1}); CaNP-K_2SO_4 (752 Ca, 100 N, 35 P and 133 K kg ha^{-1}). The experiment included five replicates per treatment in a fully randomized block design. All treatments were cut twice a year in late June or early July, and in mid-October. Percentage cover of individual plant species was estimated by visual observation in each plot in late June in the years 2005–2014.

Results: Despite inter-annual variability in the cover of the individual vascular plant species, the multivariate data analyses revealed a relatively similar response of the plant community to the different fertilizer applications throughout the ten years. With phosphorus application, no differences in botanical composition among treatments were found; however, they did differ from other treatments without phosphorus application. In the unfertilized control, there was a certain directed trend in plant species composition in response to ongoing nutrient impoverishment.

Conclusion: Species-rich grasslands of high nature conservation value were only maintained under P limitation in the control without fertilizer application (*Violion caninae*) and in the liming treatment (*Polygono-Trisetion*), but also in the treatment with liming and pure N addition. It seems that after 74 years, some stage of equilibrium of the grassland community was achieved in all treatments receiving any type of fertilizer application, but less so in the unfertilized control treatment.

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KEYWORDS
botanical composition, fertilizer applications, grassland, inter-annual variability, long-term experiment, species richness, sward, vegetation equilibrium

1 | INTRODUCTION

Fertilizer applications on grassland generally lead to increased biomass production and to changes in botanical composition in favour of highly productive plants, thereby resulting in a decrease of less-competitive species and overall species richness (Plantureux *et al*., 2005; Silvertown *et al*., 2006; Hejcman *et al*., 2010; Humbert *et al*., 2016). To determine the long-term effects of applications of fertilizers on vegetation requires long-term experiments (more than 10 years), because only long-term data series can reveal succession of the community and fluctuations of individual species in the sward. Long-term data sets are also valuable for the testing of hypotheses and for prediction of future changes (Bakker *et al*., 1996).

Many authors have previously investigated the long-term effects of fertilization on grassland, but studies have often been based on experiments of limited duration, or with interrupted observations (Crawley *et al*., 2005; Hejcman *et al*., 2014; Kidd *et al*., 2017) or with limitations on replication (Silvertown *et al*., 2006). Long-term studies require a commitment from research funders to maintain experimental sites and support the costs of data collection.

It is well documented that long-term fertilizer application in combination with a repeated defoliation management regime can lead to vegetation equilibrium, although this may take decades to be reached (Silvertown, 1980; Dodd *et al*., 1994). Vegetation equilibrium can be defined as species co-existence based on an ability of plant species to use different parts of the resource gradient by diverse phenology, ability to use light and rooting depth of plants (Lepš, 2013). The number and relative abundances of plant species in a community at equilibrium are determined by compensation dynamics, such as competition for nutrient resources and for irradiance penetrating the canopy (Tilman, 1982). Further, the equilibrium in guild composition is a dynamic one, continually perturbed by climate (Silvertown, 2006).

This study was performed on the Rengen Grassland Experiment (RGE) in the Eifel Mountains (Germany). It was established in 1941 and nowadays it is one of the oldest continuously managed long-term experiments on permanent grassland in mainland Europe. The first detailed botanical evaluation at RGE, recorded in the year 1999 (Schellberg *et al*., 1999), revealed significant effects of different fertilizer applications, not only on botanical composition (Hejcman *et al*., 2007) and on plant and soil chemical properties (Hejcman *et al*., 2010), but also on the type of phytocoenological grassland community (Chytrý *et al*., 2009). Multivariate data analysis (redundancy analysis, RDA) revealed a strong explanatory power (>60%) of fertilizer treatments (Hejcman *et al*., 2007) and of soil nutrient status (Hejcman *et al*., 2010) on plant species composition. Further, individual responses of plant species composition reflected the soil nutrient status, especially nutrient (N, P, K) limitations, and presumably an ongoing depletion of plant-available P that prevented the realization of vegetation equilibrium in the treatments without P application (Hejcman *et al*., 2010). However, this result was based on one-year data observation only, as there were no data from longer periods to support this part of the investigation. In this context, this study aimed at appraising the effect of 74 years of long-term fertilizer applications on grassland community based on assessments over a 10-year period. The study attempted to address the following research questions: (a) what was the effect of different long-term fertilizer applications on species richness and plant species composition during ten years of observation; and (b) was there any detectable trend in species richness and plant species composition over the ten years of observation?

2 | METHODS

2.1 | Study site

The fertilizer experiment was set up on the Rengen Grassland Research Station of the University of Bonn in the Eifel Mountains (Germany, 50°13′ N, 6°51′ E; elevation 475 m above sea level) in 1941 (for details, see (Schellberg *et al*., 1999)). Mean annual precipitation is 811 mm and mean annual temperature is 6.9°C (Rengen meteorological station). The soil is classified as a Stagnic Cambisol. Mean chemical properties of soils for each treatment in 2015 are presented in Table 1.

2.2 | Experimental design

The experiment is arranged in a complete randomized block design with five treatments and five replications with an individual plot size of 3 m × 5 m. The treatments are: (A) zero fertilizer treatment as a control; (B) calcium (Ca) application; (C) Ca and nitrogen (N) application; (D) Ca, N, and phosphorus (P) application; (E) Ca, N, P and potassium chloride (KCl) application; and (F) Ca, N, P and potassium sulphate (K_2SO_4) application. For a detailed description of the type of applied fertilizer with the amounts of nutrients, see Table 1. The control treatment (A) was introduced in 1998 in the vicinity of the experiment at a place that remained unfertilized but which had been mown together with all other plots since 1941. The experimental plots were mown and above-ground grassland biomass removed once a year in late summer from 1942 to 1944 and from 1950 to 1961. In the years 1945–1949, the experiment was temporarily abandoned without cutting or fertilization (Schellberg *et al*., 1999). All treatments in the experimental area have been mown twice per year (in late June/early July and in October) since 1962 (Schellberg *et al*., 1999). In response to different nutrient supplies due to the varying fertilizer applications, different grassland vegetation

TABLE 1 Soil chemical properties

Treatment abbreviations	P (CAL)	K (CAL)	pH ($CaCl_2$)	Ntot (%)	Ctot (%)	C/N	Mg ($CaCl_2$) ($CaCl_2$)
A	1.5	4.3	4.9	0.373	4.9	13.1	13.2
B	0.6	2.5	6.5	0.350	4.2	12.0	19.9
C	0.4	2.3	6.5	0.364	4.4	12.0	19.5
D	31.1	3.2	6.6	0.363	4.3	11.8	19.9
E	22.6	9.4	6.5	0.363	4.4	12.0	20.7
F	22.2	10.5	6.6	0.367	4.5	12.3	20.7

Results of basic soil chemical analysis of the 0–10 cm layer (mg/100 g of soil, according to Hejcman et al., 2010). Soil samples collected in May 2004. All analyses were conducted in accordance with standardized methods of the Association of German Agricultural Analytical and Research Institutes (VDLUFA, 1991). Numbers represent mean values of five replications (according to Hejcman et al., 2010).

TABLE 2 Supplied nutrients, alliances and biomass production

Treatment abbreviations	Applied nutrients (kg/ha)	Nutrient	Alliance	Biomass production (t ha^{-1})
A	Unfertilized control	0	*Violion caninae*	2.5
B	Ca = 718; Mg = 67	Ca	*Polygono-Trisetion*	2.9
C	Ca = 752; N = 100; Mg = 67	Ca/N	*Polygono-Trisetion*	4.9
D	Ca = 936; N = 100; P = 35; Mg = 75	Ca/N/P	*Arrhenatherion*	6.5
E	Ca = 936; N = 100; P = 35; K = 133; Mg = 90	Ca/N/P/KCl	*Arrhenatherion*	8.9
F	Ca = 936; N = 100; P = 35; K = 133; Mg = 75	Ca/N/P/K_2SO_4	*Arrhenatherion*	9.6

Amounts of nutrients (kg/ha) supplied annually to the treatments from 1941 (according to Schellberg et al., 1999), classification of vegetation into alliances according to Chytrý et al. (2009) and dry matter biomass production (according to Hejcman et al., 2010).

types (Chytrý *et al*., 2009) with different biomass productions developed during the period of the experiment (Table 2).

2.3 | Data collection

The percentage cover (%) of all vascular plant species was visually estimated every year in June in the centre (1.8 m × 3.2 m) of each plot (Hejcman *et al*., 2007) by the same research group during the ten years of data collection (2005–2014). To avoid operator bias in the data observation, the first replicate of each fertilizer treatment was assessed by all four scientists in the research group; subsequently the other plots were each monitored by a team of two. Nomenclature of vascular plant species follows the regional flora (Rothmahler *et al*., 2000). Plant species diversity was evaluated by the plant species richness, and Shannon and Simpsons species diversity indices (Begon *et al*., 2005).

2.4 | Data analysis

A linear mixed-effects model (GLM) with treatment, time (factor) and their interaction as fixed effects and replication as random effect was used to evaluate the cover of the most abundant vascular plant species and species richness. If necessary, data were log-transformed to meet ANOVA assumptions. For all univariate statistical analyses, the software Statistica 13.2 was used (Dell Inc., Tulsa, OK, 2016).

The programme Canoco 5 (Ithaca, NY) was used for the evaluation of multivariate data (Ter Braak and Šmilauer, 2012). RDA was applied as the length of the gradient was 3.2 SD (standard deviation) units. Species cover (%) data were logarithmically transformed [y=log (y+1)] for the purpose of RDA. Further, a Monte Carlo permutation test with 999 permutations in hierarchical design was used to reveal if the tested explanatory variables (environmental variables in this case) had a significant effect on plant species composition. Split-plot permutations in each plot during ten years were not used (independent across the whole plot). Whole-plot permutations were used as freely exchangeable. Year as a factor was used as covariate in all analyses. The effect of treatment was used as an explanatory variable. Results of the multivariate analysis were visualized in the form of a bi-plot ordination diagram.

As part of the RDA analysis, species response curves for all individual treatments during the years of observation were created. Year was used as an explanatory variable (quantitative) and replications

TABLE 3 A linear mixed-effects model (GLM) analysis

	Tested variable	Treatment (*df* 5)	Year (*df* 9)	Treatment ∗ Year (*df* 45)
Number of all species	*F* ratio	12.25	13.63	2.23
	P value	<0.001	<0.001	<0.001
Number of species > 1%	*F* ratio	17.82	11.63	3.74
	P value	<0.001	<0.001	<0.001
Shannon index *H*	*F* ratio	4.3	15.11	3.19
	P value	0.003	<0.001	<0.001
Simpson index *D*	*F* ratio	2.49	14.44	2.25
	P value	0.045	<0.001	<0.001
Alopecurus pratensis	*F* ratio	53.24	3.28	0.75
	P value	<0.001	0.004	0.877
Arrhenatherum elatius	*F* ratio	124.8	3.4	1
	P value	<0.001	0.003	0.398
Briza media	*F* ratio	35.82	2.08	7.51
	P value	<0.001	0.052	<0.001
Festuca rubra agg.	*F* ratio	32.36	1.69	5.5
	P value	<0.001	0.119	<0.001
Galium mollugo	*F* ratio	118.7	3.3	0.4
	P value	<0.001	0.004	1
Leucanthemum vulgare	*F* ratio	69.74	1.46	2.72
	P value	<0.001	0.192	<0.001
Lotus corniculatus	*F* ratio	53.73	1.17	1.83
	P value	<0.001	0.334	0.002
Nardus stricta	*F* ratio	91.95	0.53	0.45
	P value	<0.001	0.847	0.999
Plantago lanceolata	*F* ratio	87.27	5.03	1.14
	P value	<0.001	<0.001	0.26
Rhinanthus minor	*F* ratio	28.19	3.52	7.56
	P value	<0.001	0.002	<0.001
Trifolium pratense	*F* ratio	17.93	2.28	4.58
	P value	<0.001	0.034	<0.001
Trisetum flavescens	*F* ratio	133.3	4.4	1
	P value	<0.001	<0.001	0.427

Results of the GLM for the cover of the dominant plant species, functional groups and number of plant species. *df*, degrees of freedom; *F* ratio, *F* statistics for the test of particular analysis; *P* value, obtained probability value. Significant results of *P* values in background shading.

were used as covariates. The setting was the same as for RDA analyses.

Principal response curves (PRC) were used to analyse principal components of the treatment effects against time, expressing the treatment effects as deviations from the control treatment (reference coding). Year was used as a covariate and the interaction treatment ∗ year was used as explanatory variable. A set of hierarchical design was used in the same way as in the case of RDA. The set of species weights shown on the right side of the diagram was associated with each PRC.

The effect of treatment in a particular year (2005–2014) on plant species composition was evaluated by redundancy analysis (RDA) with 999 unrestricted permutations, where blocks were defined as a covariate.

PCA as part of the unconstrained multivariate statistical method was used to illustrate differences between treatment responses within individual years (2005–2014), where the effect of treatment was used as a supplementary variable.

To see if there is some type of temporal trend within individual treatments, variability partitioning as part of RDA was evaluated from the sum of all eigenvalues to identify residual variability in vegetation data.

3 | RESULTS

3.1 | Species richness and plant species composition

During the study period (2005–2014), a total of 90 plant species was recorded across all treatments and replicates. The effect of treatment, year and treatment ∗ year interaction was significant for the number of all species, the number of all species >1%, Shannon index *H*, Simpson index *D* and for *Rhinanthus minor* and *Trifolium pratense* (Table 3).

The effect of treatment and year was significant for *Alopecurus pratensis*, *Arrhenatherum elatius*, *Galium mollugo*, *Plantago lanceolata* and *Trisetum flavescens*. Effect of treatment and treatment ∗ year interaction was significant for *Briza media*, *Festuca rubra* agg., *Leucanthemum vulgare* and *Lotus corniculatus*. High year-to-year variability of the number of all species, the number of all species >1%, Shannon index *H*, and Simpson index *D* with a trend to decrease during the study period was found (Figure 1). The highest species richness was revealed in the control treatment (A) and in the treatment with Ca application only (treatment B).

The cover of tall grass species such as *Arrhenatherum elatius*, *Alopecurus pratensis* and *Trisetum flavescens* was supported by P application, on average, in all study years (D–F) (Figure 2). On the other hand, plant species such as *Briza media* and *Festuca rubra* agg. exhibited higher cover in the treatments without P application (A–C). Between these two species, a strong negative correlation was revealed in treatments B ($R = -0.58$; $P < 0.001$) and C ($R = -0.66$; $P < 0.001$). *Nardus stricta* was present predominantly in treatments A and C.

The tall forb *Galium mollugo* had higher cover in the treatments with P application (D–F) than in the other treatments (A–C) (Figure 3) throughout the ten years of observation. On the other hand, forbs such as *Leucanthemum vulgare*, *Plantago lanceolata* and *Rhinanthus minor* had higher cover in the treatments without P application (A–C). The cover of legumes such as *Trifolium pratense* and *Lotus corniculatus* was supported predominantly by Ca application (treatment B).

3.2 | Species response curves

The species response curves (based on RDA analysis) displayed the percentage cover development of plant species in the individual

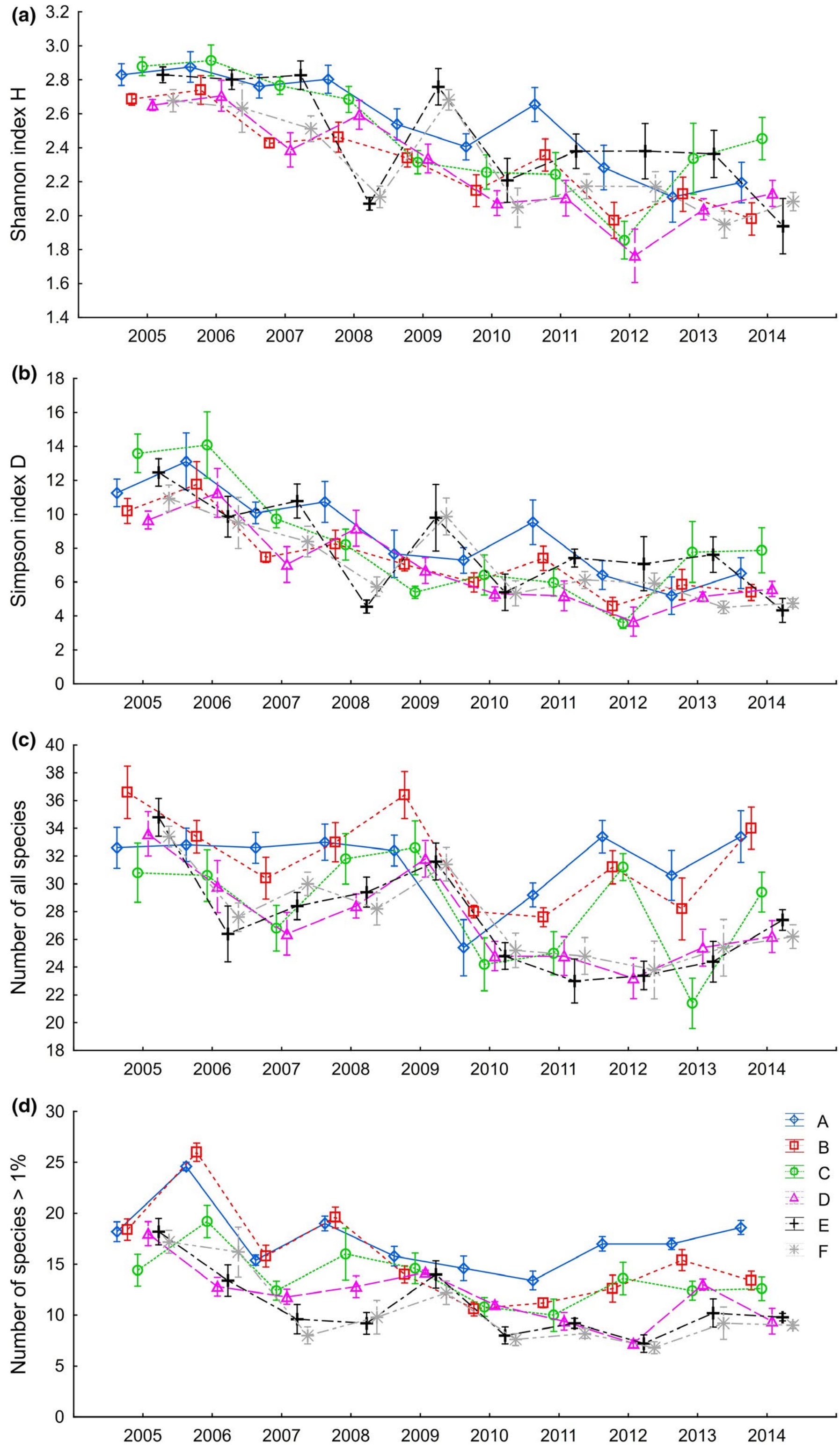

FIGURE 1 Plant diversity measurements. (a) Shannon index *H*, (b) Simpson index *D*, (c) number of all vascular plant species and (d) number of vascular plant species with cover more than 1% in the treatments over the study period 2005–2014. Treatment abbreviations and fertilizer inputs for each treatment are explained in Table 2 [Colour figure can be viewed at wileyonlinelibrary.com]

treatments (Appendices S2, S3, S4). A decrease in cover of species such as *Briza media*, *Festuca rubra* agg., *Rhinanthus minor*, *Lathyrus linifolius*, *Ranunculus nemorosus* and *Linum catharticum* and an increase in cover of species such as *Succisa pratensis* and *Hypochaeris radicata* was recorded in treatment A over the duration of the experiment (Appendix S2a).

After the initial increase in the cover of *Lotus corniculatus*, *Leucanthemum vulgare* and *Festuca rubra* agg., their successional decrease in treatment B (Appendix S2b) was revealed. The forbs *Rhinanthus minor*, *Trifolium dubium* and *Ranunculus acris* showed decreasing cover during period of the experiment.

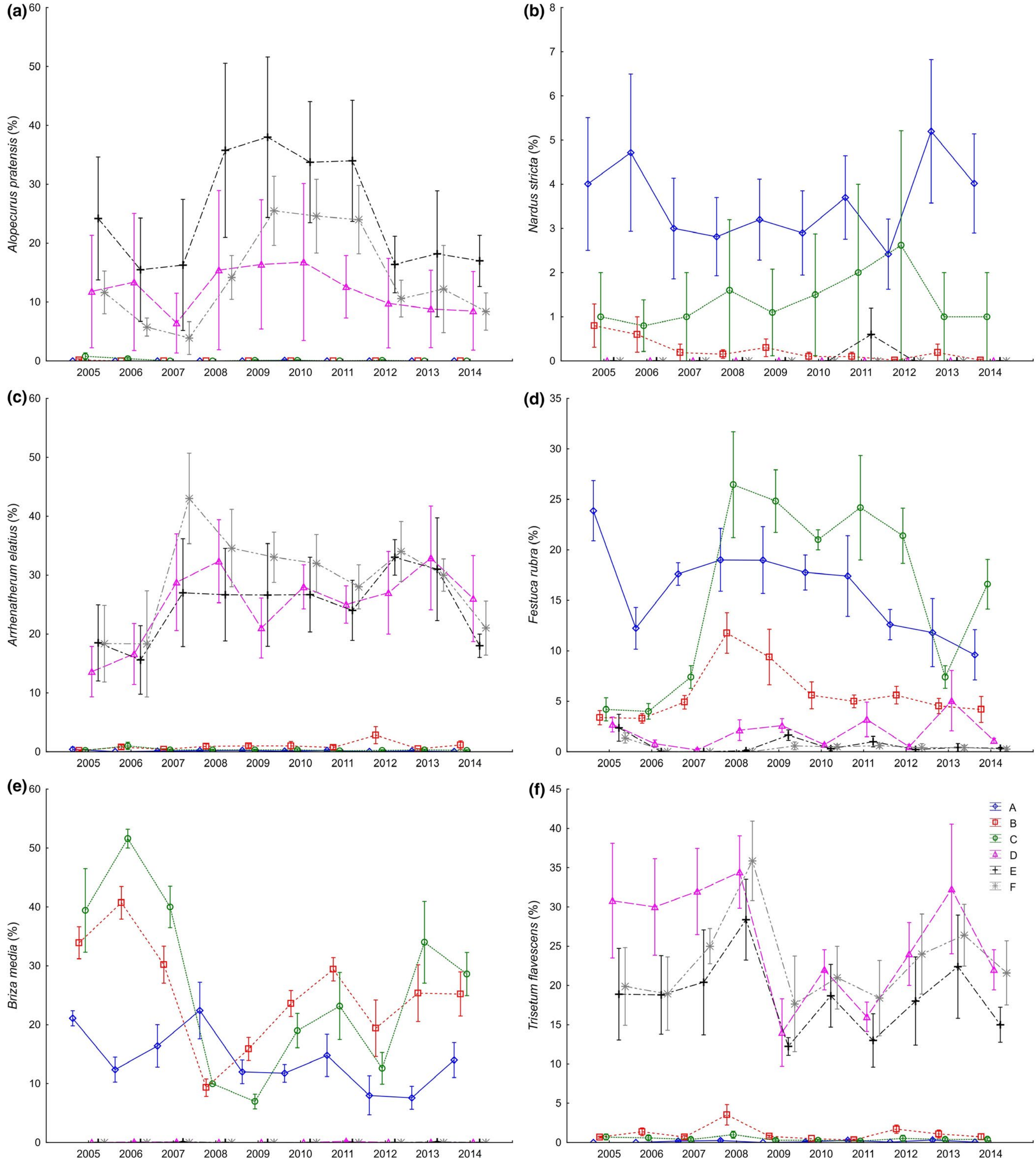

FIGURE 2 Dominant grass species. The mean cover (%) of the dominant grass species – (a) *Alopecurus pratensis*, (b) *Arrhenatherum elatius*, (c) *Briza media*, (d) *Nardus stricta*, (e) *Festuca rubra* agg. and (f) *Trisetum flavescens* in the treatments over the study period 2005–2014. Treatment abbreviations and fertilizer inputs for each treatment are explained in Table 2 [Colour figure can be viewed at wileyonlinelibrary.com]

In treatment C, a mutual complementarity was recorded between *Festuca rubra* agg. and *Briza media* (Appendix S3a). An increase in the cover of *Festuca rubra* agg. was accompanied by a decrease in the cover of *Briza media* and vice versa. The cover of species such as *Plantago lanceolata* and *Potentilla erecta* remained relatively stable during the study period.

Species such as *Taraxacum* sp., *Festuca pratensis*, *Ranunculus acris*, *Ranunculus nemorosus*, *Trifolium pratense* and *Trifolium dubium* decreased their cover in the D treatment during the course of the study (Appendix S3b). *Rumex acetosa*, *Poa trivialis* and *Holcus lanatus* showed high temporal cover variability; their cover started to

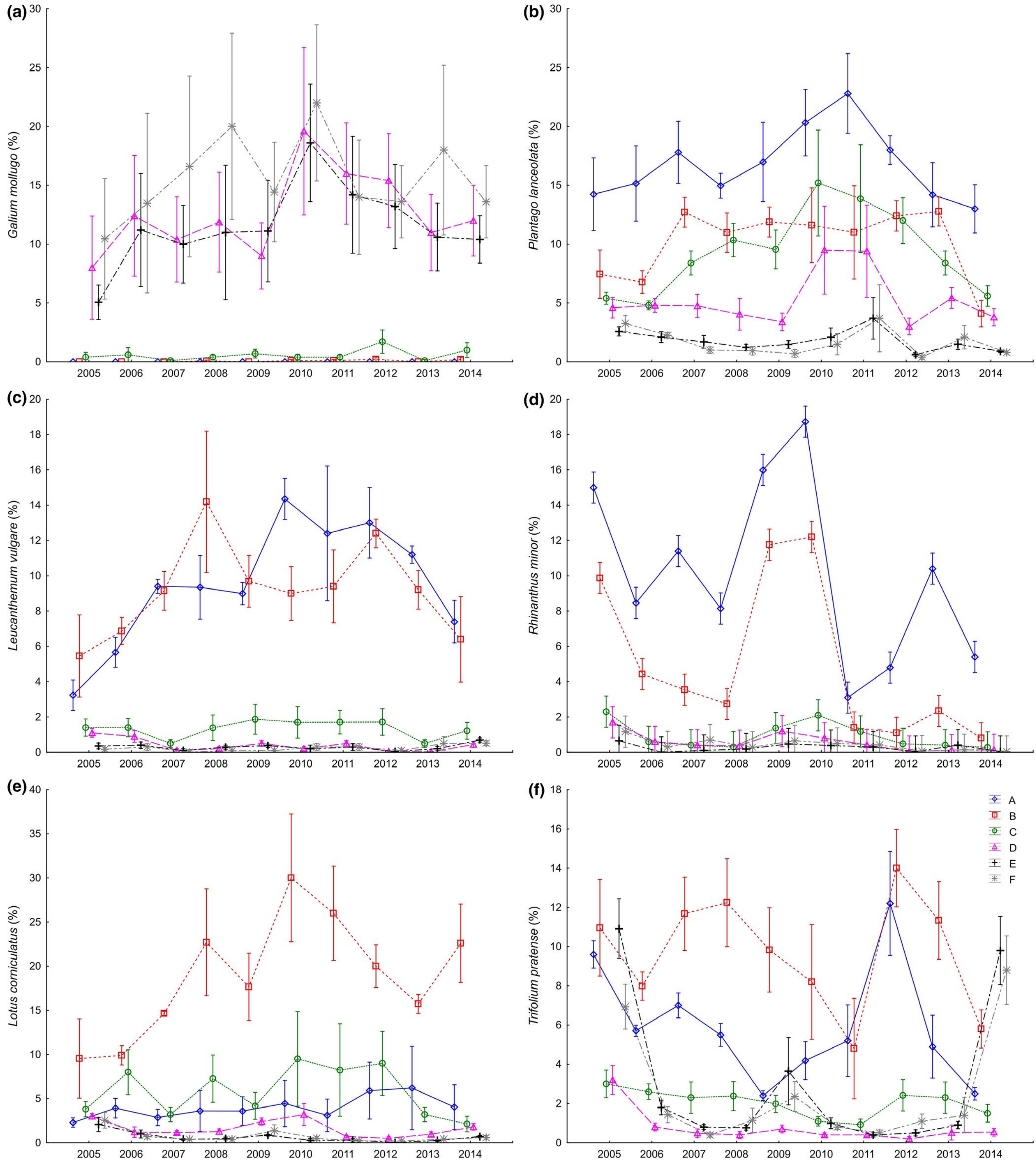

FIGURE 3 Dominant forb species. The mean cover (%) of the dominant forb species—(a) *Galium mollugo*, (b) *Leucanthemum vulgare*, (c) *Lotus corniculatus*, (d) *Plantago lanceolata*, (e) *Rhinanthus minor* and (f) *Trifolium pratense* in the treatments over the study period 2005–2014. Treatment abbreviations and fertilizer inputs for each treatment are explained in Table 2 [Colour figure can be viewed at wileyonlinelibrary.com]

increase at the beginning of the study period but then decreased. The only species to show increased cover successively over the ten-year study period was *Helictotrichon pubescens*.

Similar responses of plant species in the treatments E and F have been found during the ten yearss of the study period (Appendix S4ab). For example, *Arrhenatherum elatius* started to increase its cover at the beginning of the study period and decreased thereafter. The opposite response was found for species such as *Trifolium pratense*, *Crepis biennis* and *Trifolium hybridum*.

3.3 | Redundancy and community composition analyses of community composition

Results of RDA analyses based on vegetation data for all ten years showed that the effect of the treatments on plant species composition explained 54.25% of the variability (*F* value 67.6, *P* = 0.001) and 64.87% of the variability (*F* value 105.0, *P* = 0.001) on the first and all axes, respectively (Figure 4). The treatments that had similar plant species composition according to the first ordination axis were sorted into two main groups: treatments A–C, and all treatments with Ca, N and P application (D–F). *Lathyrus linifolius* was strictly bound to treatment A, whereas *Carex panicea* was related to treatment C. Species such as *Potentilla erecta*, *Succisa pratensis*, *Anthoxanthum odoratum*, *Linum catharticum* and *Carex pilulifera* were correlated with treatment B. All treatments with Ca, N and P application (D–F) had a similar effect on plant species composition and were related with the following tall grasses: *Poa pratensis*, *Trisetum flavescens*, *Poa trivialis*, *Alopecurus pratensis* and *Arrhenatherum elatius*.

The effect of all treatments on individual plant species in the particular years based on RDA analysis (Appendix S5) showed that the percentage of explained variability on the first axis ranged from 53.21% to 62.46% and that the percentage of explained variability of all axes ranged from 66.73% to 75.77%. It showed the strong explanatory power of fertilizer application which was relatively stable over the years.

The PCA analyses displayed the position of community composition for individual treatments in particular years (Appendix S6), which was relatively stable over the ten years and reflected the results of the other multivariate data analyses, RDA (Figure 4) and PRC (Appendix S7–S9).

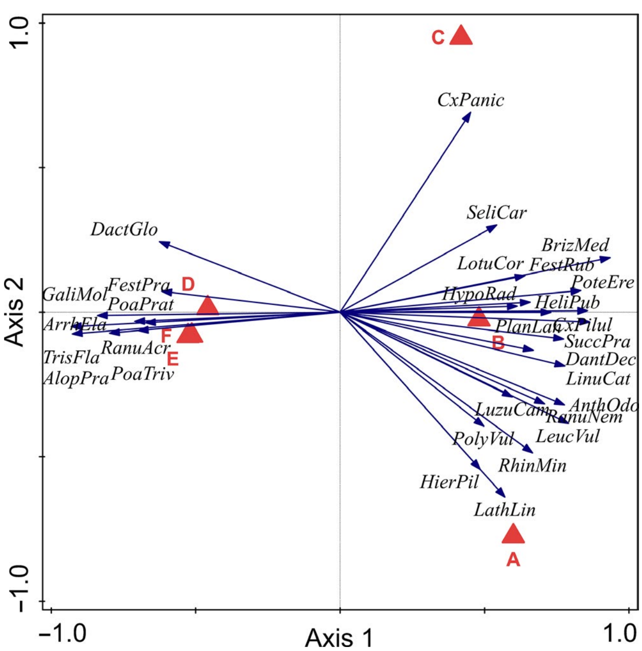

FIGURE 4 Reduncancy analysis (RDA) ordination diagram. Result of RDA analysis of plant species composition. For treatment abbreviations and fertilizer inputs for each treatment see Table 2. For plant species abbreviations see (Appendix S1) [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Principal response curves

The first PRC axis explained 76.20% (*F* value 5.7, *P* = 0.001), the second explained 8.10% (*F* value 0.7, *P* = 0.001) and the third explained 4.70% of the variability of plant species composition (*F* value 0.5, *P* = 0.001). The PRC diagrams showed differences between the control treatment and the other treatments. The first vertical axis of the PRC diagram showed the primary effect of P application. There was a similar response of plant species composition in the treatments where P application was used (D–F) and in the treatments (A–C) without P application over the study period (Appendix S7). The second axis of the PRC showed the effect of N application and so separated treatment C from the others (Appendix S8). The third axis showed the effect of Ca application without N and P applications and separated treatment B from the other treatments (Appendix S9).

3.5 | Variability eigenvalue decomposition

Based on RDA, the temporal variability eigenvalue decomposition of individual treatments within the ten experimental years showed that the control treatment (A) had lower temporal residual variability and higher temporal explained variability compared with the other treatments (B– F). The lowest temporal explained variability was found in treatments fertilized with N (treatments C– F) (Appendix S10).

4 | DISCUSSION

4.1 | Species richness and plant species composition

Long-term NPK and NP fertilization led to the grassland community shifting to becoming adapted to nutrient-rich conditions (Pierik *et al*., 2011). This had a negative impact on species richness, which has been reported in many studies (Pierik *et al*, 2011; Hejcman *et al*., 2012; Korevaar and Geerts, 2015; Čop and Eler, 2019).

The decrease in Shannon and Simpson index values in all treatments over the study period is connected with the increasing number of plant species with a cover of <1%. According to Isbell *et al.* (2015) and Gaisler *et al.* (2019), species-rich grassland communities with higher numbers of species with covers >1% are more resistant to species fluctuation. Therefore, the treatments fertilized by NP and NPK (D–F) which have lower numbers of species with cover >1% can show greater vulnerability of their grassland community to perturbations (Pressey and Taffs, 2001).

The higher cover of tall grasses such as *Alopecurus pratensis*, *Arrhenatherum elatius* and *Trisetum flavescens* in the treatments fertilized by N in combination with Ca, P and K (treatments D–F) was in accordance with previous results of one year of data from the same experiment (Hejcman *et al*., 2010) as well as with results from other experiments conducted in Central European temperate grasslands (Pavlů *et al*., 2012; Hejcman *et al*., 2014; Kidd *et al*., 2017). The variability of *Briza media* and *Festuca rubra* agg. in treatments B and C are an example of compensatory dynamic (Lepš *et al*., 2018), where the decrease in cover of one species is compensated by an increase of another species. This effect was probably the result of *Briza media* being less tolerant to drought (Dixon, 2002), whereas *Festuca rubra* agg., which has many ecotypes (Grime *et al*., 1988), is well adapted to different abiotic conditions including drought. It seems, that *Briza media* needs vegetation seasons with average or excessive precipitation to recover its dominant presence in the sward. The stability in the cover of *Nardus stricta* in the unfertilized control underlined its preference for, or adaptation to, oligotrophic acid soils (Grime *et al*., 1988; Kurtogullari *et al*., 2020).

In our experiment *Plantago lanceolata* was one of the forb species present in all treatments and the addition of P reduced its cover significantly. Although this species is associated with moderately fertile soils (Grime *et al*., 1988), it can tolerate nutrient enrichment and was present even in the fully fertilized (NPK) treatments (E and F in this study). However, *Plantago lanceolata* in nutrient-rich soil cannot fully compete for light with grasses due to its leaf morphology (Stewart, 1996). Therefore, only a few large plants of *Plantago lanceolata* were recorded (unpublished data) in the fully fertilized treatments.

Both treatments without N application (A and B) promoted the annual facultative root hemi-parasitic plant *Rhinanthus minor* and the forb *Leucanthemum vulgare*. *Rhinanthus minor* is suppressed in grasslands that have nutrient-rich soil (Jiang *et al*., 2010; Kidd *et al*., 2017) and is unable to compete in fertilized grassland where annual herbage production exceeds about 5 t ha^{-1} dry matter (DM; Hejcman *et al*., 2011). *Leucanthemum vulgare* is characterized as a species widely distributed on base-rich soil with pH >5, in grasslands with an intermediate level of productivity (Grime *et al*., 1988). It seems that increased biomass productivity (more than 3 t/ha DM) was detrimental for the competitive ability of both these species, as observed throughout the study period.

Leguminous species such as *Trifolium pratense* and *Lotus corniculatus* are generally known for their positive response to PK and negative response to high inputs of N, NP or NPK (Rabotnov, 1977; Hejcman *et al*., 2012; Čop and Eler, 2019). This negative response of both species was also confirmed for the majority of years in all treatments with N application in this experiment. The highest cover of legumes in treatment B was supported by lime application and higher soil pH (Storkey *et al*., 2015) without N fertilization.

This unique study of over ten years of continuous vegetation recording in the RGE also showed year-to-year variability in the cover of most of the vascular plant species in all treatments. Such variability has been widely reported and is commonly attributed to variations in weather conditions (Herben *et al*., 1995; Grant *et al*., 2014; Deléglise *et al*., 2015; Louault *et al*., 2017).

4.2 | Community composition

Despite year-to-year variability in the cover of individual plant species, the percentage of explained variability of RDA analyses for the first (53.2%–62.4%) and all axes (66.7%–75.8%) showed a similar response of grassland communities to the treatments within particular years. This was underlined by the results of the PCA revealing similar responses during the 10-year period. The relative stability of the plant community was also underlined by the principal response curve results based on RDA. Similarly, Dodd *et al*. (1995) found at the Park Grass Experiment an equilibrium at the guild level, whereas individual vascular plant species changed their abundances and distribution because of weather conditions.

However, in the unfertilized control (*Violion caninae* alliance), a temporal trend and low residual eigenvalue variability were shown for the development of the vegetation (Appendix S9). This was probably caused by an ongoing oligotrophication through nutrient removal, even after 64 years, as there was more plant-available P and K in the soil than in the treatments with Ca and CaN applications (Hejcman *et al*., 2010). Moreover, P is a slowly cycling nutrient and in the long-term perspective the low extractable P pool can resupply the bioavailable P pool (Roberts and Johnston, 2015). Therefore its depletion in treatments without P application is not straightforward. It seems that a stage of community equilibrium was induced by the presence of high P levels in the soil in all treatments fertilized by P (D–F), in contrast to treatments B and C. The treatments with Ca (treatment B) and CaN application (C) had greater amounts of biomass DM yield, which led to higher P and K depletion from the soil in comparison with the control treatment. Plant-available P (<0.6 mg 100 g^{-1} of soil) and K (<2.5 mg 100 g^{-1} of soil) in treatments with Ca (B) and CaN (C) applications were very low (Hejcman *et al*., 2010), and it was probably very difficult to reduce them further. Therefore, grassland communities (*Polygono-Trisetion* alliance) in these treatments were adapted to very low plant-available P and K. On the other hand, all treatments fertilized by P (treatments D–F) developed relatively stable grassland communities (*Arrhenatherion* alliance) with highly productive plant species already adapted to high levels of nutrients in the soil. Silvertown *et al*. (2006) concluded that equilibrium in plant species composition was already achieved after 40 years of fertilizer application in the Park Grass Experiment. Especially, in unfertilized grasslands, the time necessary to reach equilibrium may strongly depend on the initial nutrient status of the soil and the quantity of nutrients added per year.

5 | CONCLUSION

The main finding of this ten-year study is that although year-to-year variability in the cover of individual vascular plant species was observed, the grassland community as a whole reacted rather similar to different fertilizer application treatments, indicating that equilibrium had been reached. Surprisingly, in the unfertilized control we found the relatively strongest directional trend in plant species composition which may be interpreted as a response to ongoing nutrient impoverishment. Biomass production increased with increasing levels of fertilizer application (zero, Ca, CaN, CaNP and CaNPK) which led to a decline in species richness and evenness, especially in the numbers of species with cover >1% with increasing productivity. In the long-term, species-rich swards were only maintained in the unfertilized (*Violion caninae* alliance) and limed (*Polygono-Trisetion* alliance) treatments, but also in the treatment with pure N application and liming (*Polygono-Trisetion* alliance). On the contrary, the combined application of growth-limiting macro-nutrients always resulted in a strong diversity decline. Our long-term results confirm the crucial role of low P levels for the maintenance and restoration of species-rich mountain meadows.

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AUTHOR CONTRIBUTIONS

The research was conceived by JS. VP, LP, MH and JG collected the data. JT, LP and VP performed statistical analysis and wrote the paper. All authors discussed the results and commented on the manuscript.

DATA AVAILABILITY STATEMENT

Data are available on request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix S1. Plant species abbreviations
Appendix S2. Plant species responses (A, B)
Appendix S3. Plant species responses (C, D)
Appendix S4. Plant species responses (E, F)
Appendix S5. RDA analyses
Appendix S6. PCA analysis
Appendix S7. PRC curve – axis 1
Appendix S8. PRC curve – axis 2
Appendix S9. PRC curve – axis 3
Appendix S10. Variability decomposition

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