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# Recovery of plant species richness during long-term fertilization of a species-rich grassland

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*Abstract.* Nutrient enrichment of habitats (eutrophication) is considered to be one of the main causes of plant diversity decline worldwide. Several experiments have shown a rapid loss of species in the first years after fertilization started. However, little is known about changes in species richness in the long term. Here, we use a 50-year-old field experiment with a range of fertilization treatments in grasslands that were mown twice each year in the center of The Netherlands. We show that species richness in all plots initially declined but started to recover after ~25 years of continued fertilization. This was also true for the heavily fertilized treatment (NPK). In NPK-fertilized plots, the decline was strongest and associated with a strong divergence of plant trait composition from the control, reflecting a shift to a plant community adapted to nutrient-rich conditions. During the subsequent period of increase in species richness, the trait composition remained stable. These results show that plant species richness can, at least partially, recover after an initial diversity decline caused by fertilization.

*Key words: biodiversity; community assembly; eutrophication; field experiment; grassland; Ossekampen farm, Wageningen, The Netherlands; plant traits.*

## Introduction

Biodiversity is declining worldwide, with direct consequences for ecosystem functioning and services (Loreau et al. 2001, Hooper et al. 2005). Next to habitat loss and fragmentation, degradation of habitat quality is a major driver of biodiversity loss (Millenium Ecosystem Assessment 2005). One of the most important changes in abiotic conditions that has caused deterioration of habitats during the last decades in Europe and North America is eutrophication (nutrient enrichment of habitats by e.g., fertilization or nitrogen deposition). Experiments at spatial scales ranging from 0.2 $m^2$ to 1000 $m^2$ have shown that nutrient enrichment often causes a rapid decline of plant diversity (Tilman 1987, Berendse and Elberse 1990, Wedin and Tilman 1996, Gough et al. 2000, Stevens et al. 2004, Crawley et al. 2005) resulting in a strongly impoverished community. This relationship between nutrient availability and species richness is described in two well-known views (e.g., Wilson and Tilman 1993, Grime 2001). The first view proposes that nutrient enrichment causes a shift in competition between plants for soil resources in low productive habitats to increased aboveground competition for light in high productive habitats (e.g., Tilman 1988, Wilson and Tilman 1991). The second view proposes that both above- and belowground competition increase with increasing soil fertility (Grime 1973, 1979). In both cases species are outcompeted by strong competitors. The functional trait-based selection theory states that a specific environment selects for species with the appropriate traits for that environment, which outcompete species with traits that are less well adapted to this environment (Suding et al. 2005). Following this view, nutrient enrichment can lead to species richness decline because the new nutrient-rich environment selects for traits that are favorable under nutrient-rich conditions, such as rapid growth and tall stature, and excludes species that have traits that reflect adaptations to nutrient limitation (Keddy 1992, Díaz et al. 1998). A consequence of such a selection for plant traits is that new species with traits suitable for nutrient-rich conditions are expected to colonize the fertilized communities. Clearly, these species should be present in the local or regional species pool with sufficient dispersal possibilities (Turnbull et al. 2000, Hautier et al. 2009). This could lead to a partial or even complete recovery of species richness. These plant communities would, however, exist of different species that exhibit a different set of plant traits. In addition, this mechanism predicts a lag time in species richness recovery because colonization processes take time.

To test this hypothesis, we analyzed patterns of species richness and plant trait composition in a long-term fertilization field experiment initiated in 1958. The long experimental duration, combined with a relatively

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large plant species pool (112 species), provides a unique opportunity to study the long-term impacts of fertilization on species-rich grassland.

## Methods

### *Experimental design*

The experiment was initiated in 1958 at the Ossekampen experimental farm near Wageningen in the center of The Netherlands (51°58′15″ N; 5°38′18″ E). The mean annual precipitation in the period from 1958 to 2005 was 818 mm and the mean annual temperature was 9.7°C. At the start of the experiment, grassland plots of 40 $m^2$ (16 × 2.5 m) were established in a species-rich meadow on heavy river clay that had been mown and grazed (extensively by cattle) in alternating years (Elberse et al.1983). The total area of the experimental farm covers ~22 ha. The vegetation was typical for seminatural Central European mesophilic grasslands classified as the Arrhenaterion alliance (Elzebroek 1990, Schaminée et al. 1996). These originally species-rich grasslands have traditionally been used for haymaking for centuries (Weeda et al. 2002), and natural succession toward forests is hampered by mowing. The area surrounding the experiment used to be managed as traditional hay meadows as well, but was gradually converted to intensively used agricultural pasture after the start of land consolidation during the Second World War.

The plots are randomly distributed with a 2.5 m wide buffer area in between the plots. Plots and buffers are mown twice a year. In addition to control plots without any fertilization, fertilized and limed plots were established (Appendix A: Table A1). The fertilizers used were superphosphate (P), potassium sulphate (K), ammonium nitrate (N), and lime marl (Elberse et al.1983). In this study we refer to the NPK plots, in which the fertilizer treatment removed macronutrient limitation, as the heavily fertilized plots. The seven treatments were replicated twice resulting in 14 plots. The nutrient additions were not fully factorial, which is clearly not ideal concerning statistical analysis. However, the duration of the experiment is exceptional and therefore of great value for studying long-term effects of fertilization. Also, the size of the plots and the presence of both contrasting treatments (unfertilized and heavily fertilized plots) and intermediate fertilization treatments compensate to a certain extent the unbalanced design. The N-only treatment was initiated eight years later.

From 1958 onward, grazing was ceased and the grassland has been managed by mowing twice each year. After mowing, all aboveground biomass was removed lengthways of the plots to prevent seed rain into adjacent plots. The aboveground biomass production of the vegetation was measured twice a year when the plots were mown. The botanical composition was determined 35 times in 47 years. Fifty samples of 25 $cm^2$ were taken from each plot at regular intervals along three parallel lines by clipping. The presence of all species was recorded in each sample (Elberse et al.1983). For each species, its abundance in each plot was determined as a frequency percentage (i.e., the proportion of 50 samples in which the species was present). In addition, soil pH, organic matter content of the soil, and nutrient concentrations in the soil were measured at intervals of 4–7 years (Elberse et al. 1983). Groundwater levels were measured from 1962 until 2000 in an adjacent field.

### *Analysis of species richness patterns*

Species richness per plot was determined as the total number of species observed in the 50 samples. We calculated the Shannon evenness based on the species frequency data. The relationship between species richness and time was analyzed using repeated-measures ANOVA with time as a within-subject factor and fertilization treatment as a between-subjects fixed factor. Because of a significant interaction between time and treatment, a second order polynomial was fitted through each treatment separately. We also fitted a linear function through the data of the period of decline and increase separately (1957–1980 vs. 1981–2005 for all treatments but N and 1966–1987 vs. 1988–2005 for N) and compared the estimates based on 95% confidence intervals. A GLM was used to test whether initial species richness differed between treatments. We estimated the year with the lowest species richness and the 95% confidence intervals for each treatment using the predicted regression coefficients and confidence intervals of the second order polynomial and the basic mathematical formula that the $x$ value for the peak of a parabola ($y = ax^2 + bx + c$) equals $-b/2a$.

### *Trait composition*

We used available databases (Klotz et al. 2002, Tamis et al. 2005*a*, Flynn et al. 2006, Grime et al. 2007) to collect plant traits for each species that was recorded in the plots. We chose traits that were available for all species in the experiment and that we considered to be important for colonization, establishment, and competition (Appendix A: Table A2). We collected a number of dispersal, seed, morphology, and growth traits. We also recorded the functional group for each species (grass, forb, legume, shrub) and grazing tolerance, which was hypothesized to be important because of the former management of mowing and grazing.

Plant functional trait composition was determined by calculating the weighted abundance of all traits within each treatment for each replicate plot and each year. For traits with continuous values (e.g., seed mass) the trait value of each species was multiplied to its frequency. These values were summed for all species and divided by the sum of the species frequencies. For traits with nominal or categorical values (e.g., pollen vector) we calculated the weighted presence of each trait class by using the sum of all frequencies of the species sharing this trait class and dividing this by the sum of all frequencies.

*Multivariate analysis of the trait composition*

We analyzed whether the temporal patterns of plant trait composition differed over time and between treatments using multivariate constrained analyses (RDA) with treatment × time as explaining (dummy) variables (Lepš and Šmilauer 2003). Significance was determined using a permutation test (999 permutations) in a split-plot design. Replicate plots of the treatments were permuted freely within years, while years (whole plots) were not permuted (Lepš and Šmilauer 2003). The regression coefficients per treatment per year, which resulted from the RDA, were split into two periods (1958–1980 and 1981–2005). These regression coefficients were used in a linear regression analysis per period to test the temporal patterns of the regression coefficients. The trait data of rare species, which were present in <5% of all observations over all years and all treatments, were not included in the RDA. The N-only treatment was not included in this analysis because this treatment was initiated eight years later.

## Results

Plant species richness changed with time (repeated-measures ANOVA time: $F_{33,198} = 32.8$, $P < 0.001$), but this effect differed between treatments (treatment × time: $F_{5,127} = 2.8$, $P < 0.001$). Initial species richness did not differ between treatments (GLM: $F_{5,12} = 0.67$, $P > 0.05$). When analyzed separately, species richness declined in all treatments for a period of ~25 years (Fig. 1). However, after this period plant species richness increased in all treatments resulting in significant ($P < 0.001$) nonlinear (concave) temporal patterns of species richness (Fig. 1; Table 1). A model with a quadratic term (species richness = $a \times \text{time} + b \times \text{time}^2 + c$) fitted the observed data in all treatments significantly better ($P < 0.001$) than a linear model. The exact shape of the relationship differed between fertilization treatments. The nonlinear patterns in the heavily fertilized (NPK), N-only, and the limed treatments were significantly different from the other treatments (see Table 1). We applied linear regression analysis to the period of decline and increase separately for each treatment. The slopes of the regression lines were compared based on the 95% confidence intervals. This revealed that the differences between the nonlinear patterns were due to a significantly steeper decline in the heavily fertilized plots during the first period and a significantly stronger increase in the limed plots during the second period (see Appendix A: Table A3). When analyzed separately, the linear increase in species richness during the second period was not significant ($P > 0.05$) for control plots (see Appendix A: Table A3). The start of the recovery of the species richness differed between treatments (see Appendix A: Fig. A1) and occurred latest in the N-only treatment, which was initiated eight years later. Shannon evenness patterns were very similar to the temporal patterns in species richness (see Appendix A: Fig. A2).

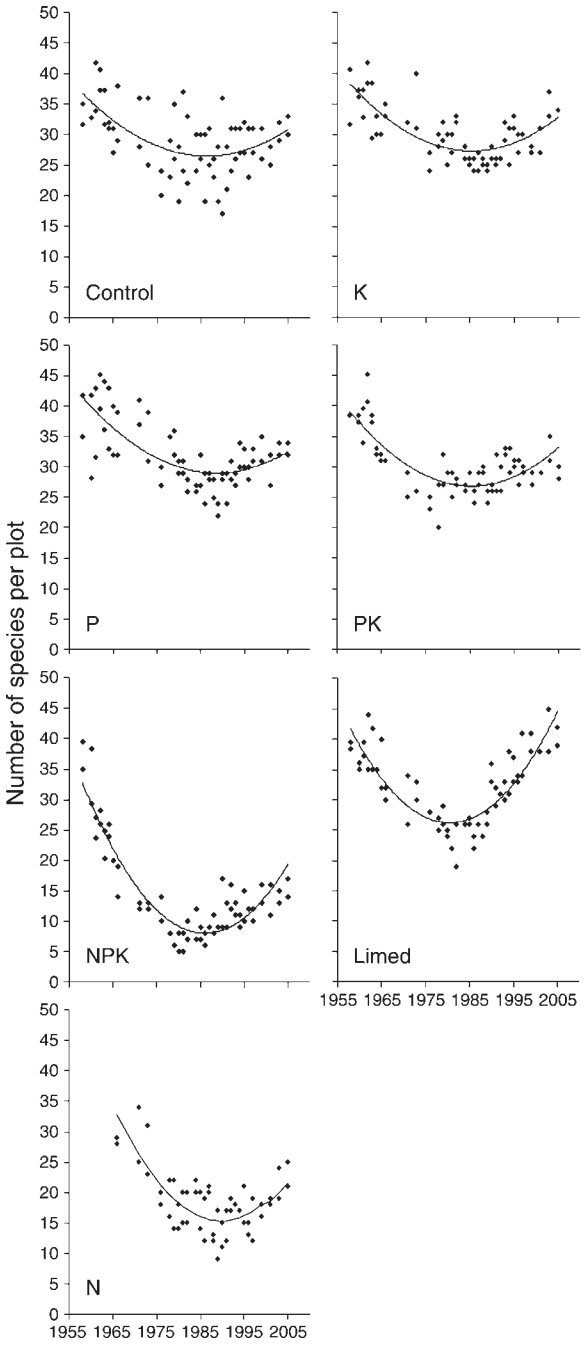


FIG. 1. Long-term changes of plant species richness in unfertilized control, K, P, PK, NPK, unfertilized limed, and N grassland plots near Wageningen, The Netherlands. Species richness declined with time in all treatments for approximately 25 years and increased afterward in all treatments, resulting in a significant nonlinear pattern ($P < 0.001$ for all treatments). See Table 1 for the regression parameters.

The composition of plant functional traits (trait composition) of the heavily fertilized plots strongly diverged from its initial composition ($F_{1,12} = 429$, $P < 0.001$) and from the other treatments (based on confidence intervals of the slopes of the regression lines;

TABLE 1. Estimation of regression parameters for the relationship between species richness and time in grassland plots near Wageningen, The Netherlands.

| Treatment | $a$ | $b$ | $c$ | $R^2$ | $F$ | df | $P$ |
|---|---|---|---|---|---|---|---|
| Control | $-0.76^{a} \pm 0.16$ | $0.013^{a} \pm 0.003$ | $37.33^{ac} \pm 1.66$ | 0.30 | 14.24 | 2, 66 | <0.001 |
| K | $-0.84^{a} \pm 0.11$ | $0.015^{a} \pm 0.002$ | $38.92^{ac} \pm 1.16$ | 0.50 | 32.45 | 2, 66 | <0.001 |
| P | $-0.85^{a} \pm 0.14$ | $0.014^{a} \pm 0.003$ | $42.32^{ab} \pm 1.40$ | 0.49 | 32.20 | 2, 66 | <0.001 |
| PK | $-0.96^{a} \pm 0.11$ | $0.017^{a} \pm 0.002$ | $40.15^{a} \pm 1.10$ | 0.60 | 49.36 | 2, 66 | <0.001 |
| NPK | $-1.88^{b} \pm 0.11$ | $0.033^{b} \pm 0.002$ | $34.71^{c} \pm 1.10$ | 0.85 | 192.22 | 2, 66 | <0.001 |
| Limed | $-1.50^{b} \pm 0.12$ | $0.032^{b} \pm 0.002$ | $43.48^{ab} \pm 1.17$ | 0.73 | 90.76 | 2, 66 | <0.001 |
| N | $-1.54^{b} \pm 0.17$ | $0.032^{b} \pm 0.004$ | $33.94^{b} \pm 1.56$ | 0.65 | 50.28 | 2, 52 | <0.001 |

*Notes:* Species richness $= a \times \text{time} + b \times \text{time}^2 + c$; values for $a$, $b$, and $c$ are given as means $\pm$ SE. The regression analysis of the N-only treatment, which was initiated eight years later, was performed relative to the year of initiation. Within columns, significant differences between treatments are denoted by lowercase superscript letters and are based on the 95% confidence intervals. Parameters $a$ and $b$ of the N, NPK, and limed plots differ significantly from the other treatment plots, but not from each other.

*data not shown*) during the first 25 years of the experiment (Fig. 2), while the trait composition in the other treatments remained relatively constant during this period (other treatments except limed plots, regression analysis; $P > 0.05$). In contrast, during the second period when species richness was increasing, the trait composition of the heavily fertilized plots remained stable (regression analysis; $F_{1,18} = 0.014$, $P > 0.05$). The trait communities in the control, K, and P plots showed only minor shifts (regression analysis; $P < 0.05$), particularly after ~30 years, whereas the limed and PK plots showed larger shifts (regression analysis; $P < 0.001$; Fig. 2). These changes, however, were much weaker than the initial divergence shown by the heavily fertilized plots (Fig. 2).

The main difference between the trait composition in the heavily fertilized plots and the other treatments (especially the control plots) was due to the strong decrease of the species associated with grazing in the heavily fertilized plots, which was part of the traditional management prior to establishing the experiment (highest regression coefficient of the RDA) (see Appendix A: Table A4 and Fig. A3). The decline of slow growing species adapted to nutrient poor systems caused an additional decrease of species richness in the heavily fertilized plots (see Appendix B: Table B1).

## DISCUSSION

Our results provide evidence for a partial recovery of plant species richness during long-term fertilization. To our knowledge, this has not been shown before. Particularly the species richness increase observed in the heavily fertilized (NPK) plots strongly contrasts with the temporal decline in species richness observed in other fertilization studies. There are several explanations for this discrepancy. First, the duration of most studies varies between 4 and 18 years (Tilman 1987, Wedin and Tilman 1996, Gough et al. 2000), which may not be sufficiently long to allow new species to colonize and establish. An obvious exception is the Park Grass Experiment (for a review, see Silvertown et al. 2006). This experiment also shows that fertilization leads to a reduction of species richness, particularly when fertilized with both N and P (Crawley et al. 2005). However, a recovery of species richness such as in our study has not been observed in the Park Grass Experiment. A second reason can be the small size or even absence of a local species pool of species adapted to the new conditions in other studies. This may have prevented the establishment of species adapted to the new environment in other experiments. In our study area, the former management of grazing and mowing and the different fertilization treatments resulted in a diverse species pool on a small scale, including species adapted to nutrient-rich conditions. Third, in our experiment, which was established on a well-buffered heavy clay soil, the soil pH showed only a minor decrease during the first 20 years (except for the limed plots) and remained constant afterward (see Appendix A: Fig. A5). In contrast, other experiments like the Park Grass Experiment have reported a strong decrease in soil pH associated with nitrogen (ammonium in particular) fertilization (Crawley et al. 2005), and this can strongly inhibit species richness in fertilized plots (Grime 2001, Clark et al. 2007).

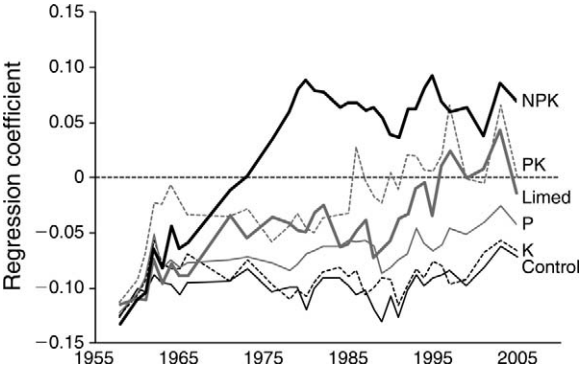


FIG. 2. Long-term changes of the trait community for each treatment (except N which started eight years later) analyzed with the multivariate analysis technique RDA. The trait community changed rapidly in the NPK plots during the first 20 years but stabilized afterward. The trait composition in the other treatments remained relatively stable during approximately the first 30 years but started to change afterward. The analysis showed a significant overall difference between the treatments over time ($F = 374.1$, $P = 0.001$).

Species loss in the heavily fertilized plots was associated with a strong shift in trait composition, confirming our hypothesis that a shift in the environmental conditions due to fertilization would select for different sets of traits, eliminating traits that are not favorable under the new conditions. In addition, the trait composition remained similar when species richness increased, strongly suggesting that only species sharing these traits were able to colonize and persist in these plots. Only two species that were not recorded at the start of the experiment (*Heracleum sphondylium* L. and *Anthriscus sylvestris* (L.) Hoffm.) at any of the parcels of the experimental farm, including the experimental plots (i.e., the local species pool), colonized the heavily fertilized plots. The other species that contributed to the increase in species richness were not sampled in the heavily fertilized plots, but were occasionally recorded in at least one of the other nearby experimental plots.

Species richness also declined in the other treatments, although to a lesser extent than in the heavily fertilized treatment. This is in line with the Park Grass Experiment, which also showed a gradual loss of species (Silvertown 1980). The importance of grazing tolerance in the trait analysis could suggest that this overall decline in species richness was due to the disappearance of grazing-tolerant species, although here it mainly reflected a shift in dominance from small-statured, relatively grazing-tolerant species toward tall-growing, less grazing-tolerant species in the fertilized plots (see Appendix A: Fig. A3). Loss of a few grazing-tolerant species did occur in all treatments during the first years after the start of the experiment (e.g., *Cynosurus cristatus* L. and *Lolium perenne* L.), but changes in later years were not related to grazing tolerance (see Appendix A: Fig. A4 for control plots).

Apart from the heavily fertilized plots, most other treatments also showed an increase in species richness in the second half of the experiment. However, in these cases the increase of species richness was not associated with a strong shift in trait composition. The increase in species richness found in limed plots, which was more pronounced than in the other treatments, may be explained by the increase in soil pH caused by liming. An increase of pH generally enhances the environmental suitability for a wider range of plant species (Gough et al. 2001, Grime 2001).

The fact that different experimental treatments showed rather similar temporal patterns of species richness suggests that these results were driven by an external factor. Clearly at the start of the experiment, the mowing regime changed from once to twice a year, and this may have caused a decline in species richness. However, this does not explain the increase in richness that occurred around 25 years later. It is possible that environmental changes occurred around that time in the surroundings of the experiment. However, important factors known to regulate diversity, such as soil pH and groundwater level, did not show consistent changes after 1980. Reduced sulphate and nitrogen emissions in Europe during the 1980s and 1990s (Erisman et al. 2003) may have allowed species richness to recover, but this should also be reflected in decreased biomass production and we found no evidence to support this (Appendix A: Table A5 and Fig. A6). Alternatively, our results may have been affected by changes in the fertilization scheme that occurred in 1981 and 1986 (Appendix A: Table A1). However, this is unlikely because the plots fertilized with N only, for which the amount was not adjusted, showed a similar temporal pattern. Moreover, the start of the recovery differed substantially between treatments (see Appendix A: Fig. A1).

In conclusion, our results clearly show that at least a partial recovery of plant diversity after biodiversity loss due to eutrophication is possible, when species that are adapted to the new conditions are able to colonize the area. Our results also show that this is a very slow process, which may take several decades. These results stress the importance of long-term studies, which take dispersal limitations into account, to unravel mechanisms playing a role in community assembly after changing environmental conditions. It is important to note that our study was carried out in hay meadows. Although this is a widespread vegetation type with a long tradition and, at least historically, relatively high species richness in Europe, it remains to be established if long-term species recovery also occurs in other systems.

Finally, although we show that species richness can recover after eutrophication, which may limit the negative effects of biodiversity loss on ecosystem processes (Hooper et al. 2005, Millenium Ecosystem Assessment 2005), it should be noted that the species responsible for the recovery are not necessarily the same as the ones that disappeared. Typically, species characteristic of nutrient-poor conditions decline after eutrophication (Tamis et al. 2005*b*). Conservation of these species, many of which have become increasingly rare in the last decades, requires restoration measures including a reduction of soil fertility.

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## APPENDIX A

Supporting fertilization scheme, statistical results, graphs, and species information (*Ecological Archives* E092-117-A1).

## APPENDIX B

A table showing species names, life forms, and trends in the experimental plots (*Ecological Archives* E092-117-A2).