

# Long-term effect of increasing nitrogen level on plant species composition in an alluvial meadow

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## Abstract

To understand the dynamic of grassland productivity and ecosystem services, long-term grassland experiments are essential. A fertilization experiment with constant P (41.90 kg ha<sup>-1</sup> year<sup>-1</sup>) and K (99.62 kg ha<sup>-1</sup> year<sup>-1</sup>) and an increasing N level (0, 40, 80, 120 kg N ha<sup>-1</sup> year<sup>-1</sup>) was set up in an alluvial wet meadow in Admont (Austria) in 1944. A non-fertilized treatment from an experiment established in the immediate vicinity in 1946 was used as a control. Detailed botanical evaluation was conducted for all treatments in June 2015: (1) unfertilized control, (2) PK, (3) N<sub>40</sub>PK, (4) N<sub>80</sub>PK and (5) N<sub>120</sub>PK. A significant effect of treatment on plant species composition, species richness and sward height was found. For example, *Poa trivialis*, *Glechoma hederacea* and *Aegopodium podagraria* occurred predominantly on the two most N fertilized treatments; *Alopecurus pratensis*, *Arrhenatherum elatius*, *Trisetum flavescens* and *Plantago lanceolata* preferred moderate N fertilization. PK fertilization promoted legumes *Trifolium pratense* and *Vicia cracca* as well as *Leontodon hispidus*. Species with low nutrient demand, *Anthoxanthum odoratum* and *Luzula campestris*, were promoted by non-fertilization. The moderately fertilized sward with the 3-cut regime supported plant species richness.

**Keywords:** compressed sward height, fertilization, grassland vegetation, species richness, three-cut management

## Introduction

The negative effect of high nitrogen fertilization on grassland vegetation has been studied in many experiments; however, due to different climatic and soil conditions as well as different management and plant community the results are not straightforward. Generally, nitrogen fertilization increases the production of aboveground biomass, but it is often responsible for reducing plant species richness and promoting species that are better competitors for light. According to Humbert *et al.* (2016) sustained application of low to moderate levels of N over time has negative effects on plant species richness similar to short-term application of high N doses. To determine how different rates of N fertilizer (along with a constant rate of PK fertilizer) affect plant species richness and sward height we analysed data from the Admont (Austria) long-term experiment.

## Materials and methods

The long-term fertilization experiment was set up in 1944 in an alluvial wet meadow in Admont, province of Styria (Austria), (47°34'52" N, 14°27'4" E; altitude of 635 m a.s.l.). The soil type is a Gleyic Fluvic Dystric Cambisol. Mean annual air temperature is 6.8°C and the average annual precipitation is 1227 mm. All treatments (apart from control treatment) were fertilized with constant P (41.90 kg ha<sup>-1</sup> year<sup>-1</sup>) and K (99.62 kg ha<sup>-1</sup> year<sup>-1</sup>) and an increasing N level (0, 40, 80, 120 kg N ha<sup>-1</sup> year<sup>-1</sup>). A control treatment was established in the immediate vicinity in 1946 and has not been fertilized at all. The treatments applied were: (1) unfertilized control (Co), (2) PK, (3) N<sub>40</sub>PK, (4) N<sub>80</sub>PK and (5) N<sub>120</sub>PK. All treatments were cut regularly three times a year (around 25 May, 20 June and 30 September). The experiment was established in four blocks, using rectangular plots of 4.1 m×5.0 m. The percentage cover of all vascular plant species was recorded visually in each experimental plot in June 2015. The nomenclature of the plant

species follows Fischer *et al.* (2008). The species richness was defined by the total number of vascular plant species in each plot. Compressed sward height was measured with a rising plate meter (Corell *et al.*, 2003) before the first cut. Redundancy analysis (RDA) in the CANOCO 4.56 program was used to evaluate multivariate vegetation and ANOVA for univariate data.

## Results and discussion

Different levels of fertilization significantly influenced plant species richness (Figure 1a). The lowest total number of plant species was recorded in N<sub>120</sub>PK and Co treatments with mean values of 26.3 and 28.8, respectively. In contrast, the highest mean number of species was in PK, N<sub>40</sub>PK and N<sub>80</sub>PK treatments with mean values of 37.8, 37.0 and 36.0 respectively. Sward height before the first cut was significantly influenced by treatment (Figure 1b). The lowest mean sward height was measured in Co treatment (7.4 cm), whereas the highest mean height was in N<sub>120</sub>PK (31.1 cm) and N<sub>80</sub>PK (29.0 cm) treatments.

The lowest mean number of plant species and the shortest sward height in the Co treatment were likely influenced by nutrient depletion in the soil after 71 years of three-cut management with the removal of cut biomass. In the most fertilized treatment (N<sub>120</sub>PK), despite being cut three times a year, the sward attained such height that it hindered light penetration, suppressing the growth of shorter species (Francksen *et al.*, 2022). This result is in agreement with the results of most of the studies dealing with fertilization, yield and diversity issues.

In the RDA based on the vegetation data the effect of the treatments on plant species composition explained 68.9% of the variability ( $F=6.7$ ,  $P=0.001$ ) on all constrained axes. Four groups of treatments with similar plant species composition were recognised on the ordination diagram (Figure 2). The first group (N<sub>120</sub>PK and N<sub>80</sub>PK treatments) included mainly species requiring nutrient-rich soils, for example *Poa trivialis*, *Glechoma hederacea* and *Aegopodium podagraria*. The second group (N<sub>40</sub>PK treatment) included species that prefer moderately nutrient-rich soils, *Trisetum flavescens*, *Arrhenatherum elatius*, *Alopecurus pratensis* and *Plantago lanceolata*. The third group (PK treatment) was especially associated with legumes (around 30%) *Trifolium pratense* and *Vicia cracca*, and also to *Leontodon hispidus* and *Achillea millefolium*. The fourth group (Co treatment) included species occurring predominantly on nutrient-poor soils, such as *Anthoxanthum odoratum* and *Luzula campestris*.

## Conclusions

The three-cut regime reduced the height of the moderately N fertilized sward to the extent that shading of the sward was relatively low, thus allowing the survival of low species. Conversely, long-term high fertilizer doses as well as long-term nutrient removal without fertilization led to species decline.

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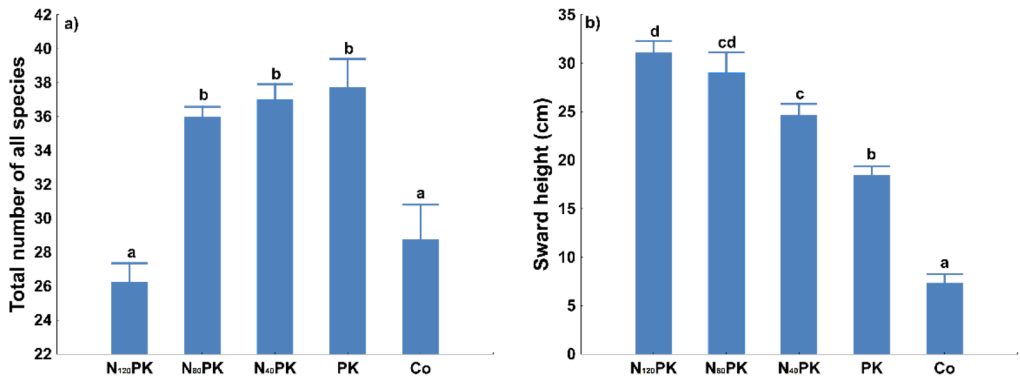


Figure 1. Effect of fertilization on (a) species richness and (b) compressed sward height before the first cut. For treatment abbreviations see Materials and methods section. The post-hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments, which are indicated by different small letters. Error bars represent standard error of the mean.

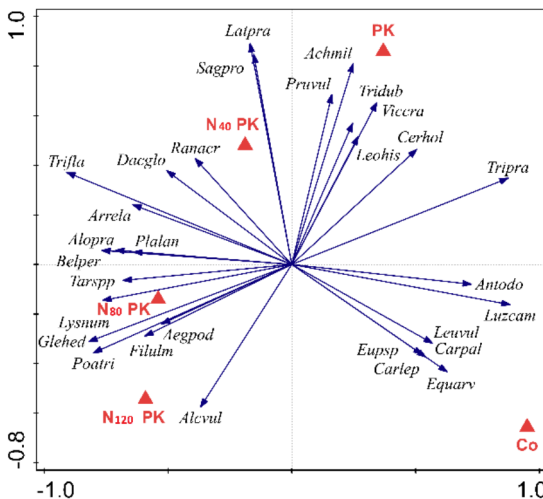


Figure 2. Ordination diagram showing the results of RDA of vegetation data. For treatment abbreviations see Materials and methods section. Species abbreviations: Achmil, *Achillea millefolium*; Aegpod, *Aegopodium podagraria*; Alcvul, *Alchemilla vulgaris*; Alopra, *Alopecurus pratensis*; Antodo, *Anthoxanthum odoratum*; Arrela, *Arrhenatherum elatius*; Belper, *Bellis perennis*; Carlep, *Carex leporina*; Carpal, *Carex palescens*; Cerhol, *Cerastium holosteoides*; Dacglo, *Dactylis glomerata*; Equiset, *Equisetum arvense*; Eupsp, *Euphrasia* sp.; Filulm, *Filipendula ulmaria*; Glehed, *Glechoma hederacea*; Lathyr, *Lathyrus pratensis*; Leohis, *Leontodon hispidus*; Leuvul, *Leucanthemum vulgare*; Luzcam, *Luzula campestris*; Lysnum, *Lysimachia nummularia*; Plalan, *Plantago lanceolata*; Poatri, *Poa trivialis*; Pruvul, *Prunella vulgaris*; Ranacr, *Ranunculus acris*; Sagpro, *Sagina procumbens*; Tarspp, *Taraxacum* spp.; Tridub, *Trifolium dubium*; Trifla, *Trisetum flavescens*; Tripa, *Trifolium pratense*; Viccra, *Vicia cracca*.