

II *Molinia caerulea* responses to N and P fertilisation in a dry heathland ecosystem (NW Germany)

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Abstract In the present study we analysed whether airborne N pollution may constitute one important driver for the encroachment of *Molinia caerulea* in dry heathland ecosystems. Based on full-factorial field experiments (in 2006 and 2008) and complementary greenhouse experiments (in 2008), we quantified growth responses of *Molinia caerulea* to N and P fertilisation (50 kg N ha⁻¹ year⁻¹, 20 kg P ha⁻¹ year⁻¹). Aboveground biomass production of *Molinia caerulea* was limited by P in 2006, but by N in both experiments in 2008. In the greenhouse experiment, N addition caused a sixfold increase of the biomass of vegetative tillers, and in all experiments the biomass and numbers of flowering tillers showed a significant increase due to fertilisation. Our experiments indicated that growth of *Molinia caerulea* was primarily limited by N, but in dry heaths the kind of nutrient limitation may be mediated by other factors such as water availability during the vegetative period. Shifts in biomass allocation patterns resulting from N fertilisation showed that *Molinia caerulea* encroachment in dry heaths is not only attributable to increased leaf biomass, but also due to higher investments in

reproductive tissue that allow for increased seed production and thus accelerated encroachment of seedlings in places where the dwarf shrub canopy has been opened after disturbance.

Keywords Biomass allocation · N:P ratio · Nutrient limitation · Phosphorous supply · Productivity

Introduction

One of the major threats to semi-natural ecosystems and their biodiversity in recent decades has been the increase of airborne nitrogen pollution (Bobbink 1998; van Diggelen and Marrs 2003). Atmospheric N loads affect ecosystem nutrient levels and cycles, the growth of plants and their competition and, ultimately, may cause shifts in plant community composition and biodiversity loss (Stevens et al. 2006). In heathlands, atmospheric N deposition has contributed to an increased aboveground productivity, accelerated nutrient cycles and shifts in the community resilience (van Rheenen et al. 1995; Schmidt et al. 2004; Calvo et al. 2005). Moreover, enhanced N levels are considered one important driver for the replacement of dwarf shrubs by grasses such as *Deschampsia flexuosa* and *Molinia caerulea* (Marrs 1993; Bobbink et al. 1998).

Encroachment of *Molinia caerulea* was initially observed in wet heaths and bogs and was first

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recognised in the Netherlands in the late 1970s/early 1980s, where N deposition was on average 2–8 times higher than in other European countries (Bobbink et al. 1998). Fertilisation experiments aiming at an understanding of the underlying mechanisms showed that stands of *Erica tetralix* are replaced by *Molinia caerulea* swards at high nitrogen levels (Aerts and Bobbink 1999). A transition to *Molinia*-dominated swards occurred when N input rates exceeded values of 17–22 kg ha⁻¹ year⁻¹ (Bobbink et al. 1998). The main factors explaining the vigour of *Molinia caerulea* in wet heaths and bogs at high N levels were (i) a significantly improved productivity, (ii) a high percentage biomass allocation to the roots and (iii) plasticity in the spatial arrangement of leaves over its tall canopy (Aerts et al. 1991).

In dry heathlands, where *Calluna vulgaris* is the main competitor of *Molinia caerulea*, competitive relationships are more complicated, and competition experiments have produced contradictory results (Aerts and Bobbink 1999). In fertilisation experiments of Aerts and Heil (1993) *Calluna vulgaris* was the superior competitor in different nutrient treatments, even at input rates of 200 kg N ha⁻¹ year⁻¹. However, the competitive superiority of *Calluna vulgaris* in this experiment is not in agreement with the experimental results of Heil and Bruggink (1987), nor can it explain the large-scale replacement of *Calluna vulgaris* by *Molinia caerulea* observed in many dry lowland heaths of NW Europe during the last decade (Härdtle et al. 2009). Aerts and Heil (1993) contend that encroachment of *Molinia caerulea* in dry heaths is probably also triggered by stress and disturbance factors, such as senescence, frost, drought, or heather beetle attacks, which weaken the competitive power of *Calluna vulgaris*. Similarly, the replacement of *Calluna vulgaris* by *Deschampsia flexuosa* only occurs when the *Calluna*-canopy is opened as a result of disturbance (Hölzel 2005) or the application of high-intensity management measures (Werger et al. 1985).

Besides the competition experiments mentioned above, *Molinia caerulea* revealed no consistent response pattern to experimental fertilisation with N and P. In some (field and pot) experiments, *Molinia caerulea* growth was limited by N (Thornton 1991; Güsewell et al. 2003; van Heerwaarden et al. 2005), whereas in other cases *Molinia* showed a marked response to P fertilisation (Heil and Bruggink 1987),

or biomass productivity increased significantly only when both N and P were applied (Roem et al. 2002). It is, thus, conceivable that additional factors such as water supply or mycorrhizal infection may mediate experimental outcomes (Aerts and Bobbink 1999). In summary, mechanisms controlling the encroachment of *Molinia caerulea* in dry heaths are not yet well understood, partly due to inconsistencies in experimental results, and partly due to insufficient evidence from experiments analysing growth responses to N or P fertilisation at dry heath sites.

The present study aims at contributing to a better understanding of mechanisms underlying the ongoing process of *Molinia caerulea* encroachment in dry lowland heaths. We hypothesise that growth of *Molinia caerulea* in dry heaths is primarily limited by N, but a shift to P limitation is conceivable as a result of continuing high airborne N loads. Therefore, we analysed growth responses of *Molinia caerulea* to N, P, and N + P fertilisation in a 2-year field experiment and a complementary greenhouse experiment. We focused on growth variables such as biomass of flowering and vegetative tillers as well as the nutritional status of *Molinia caerulea* in order to assess shifts in its competitive performance as affected by fertilisation. The following questions were addressed: (i) Is the aboveground biomass productivity of *Molinia caerulea* limited by N, P or N and P? (ii) Are biomass allocation patterns to flowering and vegetative tillers affected by fertilisation?

Materials and methods

Study area

Field experiments were conducted in the Lüneburger Heide nature reserve (Lower Saxony, NW Germany; 53°15'N, 9°58'E, 105 m a.s.l.), the site with the largest complex of dry heathlands in NW Germany (about 5,500 ha). The study area is characterised by Pleistocene sandy deposits, from which predominantly nutrient-poor podzols developed. The climate is of a humid suboceanic type. Mean precipitation is 811 mm year⁻¹ and the mean temperature is 8.4°C (Niemeyer et al. 2005). The background deposition in the study area was determined to be 23 kg N ha⁻¹ year⁻¹ and less than 0.3 kg P ha⁻¹ year⁻¹ (Härdtle et al. 2007).

Complementary greenhouse experiments took place in greenhouses of the Department of Wood Science (University of Hamburg, Germany).

Experimental design

Field experiment: In March 2006, a first series of 10 replicate plots 10 m × 10 m in size was randomly selected in the study area. Replicate plots were scattered in the nature reserve Lüneburger Heide and thus were spaced apart by at least 100 m (and up to several kilometres). The surface of all replicate plots (i.e. sites where experiment were carried out) was even (i.e. no inclination, no unevenness). *Molinia caerulea* (henceforth referred to as *Molinia*) was mono-dominant (cover 60–90%) in all plots, and ranged in age from 5 to 6 years (according to the management plans of the “Verein Naturschutzpark”). In each of the 10 plots 24 tussocks of *Molinia* were selected by random (distance between tussocks more than 1 m). An area of 0.25 m × 0.25 m surrounding each tussock was defined as subplot and marked with poles. Respectively, 6 subplots per plot were randomly assigned to one of the four treatments: control, N, P, and N + P. All plots were fenced to prevent grazing.

In the N subplots, 50 kg N ha⁻¹ year⁻¹ was applied as NH₄NO₃. In the P subplots, 20 kg P ha⁻¹ year⁻¹ was applied as NaH₂PO₄, and the N + P subplots received a combination of both. In the control subplots, only distilled water was added. Solutions were applied weekly from April through August. The quantities of nutrients applied on each occasion were dissolved in 0.25 l distilled water per subplot and added using watering cans.

In March 2008, a second series of 10 replicate plots was selected and the experiment was repeated in 2008 according to the procedure described above (allowing for a comparison of growth responses of both study years).

Greenhouse experiment: Seeds of *Molinia* of 20 different seed families were collected in the study area in September 2007 (i.e. seeds were collected from individuals spaced apart at least by 50 m). Seedlings were raised in germination dishes during winter and, respectively, 16 individuals planted into pots (12 × 12 × 12 cm³ in size) at the beginning of May

2008 (using soil material collected from the upper humus horizon in the study area). Ten replicates were used per treatment (i.e. N, P, N + P, control), resulting in a total of 40 pots. In the greenhouse experiment we applied 48 kg N ha⁻¹ year⁻¹ as NH₄NO₃, and 4 kg P ha⁻¹ year⁻¹ as NaH₂PO₄ (with regard to the surface of the pots). N + P pots received a combination of both. Fertiliser applications took place in equal time intervals as in the field experiment.

Molinia responses to fertilisation

For the analyses of growth responses to fertilisation we focused on morphological traits that are considered important for the competitive performance of *Molinia* (Tomassen et al. 2003, 2004). Accordingly, we quantified the aboveground biomass production of vegetative tillers (i.e. vegetative tillers with accompanying leaves) and flowering tillers (i.e. tillers with flower stalks and panicles and accompanying leaves). Biomass production was calculated by means of allometries in order to avoid a destructive harvest.

In the field experiment, we determined the number of tillers, the length and width of the leaves as well as the length of culms (of flowering tillers) for each of the 6 tussocks selected per subplot. Numbers of tillers were counted within a ring 20 cm in diameter applied to the tussocks.

In the greenhouse experiment, the same variables were measured for each individual (total *n* of individuals: 640). For all variables means were calculated for each of the 40 pots, based on the measurements of all individuals per pot (i.e. means of 16 individuals).

Measurements were taken when plants were still in a fresh and green status (i.e. at the end of August in 2006/beginning of September in 2008). In order to validate biometric-dry weight relationships 10 tussocks located in the vicinity of the plots were randomly selected, harvested and also analysed for the parameters mentioned above (i.e. biometric data as well as dry weights of vegetative and flowering tillers including weights of leaves and culms). *R*² of the resulting regression model used for the prediction of the biomass dry weight was 0.987 (with *P* < 0.001).

In order to determine tissue N and P concentrations, five vegetative and five flowering tillers of each

of the six tussocks per treatment and plot (in the field experiment) and per pot (in the greenhouse experiment) were pooled and used as a mixed sample (on the same date as biometric measurements were taken). Prior to chemical analyses, samples were air dried, sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany) and re-dried at 105°C before weighing. N contents were analysed with a C/N-analyser (Vario EL; Elementar, Hanau, Germany). For P-determination, samples were dissolved in an $\text{HNO}_3\text{-HCl-H}_2\text{O}_2$ solution (Lambert and Hill 1998) and digested using a microwave (MLS-ETHOS; MLS-GmbH, Leutkirch, Germany). Digests were analysed by means of an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Soil chemical analyses (field experiment)

Soil samples were taken immediately before and after the fertilisation treatments (i.e. in April and August). At each subplot, three soil samples (100 cm³ each) of the upper mineral horizon were taken (sampling depth 5–10 cm), thoroughly mixed and stored at –20°C until analysis. All soil samples were sieved (<2 mm), ground with a mixer mill (MM 400, Retsch, Haan, Germany), and examined for total N and P as well as for plant-available P (PO_4^{3-}) and pH. Total N and P were determined according to the procedure for biomass samples (see above). For the determination of plant-available P, PO_4^{3-} was extracted from 5 g dry soil by shaking for 90 min in 250 ml double lactate (DL) solution (Hoffmann 1997). Samples were filtered, and extracted PO_4^{3-} was measured with an ICP-OES.

Data analysis

In the field experiment treatment effects on biomass production, tissue N and P contents, and soil nutrient concentrations were tested using Generalized Linear Models (GLM). Each GLM included the main effects of block (i.e. plot), N, P and the N × P interaction. Results of the greenhouse experiment were evaluated by means of a one-way ANOVA with post-hoc Tukey's test. Analyses were conducted with SPSS 16.0 (SPSS Inc., Chicago, IL). Data were log-

transformed prior to analyses and the calculation of means and standard errors.

Results

Fertilisation effects on growth of *Molinia*

Field experiment

In 2006, the biomass of both flowering and vegetative tillers increased significantly in the P treatment, whereas neither significant responses to N fertilisation nor N × P interactions were found (Fig. 1a, b; Table 1). Biomass increase in 2006 was due to an increase in both the total number of tillers (i.e. flowering and vegetative tillers; Table 1) and the size of leaves (i.e. leaf length: control 24.0 cm (SE 1.4); N treatment 24.6 cm (SE 1.7), P treatment 24.9 cm (SE 1.3), N + P treatment 25.1 cm (SE 1.4); data on leaf size not shown in Table 1). By contrast, in 2008 the biomass of flowering tillers as well as the number of tillers increased significantly in the N treatment, but no significant effects of the P treatment or of the N × P interactions were found (Fig. 1a, b; Table 1). The biomass increase of flowering tillers only was attributable to an increase in the number of tillers (Table 1), whereas the size of leaves was not significantly affected (leaf length: control 25.5 cm (SE 1.5); N treatment 24.4 cm (SE 1.7), P treatment 23.1 cm (SE 1.5); N + P treatment 25.1 cm (SE 1.5); data on leaf size not shown in Table 1). The biomass of vegetative tillers was not significantly affected by the treatments in 2008.

Greenhouse experiment

In the N and N + P treatment the biomass of vegetative tillers was about sixfold and fivefold higher, respectively, than in the control (leaf length: control 8.5 cm (SE 0.3); N treatment 18.4 cm (SE 0.4), P treatment 8.7 cm (SE 0.2); N + P treatment 19.0 cm (SE 1.0); data on leaf size not shown in Table 1). Flowering tillers were only developed in the N and N + P treatment (Fig. 1c, d). The P treatment had no significant effects on the biomass of both vegetative and flowering tillers.

Fig. 1 Effects of treatments (control, N, P, N + P) on biomass production of vegetative tillers (a and c) and flowering tillers (b and d) of *Molinia caerulea* in the field experiment (2006 and 2008) and the greenhouse experiment, respectively (means + 1SE; biomass data refer to one *Molinia* tussock in the field experiment and to 16 individuals per pot in the greenhouse experiment; in a and b significant differences according to the GLM, see Table 1)

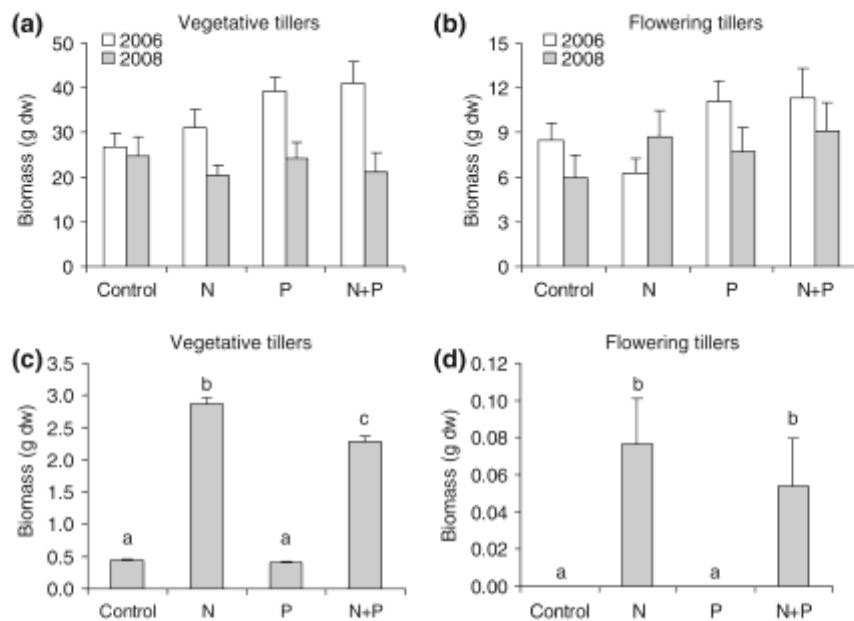


Table 1 Results of the GLM analyses of fertilisation effects on biomass production (flowering and vegetative tillers), number of flowering and vegetative tillers, tissue N and P contents, and tissue N:P ratios of *Molinia caerulea* (field experiment)

Year	Source	df	Biomass of flowering tillers		Biomass of vegetative tillers		Number of flowering tillers		Number of vegetative tillers		Tissue N content		Tissue P content		Tissue N:P ratio	
			F	P	F	P	F	P	F	P	F	P	F	P	F	P
2006	N	1	0.72	0.42	1.21	0.30	0.70	0.43	0.61	0.45	8.58	0.02	1.59	0.24	5.91	0.04
	P	1	16.39	0.003	25.10	0.001	20.24	0.001	55.02	0.000	1.22	0.30	46.51	0.000	68.61	0.000
	N × P	1	0.91	0.37	0.34	0.58	1.59	0.24	0.58	0.47	2.50	0.15	1.95	0.20	0.20	0.66
	Block	9	7.81	0.40	5.75	0.05	2.78	0.30	5.48	0.05	9.73	0.03	8.13	0.03	4.86	0.02
2008	N	1	5.34	0.046	4.46	0.06	5.12	0.049	11.08	0.009	0.12	0.73	2.72	0.13	4.33	0.07
	P	1	0.70	0.42	0.01	0.93	2.87	0.12	0.01	0.91	0.11	0.75	6.41	0.03	38.88	0.000
	N × P	1	0.21	0.66	0.07	0.80	0.60	0.46	0.38	0.56	0.06	0.81	4.29	0.07	7.61	0.02
	Block	9	11.67	0.41	6.74	0.10	15.91	0.25	0.56	0.81	2.42	0.18	4.48	0.01	4.98	0.03

F-ratios and significance values for nitrogen (N), phosphorus (P), the interaction between N and P, and block effects are given (significant results in bold)

df degrees of freedom

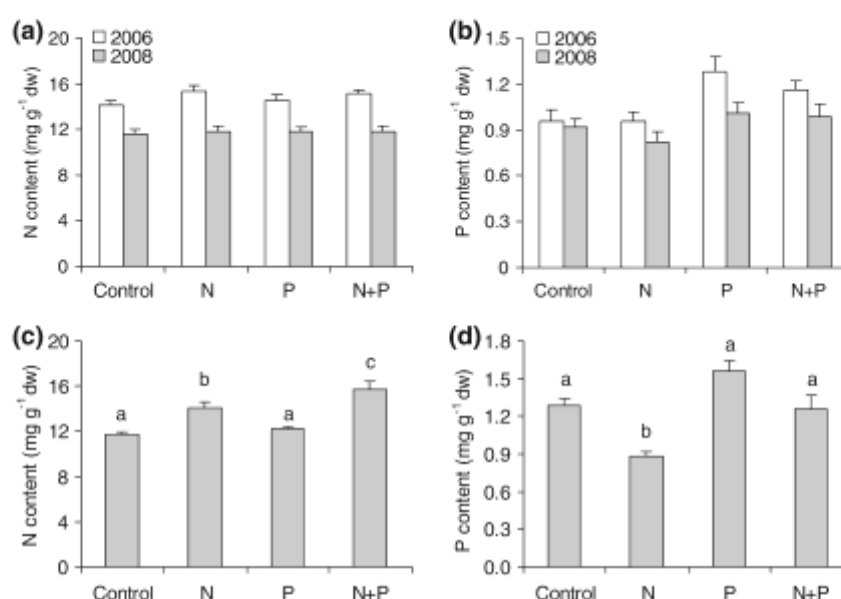
Fertilisation effects on the nutritional status of *Molinia*

Field experiment

In 2006, the N and P contents of the biomass increased due to the N and P treatment, respectively (Fig. 2a, b; Table 1), and both treatments influenced the biomass

N:P ratios (control: 15.2, N treatment: 16.4, P treatment: 11.8; data not shown in Fig. 2). In the control, the biomass N content was 14.1 mg g⁻¹ (vs. 15.4 mg g⁻¹ in the N treatment), and the P content was 0.96 mg g⁻¹ (vs. 1.23 mg g⁻¹ in the P treatment; Fig. 2). In 2008, the N treatment had no effect on the N biomass content and biomass N:P ratios, but the P treatment caused a significant increase in the

Fig. 2 Effects of treatments (control, N, P, N + P) on biomass N (**a** and **c**) and P contents (**b** and **d**) of *Molinia caerulea* in the field experiment (2006 and 2008) and the greenhouse experiment, respectively (means + 1SE; in **a** and **b** significant differences according to the GLM, see Table 1)



biomass P content (control: 0.92 mg g⁻¹; P treatment: 1.01 mg g⁻¹), and a decrease of N:P ratios from 12.7 to 11.8 (control vs. P treatment).

Greenhouse experiment

Biomass N contents were significantly higher in the N and N + P treatment. P addition had no effect on biomass P contents, but influenced the N content negatively. In the N and P treatment biomass N:P ratios were equivalent to 16.0 and 7.8, respectively (control: 9.1; data not shown in Fig. 2).

Fertilisation effects on soil chemical properties (field experiment)

With the exception of pH and N_t (in August 2008), treatments had no significant effects on the soil chemical properties measured (Table 2).

Plant-available PO₄³⁻ ranged between 5.4 and 12.9 mg l⁻¹ (minimum in the control in August 2006), but treatments had no significant affect on the availability of P (in 2006 and 2008). N_t ranged between 1.80 and 2.25 g l⁻¹ in both years and on both sampling dates (i.e. April and August). P_t was between 0.13 and 0.20 g l⁻¹ (both years and both sampling dates; Table 2). The soil N:P ratio was lowest in the control in April in 2008 (10.3) and highest in the N treatment in April in 2008 (16.4; Table 2).

Discussion

Fertilisation effects on growth of *Molinia*

In the greenhouse experiment and the field experiment in 2008 growth of *Molinia* was limited by N. N limitation was particularly evident in the greenhouse experiment, since biomass productivity increased about sixfold and only treatments with N developed generative tillers. However, our hypothesis was not confirmed by the field experiment in 2006. This indicates that additional variables must have affected responses of *Molinia* to fertilisation in the field experiment. We hypothesise that different responses of *Molinia* in the field experiment were attributable to differences in the water availability in the course of the respective vegetation period. The year 2006 was characterised by a severe drought event in July (precipitation of 27 mm in 2006 compared to 178 in 2008), accompanied by elevated July temperatures (mean daily temperature of 21.0°C in 2006 compared to 16.4°C in 2008). According to van Meeteren et al. (2007), microbial P mineralisation, and thus P availability, distinctly decreases when soils start to dry out. This may apply to sandy heath soils in particular, since their water storage capacity is comparatively low (Brady and Weil 2001). It is, therefore, likely that the severe drought in July 2006 worsened the P supply of *Molinia*,

Table 2 Means of soil pH, plant-available PO_4^{3-} (in mg l^{-1}), total N (N_t), total P (P_t ; both in g l^{-1}) and N:P ratios of the upper mineral horizon in the control (C), and treatments (N, P, N + P; +1 SE in brackets; field experiment)

Year	2006					2008					
	Treatment	C	N	P	N + P	Sign	C	N	P	N + P	Sign
pH	April	3.3 (0.03)	3.4 (0.04)	3.4 (0.03)	3.4 (0.03)		3.2 (0.03)	3.2 (0.04)	3.2 (0.03)	3.2 (0.03)	
	August	3.4 (0.03)	3.6 (0.03)	3.6 (0.04)	3.6 (0.03)	**N, ***p	3.5 (0.03)	3.5 (0.02)	3.5 (0.03)	3.4 (0.03)	*N
PO_4^{3-}	April	7.5 (7.2)	11.4 (3.9)	9.7 (1.6)	6.7 (1.6)		11.5 (1.6)	10.9 (1.2)	11.7 (1.6)	10.3 (1.4)	
	August	5.4 (2.7)	12.9 (3.8)	12.9 (2.8)	11.1 (1.5)		9.6 (1.0)	11.0 (1.4)	10.8 (1.4)	11.5 (1.2)	
N_t	April	1.80 (0.22)	2.14 (0.43)	1.96 (0.22)	1.87 (0.19)		2.03 (0.12)	2.09 (0.18)	1.99 (0.18)	2.06 (0.19)	
	August	2.25 (0.45)	2.23 (0.39)	2.16 (0.38)	1.87 (0.23)		1.99 (0.17)	2.05 (0.19)	2.08 (0.21)	2.20 (0.21)	*P
P_t	April	0.14 (0.02)	0.15 (0.03)	0.13 (0.02)	0.13 (0.01)		0.20 (0.01)	0.18 (0.01)	0.18 (0.01)	0.19 (0.01)	
	August	0.15 (0.03)	0.15 (0.03)	0.15 (0.02)	0.14 (0.01)		0.17 (0.01)	0.18 (0.01)	0.18 (0.01)	0.18 (0.01)	
N:P	April	13.3 (1.1)	14.0 (1.2)	14.9 (0.9)	14.0 (1.1)		10.3 (0.5)	16.4 (1.3)	10.8 (0.5)	10.5 (0.7)	
	August	15.2 (0.9)	15.2 (1.2)	15.1 (1.1)	13.7 (1.0)		11.6 (0.7)	11.6 (0.7)	11.6 (0.6)	12.2 (0.7)	

Significant impacts (sign) of the N and P treatment and N × P interactions (according to the GLM analyses) were given (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

resulting in a positive response to P fertilisation. This interpretation is supported by very low values of plant-available PO_4^{3-} found in the control in August 2006 (5.4 mg l^{-1} compared to 9.6 mg l^{-1} found in the control in August 2008).

By contrast, growth of *Molinia* was limited by N in the greenhouse experiment and the field experiment in 2008, probably due to a sufficient water supply *Molinia* experienced in the greenhouse and the humid summer in 2008, respectively. This finding is in agreement with other pot experiments (with sufficient water supply), in which a positive short-term response of *Molinia* to N fertilisation has been demonstrated (deAldana and Berendse 1997; Güsewell et al. 2003; van Heerwaarden et al. 2005). Hence, variable responses of *Molinia* to N and P fertilisation reported from field experiments might be partly due to differences in the water supply *Molinia* experienced in the course of these experiments.

In all experiments *Molinia* responded with an increased biomass (and number) of flowering tillers produced per tussock. This means that fertilisation increases the reproductive performance of *Molinia*, whereas an increase in leaf biomass did not prove to be a consistent response pattern in our experiments. Pot experiments by Thornton (1991) showed that enhanced biomass productivity of *Molinia* through improved nutrition was achieved by an increase in both the number of tillers produced and the size of

various plant parts (such as leaves and basal internodes). In his experiments, high N and high P supply increased the percentage of flowering tillers in particular (from 27% at low N to 60% at high N supply; Thornton 1991), which is in agreement with findings in our field experiment (increase of flowering tillers by 30 and 45% in 2006 and 2008, respectively). In principle, ‘secondary tillering’ as a result of improved nutrient supply seems to be a characteristic growth pattern of *Molinia*, since plants then regularly exhibit a higher number of tillers compared to the total number of buds that were developed at the beginning of the vegetation period (Thornton 1991). As *Molinia* has been proven to shift from an N recycling strategy to reserve formation during growth under high N conditions (van Heerwaarden et al. 2005), plants tend to develop a higher proportion of generative tillers, in which seeds represent important stores for N (Aerts and De Caluwe 1989). Variable response patterns (in terms of a variable number of vegetative tillers) to fertilisation may also be attributable to a trade-off *Molinia* experience with increasing nutrient supply. On the one hand, an increasing biomass allocation to leaves will enhance the competitive performance of plants due to an improved aboveground competitiveness for light. On the other hand, a high aboveground biomass will increase transpiration rates and thus the plant’s water requirements (van Heerwaarden et al. 2005). To meet this

evaporative demand, *Molinia* has to produce a higher number of cord roots that facilitate the transport of water to the aboveground plant parts (Berendse and Aerts 1984; van Heerwaarden et al. 2005). As a consequence, higher biomass allocation to the roots will be required.

In summary, our experiments showed that *Molinia* increased its investment in reproductive tissue under improved nutrient availability. This, in turn, allows for higher seed production and may accelerate the encroachment of seedlings in places where the dwarf shrub canopy has been opened due to management or other disturbance factors (Aerts and Heil 1993). Compared to *Calluna vulgaris*, *Molinia* is the superior competitor during the establishment phase of both species (e.g. after the application of high-intensity management measures), since then (aboveground and belowground) biomass increment rates of *Molinia* by far exceed those of *Calluna vulgaris* under high N availability (Aerts and Bobbink 1999).

Fertilisation effects on the nutritional status of *Molinia*

N treatment increased the tissue N content (greenhouse and field experiment in 2006) and the P treatment the tissue P content of *Molinia* (field experiment), whereas N × P interactions were not found (with the exception of the greenhouse experiment). In addition, both treatments affected tissue N:P ratios. This is in agreement with findings from other experiments (Morton 1977; Berendse et al. 1987a; Tomassen et al. 2004), although a strong increase in aboveground biomass productivity following N fertilisation may also result in unchanged leaf N contents (Tomassen et al. 2004). In the greenhouse experiment, however, N supply of *Molinia* was sufficient despite its high growth rates. The highest concentrations found in the N + P treatment may be attributable to a significantly lower biomass production (compared to the N treatment). In contrast to findings of Berendse et al. (1987b) and Tomassen et al. (2004), pre-senescent biomass P contents and corresponding N:P ratios of *Molinia* were high and low, respectively, in our experiments. This indicates that in our study both tissue nutrient contents and N:P ratios were limited means of predicting *Molinia* responses to N and P fertilisation, particularly as other factors such as drought events might mediate

experimental outcomes. According to Tomassen et al. (2004), aboveground biomass of *Molinia* may be stimulated through N addition even at tissue N:P ratios above 40, which contradicts the finding of Koerselman and Meuleman (1996) that plant growth tends to be limited by P when tissue N:P ratios exceed a threshold of 16. This may also demonstrate the variability of *Molinia* responses to fertilisation in relation to its pre-treatment tissue N:P ratios.

Regarding tissue N and P contents of *Molinia* in the field experiment, we found a significant block effect related to the treatments applied. This indicates that *Molinia* responses to fertilisation (in terms of biomass nutrient contents) were also modified by the variability of site conditions inherent to heathlands and, thus, to our experimental sites. This may be due to differences in soil texture and humus contents, but also to differences in the chemical properties of the experimental sites prior to treatment application (see below).

Fertilisation effects on soil chemical properties

Fertilisation treatments had almost no effects on the soil chemical properties measured (with the exception of soil pH and N_i in August 2008). This may be explained by the principle pathways which nutrients could have taken after addition (i.e. uptake by soil microbes, leaching, soil adsorption, uptake by plants). Based on the results of a long-term N addition experiment in heaths, Power et al. (1998) stated that soil microbes are responsible for the short-term absorption of a high proportion of experimentally added N (see also Johnson et al. 1998). As both total N and P stores of the humus horizons by far exceed the quantities of N and P experimentally added (Härdtle et al. 2006), N_t and P_t (as well as soil N:P ratios) were not significantly affected by the N treatment. In addition, up to 20% of the N added to the system can be leached with seepage water (Härdtle et al. 2007), but leaching rates may vary considerably in relation to the dose and frequency of nutrient additions, sampling dates, and meteorological conditions prevailing during the course of the experiment (Aerts and Chapin 2000).

As regards plant-available PO_4^{3-} , we expected increased values at least in the subplots subjected to P fertilisation. However, the sandy podzols are a P-poor substrate and have low P fluxes related to mineral weathering, but exhibit high immobilisation

rates for P (van Meeteren et al. 2007). This effect, in combination with a rapid uptake of plant-available P by plant roots, may explain why concentrations of plant-available PO_4^{3-} were not significantly affected by the treatments. In 2006, the desiccation of the upper humus layer due to drought might have strengthened the P shortage, at least in the control subplots (van Meeteren et al. 2007).

Conclusions

In summary, high input rates of N may promote *Molinia* encroachment in dry heathland ecosystems, although additional factors such as water availability (in the course of the vegetation period) might mediate *Molinia* responses to improved nutrient availability. In our experiments, *Molinia* showed an increased investment in reproductive tissue resulting from fertilisation. This, in turn, allows for higher seed production and may accelerate the encroachment of *Molinia* seedlings in places where the dwarf shrub canopy has been opened due to management or other disturbance factors. Management of dry heathlands should aim at the removal of flowering tillers of *Molinia* to avoid its encroachment due to the dispersal of seeds. Low-intensity grazing and low-intensity mow might be the best means to counteract the development of generative tillers and thus an ongoing encroachment of *Molinia* resulting from an increasing seed set under high airborne N loads.

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