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Ecosystems 13 (2010): 317-327



N:P Ratio and the Nature of Nutrient Limitation in *Calluna*-Dominated Heathlands

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ABSTRACT

There is growing evidence from different sources that prolonged high N deposition causes a shift from nitrogen (N) limitation to nitrogen and phosphorus (P) co-limitation or even P limitation in many terrestrial ecosystems. However, the number of ecosystems where the type of limitation has been directly tested by longer-term full-factorial field experiments is very limited. We conducted a 5-year fertilization experiment with N and P in the Lüneburger Heide (NW Germany) to test the hypothesis that, following decades of elevated atmospheric N inputs, plant growth in dry lowland heaths may have shifted from N to N–P co-limitation or P limitation. We also tested whether the plant tissue N:P ratio reflects the type of nutrient limitation in a continental lowland heathland. Experimental plots dominated by *Calluna vulgaris* received regular additions of N (50 kg N ha⁻¹ y⁻¹), P (20 kg P ha⁻¹ y⁻¹), a combination of both, or

water only (control) from 2004 to 2008. Over the whole study period, a highly significant positive N effect on shoot length was found, thus indicating N limitation. We conclude that a clear shift from N limitation to N–P co-limitation or P limitation has not yet occurred. Tissue N:P ratios showed a high temporal variability and no relationship between tissue N:P ratio and the shoot length response of *Calluna* to nutrient addition was found. The N:P tool is thus of limited use at the local scale and within the range of N:P ratio observed in this study, and should only be used as a rough indicator for the prediction of the type of nutrient limitation in lowland heathland on a larger geographical scale with a broader interval of N:P ratio.

Key words: *Calluna vulgaris*; fertilization experiment; nitrogen deposition; nitrogen saturation; plant growth; phosphorus limitation.

Received 28 August 2009; accepted 25 January 2010;
published online 17 February 2010

Author contributions: SAP, AM, and WH designed the study; GvO, KF, UF, AM, AK, and NB performed the research; GvO, SAP, KF, UF, AM, AK, and WH analysed the data; SAP, AM, and AK contributed new methods; GvO, SAP, KF, and WH wrote the article.

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INTRODUCTION

Nitrogen (N) was until recently generally considered to be the principal limiting nutrient for plant growth in many terrestrial ecosystems. However, human activities have dramatically increased the mobility and deposition of reactive forms of N in recent decades (Galloway and others 2004). For

large parts of Europe and North America there is growing evidence that increased N deposition alters the N status of forests, ultimately leading to N saturation (Aber and others 1998; Dise and others 1998; de Schrijver and others 2008). Aber and others (1998) used forests as an example to describe four stages of N saturation. Early stages comprise increased aboveground biomass production, increased foliar N concentrations, and an accumulation of N in the humus layers of the soil. In advanced stages of N saturation, N is leached and aboveground biomass productivity is reduced. From the analyses of two large-scale European databases, de Schrijver and others (2008) deduced that more than 25% of the European forests included in these databases have reached an advanced stage of N saturation as the N losses with seepage water exceeded $5 \text{ kg ha}^{-1} \text{ y}^{-1}$.

In non-forested terrestrial ecosystems under high N deposition, nitrogen dynamics are much less well studied. In heathland and moorland ecosystems dominated by *Calluna vulgaris* (henceforth referred to as *Calluna*) early responses to increased N deposition are similar to those observed in forests and include increased plant growth as well as a build up of nitrogen stores in the aboveground biomass and humus layers (Power and others 1998a; Carroll and others 1999; Cuesta and others 2008). Prolonged exposure to high N inputs results in an acceleration of the life cycle of *Calluna* accompanied by a changing sensitivity to biotic and abiotic stresses (Power and others 1998b). Despite the limited observational or experimental evidence of strongly increased N leaching from heathland and moorland ecosystems (but see Schmidt and others 2004), a model simulation study conducted by Evans and others (2006) showed that the effects of enhanced N deposition on heathland systems are, in the long term, fundamentally comparable to those on forests.

As N saturation advances, it has been hypothesized that plant growth in terrestrial environments becomes (co-)limited by another key element, phosphorus (P) (Verhoeven and Schmitz 1991; Aerts and Chapin 2000; Menge and Field 2007). This shift from N to P limitation may arise because P is required for plant growth in relatively large quantities, and rates of atmospheric P deposition are generally low. In addition, N inputs can decrease the availability and plant uptake of P via effects on mycorrhizae (Gundersen 1998; Turner and others 2003). Generally, the probability of a shift to P limitation resulting from increased N availability should be highest in soils derived from parent material with low P levels, in more acid

soils, and in more weathered soils (Gress and others 2007).

The shift from N to P limitation due to prolonged high N deposition has been inferred from various measures used to determine the type of nutrient limitation. Nutrient addition experiments with a factorial design are generally considered the most straightforward approach to determining nutrient limitation of plant growth (Aerts and Chapin 2000). For example, the results of a 1-year fertilization experiment showed that the growth of *Sphagnum* was N limited at a low-deposition ombrotrophic bog in northern Sweden, whereas it was P limited at a site with moderate deposition in southern Sweden (Aerts and others 1992). The authors concluded that a shift from N to P limitation of the *Sphagnum* layer had occurred because of the recent increase in N deposition. These findings were confirmed by Limpens and others (2004), who observed that P limits *Sphagnum* growth at sites with moderate to high N deposition in the Netherlands and Ireland.

An alternative approach for the analysis of nutrient limitation patterns is to use the nutrient ratios in plant tissues, or ecological stoichiometry (Güsewell 2004). N:P ratios are closely related to the actual nutrient availability, representing a quick and simple alternative to fertilization experiments. N:P ratios have in recent years been used as diagnostic indicators of nitrogen saturation and limitation of vegetative growth by these nutrients (Tessier and Raynal 2003). Bragazza and others (2004) analyzed the N:P ratio in *Sphagnum* plants sampled from ombrotrophic mires in 11 European countries, representing a broad gradient of atmospheric N deposition. The N:P ratios increased steeply at low N deposition, but above a threshold of approximately $10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ a saturating trend was observed. This has been interpreted as a shift from N limited conditions to P (co-)limited conditions.

Koerselman and Meuleman (1996) proposed that critical aboveground biomass N:P ratios could be used to predict N and/or P limitation at the community level. Based on studies of European wetlands, critical N:P ratios were found to be below 14 for N limitation and above 16 for P limitation. However, the generality of these threshold values has frequently been questioned (Tessier and Raynal 2003; Øien 2004; Soudzilovskaia and others 2005). Having reviewed a wide range of field fertilization experiments, Güsewell (2004) proposed that biomass production is N limited at N:P ratios below 10 and P limited at N:P ratios above 20, whereas within this range, the type of nutrient limitation is not unequivocally related to the N:P ratio.

Although there is growing evidence from different sources that chronic N inputs may be causing a shift from N to N–P co-limitation or even P limitation, the generality of this pattern warrants further testing because the number of ecosystems where the type of limitation has been directly tested by longer-term full-factorial experiments in the field is relatively small and alternative approaches (such as tissue N:P ratio) are not unambiguous (Güsewell 2004; Gress and others 2007). Furthermore, the emergence of P limitation will be affected by factors that influence the supply of plant-available P (Gress and others 2007). The most important of these are inherent soil properties, although management regime (Verhoeven and others 1996; Härdtle and others 2009; Akselsson and others 2008) and climatic conditions (Sardans and Peñuelas 2007; van Meeteren and others 2007) also have a major impact on P budgets and availability.

The Lüneburger Heide region in northwest Germany is characterized by nutrient-poor podsolc soils. Due to a long tradition of historical heathland farming and current management measures, dry lowland heaths dominated by *Calluna* are still the predominant vegetation type (Mück 1998; Niemeyer and others 2007). The N deposition rates measured in the Lüneburger Heide are similar to those reported for heathland ecosystems of central Europe and have exceeded the critical loads for dry heathlands for at least 30 years (Matzner 1980; Engel 1988; Härdtle and others 2007). This article describes the results of a 5-year full-factorial field experiment with N and P in the Lüneburger Heide. In this study, we test the hypothesis that growth of *Calluna*-dominated ecosystems in areas with a long history of N deposition in excess of critical loads may have shifted from N to N–P co-limited or even P limited. Furthermore, we examine whether tissue N:P ratios reflect the nature of nutrient limitation on *Calluna* growth. In addition to the field experiment, we conducted a 1-year greenhouse experiment to test the nature of nutrient limitation of *Calluna* seedlings under controlled conditions.

MATERIALS AND METHODS

Study Area

The field study was 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 5500 ha). The study area is characterized by Pleistocene sandy deposits. Prevailing soil types are nutrient-poor podzols, with pH (H₂O)

values in the topsoil ranging between 3.0 and 3.5. The climate is of a humid suboceanic type. Mean precipitation is 811 mm y⁻¹ and the mean temperature is 8.4°C (Niemeyer and others 2005). The background deposition in the study area was determined to be 23 kg N ha⁻¹ y⁻¹ and 0.3 kg P ha⁻¹ y⁻¹ (Härdtle and others 2007). The complementary greenhouse experiment took place in greenhouses of the Federal Research Institute for Rural Areas, Forestry and Fisheries (vTI, Hamburg, Germany).

Experimental Design

In the field experiment, a series of 10 replicate blocks was selected in the study area in June 2004. A pilot survey identified suitable areas with *Calluna* cover greater than 95%, *Calluna* stands aged from 10 to 12 years and similar abiotic site conditions. Within these areas, blocks were randomly located on maps. Each of the 10 blocks was divided into four 1.4 m × 1.4 m plots, with a 0.5-m wide buffer strip in between the plots. Each of the four plots per block was assigned to one of four treatments: N, P, N + P, and control. All blocks were fenced to prevent grazing.

In the N plots, 50 kg N ha⁻¹ y⁻¹ was applied as NH₄NO₃. In the P plots, 20 kg P ha⁻¹ y⁻¹ was applied as Na₂HPO₄·2H₂O, and the N + P plots received a combination of both. In the control plots only distilled water was added. Applications started in July 2004; nutrients were dissolved in 2-l distilled water per plot and applied fortnightly using watering cans until October 2004 (that is, on eight occasions). In the following 4 years, treatments began in May and continued through October, with solutions applied on 10 occasions each year. In 2006, the number of blocks was reduced from 10 to 9, because *Calluna* died off in one area during an exceptionally dry summer period.

As the response of individual plant species to nutrient treatments may differ from the overall community response, it is generally necessary to differentiate the nutrient limitation at the species level and at the community level (Aerts and Chapin 2000). However, in the plots analyzed in the present study *Calluna* formed monospecific stands. Thus, responses of *Calluna* to fertilization may be considered representative for the total community response in this system.

For the greenhouse experiment, seeds of 20 different *Calluna* individuals spaced at least 50-m apart (that is, 20 seed families) were collected in the study area in September 2007. Seedlings were raised in germination dishes during winter and, respectively,

16 individuals were planted into pots ($12 \times 12 \times 12 \text{ cm}^3$ 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 (that is, N, P, N + P, control), resulting in a total of 40 pots. In the greenhouse experiment, we applied $48 \text{ kg N ha}^{-1} \text{ y}^{-1}$ as NH_4NO_3 , and $4 \text{ kg P ha}^{-1} \text{ y}^{-1}$ as Na_2HPO_4 . N + P treatment pots received a combination of both. In the controls, only distilled water was added. Solutions were applied weekly from May through September.

Sampling and Chemical Analyses

In the field experiment, the length of the current year's shoots of *Calluna* was recorded in October 2004, 2005, 2006, and 2008 as a response variable to fertilization. In each plot, five plants were randomly selected at the start of the growing season. On each of these plants, five stems were chosen at random and tagged with a plastic band. In October, the length increment of five main shoots and the accompanying longest side shoots per stem were measured using a calliper gauge.

On one occasion before (July 2004) and on four occasions after the start of the experiment (October 2004, 2005, 2006, 2008) 20 current year's shoots per plot were collected from the top of randomly selected *Calluna* plants to determine tissue N and P concentrations. Prior to chemical analyses, biomass samples were air dried, ground with a ball mill (Pulverisette 7; Fritsch, Idar-Oberstein, Germany), and re-dried at 105°C before weighing. N contents were analyzed with a C/N-analyzer (Vario EL; Elementar, Hanau, Germany). For P-determination, samples were dissolved in an $\text{HNO}_3\text{-HCl-H}_2\text{O}_2$ solution (Lamble and Hill 1998) and digested using a microwave (MLS-ETHOS; MLS-GmbH, Leutkirch, Germany). Digests were analyzed by means of an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Immediately before the experiment was started in July 2004, three samples (100 cm^3 each) of the soil organic layer and the A horizon were taken in each plot and were analyzed for plant-available N and P to establish whether there were pre-existing differences in nutrient availability prior to the start of treatment additions. The soil samples were thoroughly mixed and stored at -20°C until analysis. Samples were extracted with 0.0125-M CaCl_2 and analyzed immediately for plant-available N (NH_4^+ , NO_3^-) using a spectrophotometer (Spectroquant VEGA 400; Merck, Darmstadt, Germany). For PO_4^{3-} analysis, soil samples were air dried and

sieved ($<2 \text{ mm}$). PO_4^{3-} was extracted from 5-g dry soil by shaking for 90 min in 250-ml double lactate (DL) solution. Samples were filtered, and extracted PO_4^{3-} was measured with an ICP-OES (Hoffmann 1997).

The main part of the soil analyses took place during the second year. In May and October 2005, soil samples were taken and treated as described above, and subsequently analyzed for plant-available N and P, as well as for total C, N, and P. Soil analyses were carried out to quantify the effects of N and P treatments on both plant-available and total N and P pools, and to establish whether current N:P and C:N ratios in the humus layers differ from those recorded at the site 25 years previously. For analysis of NH_4^+ , 10-g fresh soil was shaken for 60 min with 0.0125-M CaCl_2 (Hoffmann 1997). NO_3^- was extracted by shaking 10-g fresh soil for 10 min in 100-ml Aqua bidest (Allen 1989). The samples were filtered and analyzed immediately using an ion chromatograph (IC-DX 120; Dionex, Idstein, Germany). PO_4^{3-} was determined as described above. To examine total C, N, and P concentrations, soil samples were prepared and analyzed in the same way as biomass samples.

In the greenhouse experiment, the aboveground biomass was used to assess response to fertilization. In September 2008, the 16 *Calluna* plants per pot were harvested and weighed after drying for 12 h at 80°C . To determine tissue N and P concentrations, samples were sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany), ground with a mixer mill (MM 400, Retsch, Haan, Germany) or cut with scissors (depending on sample size) and re-dried at 105°C before weighing. Total N concentrations were determined as described above. For P-determination, samples were incinerated in a muffle furnace (N7; Nabertherm, Lilienthal, Germany) at 550°C for 3 h. The samples were dissolved in an HCl solution (Schlichting and others 1995) and analyzed with an ICP-OES.

Data Analysis

In the field experiment, the effects of N and P addition on shoot increment and tissue N and P concentrations were tested using repeated measures Linear Mixed Models (LMM). Each LMM included the treatment as the fixed effect (N, P, and N \times P interaction), and year and block as random effects (year, block, and year \times block interaction; all as type-III effects). Analyses were undertaken using restricted maximum likelihood (REML) methodology implemented by the Proc Mixed procedure of SAS 9.1 (SAS Institute Inc., Cary, NC). The effects of

N and P addition on soil nutrient concentrations were tested using Generalized Linear Models (GLM). Each GLM included the main effects of block, N and P, and the N \times P interaction.

The N:P ratio may vary considerably within the individuals of a species when these are sampled at different sites (Güsewell 2004). Not only the mean tissue N:P ratios of all plots, but also the N:P ratios of single plots may be important for testing the hypothesis that the tissue N:P ratio is a suitable indicator of the type of nutrient limitation. We, therefore, tested the correlations between tissue N:P ratio of the control plots and the relative change in current year's shoot length of the nutrient treatments, compared to control plots, using Pearson's correlation analysis.

Results of the greenhouse experiment were evaluated by means of one-way ANOVA. To allow for the visualization of changes in aboveground biomass, nutrient concentration and content in a single graph, the approach of Timmer and Stone (1978) was adopted. Aboveground biomass per pot (that is, of 16 *Calluna* plants) was multiplied by tissue nutrient concentration to yield nutrient content of the 16 *Calluna* plants. Trajectories of changes in nutrient concentration and nutrient content after fertilization are plotted in nutrient content—nutrient concentration space. The direction of change allows one to conclude whether increased nutrient content is because a nutrient is limiting (that is, increases in both nutrient concentrations and biomass) or whether it is just due to luxury consumption (increased nutrient accumulation without any gain in biomass). With the exception of the correlation analysis (no transformation), data were log-transformed prior to the analyses. With the exception of the repeated measures LMM all analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL).

RESULTS

In the field experiment, shoot length response of *Calluna* to fertilization showed a similar pattern from the second to the fifth year, whereas the first year deviated from this pattern (Figure 1). In the first year, shoot length was increased only by adding N + P simultaneously, whereas shoot length was decreased by adding N only. In the following years, there was a positive response in the N and N + P plots. Repeated measures LMM analysis revealed that there was a highly significant N effect on shoot length over the whole study period ($F_{1,111} = 13.3$; $P < 0.001$). No significant P effect ($F_{1,111} = 1.4$; $P > 0.05$) or interaction effect of N

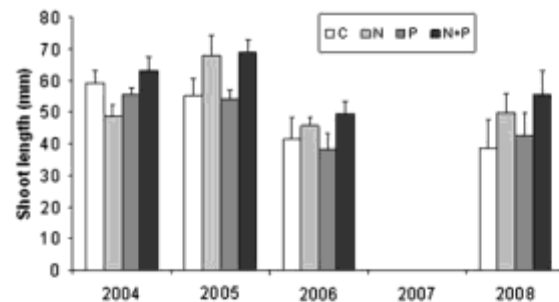


Figure 1. Effects of fertilization on current year's shoot length of *Calluna vulgaris* in the field experiment (2004–2006 and 2008; means and standard errors). C control, N nitrogen, P phosphorus.

and P was found ($F_{1,111} = 2.7$; $P > 0.05$). The mean shoot increment in the control plots was very similar in 2004 and 2005, whereas shoot length was 25% lower in 2006 and 2008 (Figure 1).

In the control plots, N concentrations of current year's shoots increased, and P concentrations decreased throughout the first 3 years of the experiment (Figure 2). As a consequence, the mean tissue N:P ratio increased considerably from 11.1 (2004, range 9–14) to 13.4 (2005, range 10–17) and then 16.9 (2006, range 13–19) (Figure 3). Indeed, in 2006, the vast majority of control plots (8 out of 9 plots) had N:P ratios above 16. In 2008, both the N and the P concentrations showed relatively low values, with an intermediate mean tissue N:P ratio of 13.1 (Figure 2; range 11–17, Figure 3). No significant correlation was found between the N:P ratio of the control plots and the relative change in current year's shoot length of the nutrient treatments, compared to control plots (r between |0.011 and |0.45|; see also Figure 3).

Neither the current year's shoot N and P concentrations nor N:P ratios differed significantly between the treatments before the start of the experiment (data not shown). Tissue N concentrations were significantly increased by the N treatment ($F_{1,111} = 44.4$; $P < 0.0001$) and the interaction between N and P ($F_{1,111} = 4.8$; $P = 0.03$), whereas the P treatment had no significant effect ($F_{1,111} = 2.4$; $P > 0.05$). The P concentrations increased significantly with P addition ($F_{1,111} = 111.5$; $P < 0.0001$), but no N or interaction effect was observed ($F_{1,111} = 0.1$ and $F_{1,111} = 0.02$, respectively; both $P > 0.05$). The addition of N significantly increased the tissue N:P ratio ($F_{1,111} = 5.5$; $P = 0.02$), whereas the addition of P strongly and significantly decreased the tissue N:P ratio ($F_{1,111} = 96.5$; $P < 0.0001$). The N:P ratio

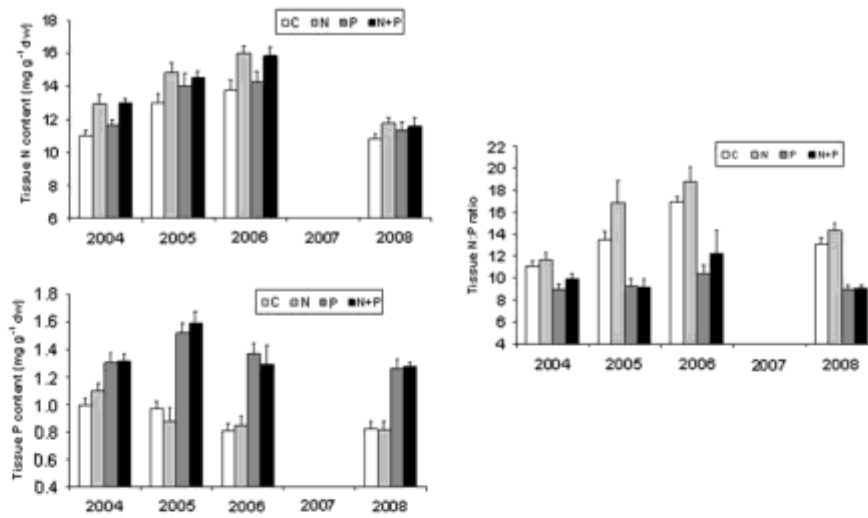


Figure 2. Effects of fertilization on tissue N and P concentrations and N:P ratios of *Calluna vulgaris* in the field experiment [October 2004–2006 and 2008; means and standard errors in mg g^{-1} dry weight (dw)]. C control, N nitrogen, P phosphorus.

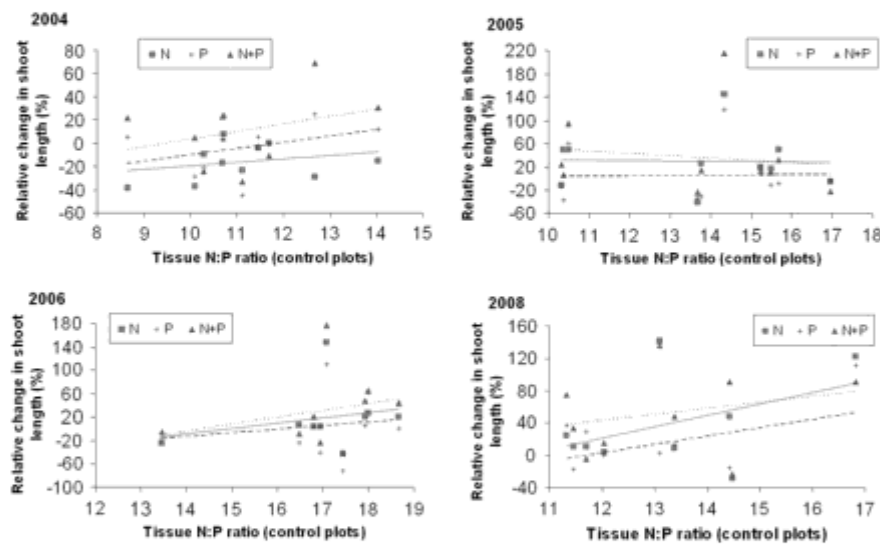


Figure 3. Relationship between tissue N:P ratio of *Calluna vulgaris* (in control plots) and the relative change in current year's shoot length in nutrient treated plots, compared to controls in the field experiment (2004–2006 and 2008): N nitrogen, P phosphorus. Each point represents the mean value of shoot length, measured on five plants per plot (see text). Number of plots in October 2004 and 2005: 10, in October 2006 and 2008: 9. Trend lines: solid line: N treatment; dashed line: P treatment; dotted line: N + P treatment.

was not significantly affected by the N and P interaction ($F_{1,111} = 0.5$; $P > 0.05$).

Soil extractable N and P concentrations were not significantly different between any of the treatments before the start of the experiment (data not shown). The first year's N and P treatments had no significant effect on the NH_4^+ concentrations of the O-horizon at the beginning of the second growing season (May 2005; Table 1). However, significant N

and interaction effects were found for the A-horizon. In October 2005, NH_4^+ concentrations were significantly higher in both soil horizons in N-treated plots. Nitrate concentrations were generally lower than NH_4^+ concentrations and did not differ between treatments. Plant-available PO_4^{3-} was significantly higher in the O-horizon of P-treated plots in May and October 2005. Phosphate concentrations were also highest in the A-horizon of P-

Table 1. Mean Concentrations of Plant-Available NH_4^+ , NO_3^- , PO_4^{3-} , and Total N (N_t), P (P_t) (in mg kg^{-1}) as well as Mean N:P and C:N Ratios in the O-Horizon (O) and A-Horizon (A)

		May 2005					October 2005				
		C	N	P	N + P		C	N	P	N + P	
NH_4^+	O	8.3	9.8	8.8	10.3	$\text{N}^* \text{N} \times \text{P}^{**}$	12.8	17.4	12.7	18.4	N^{**}
	A	0.8	2.1	1.6	1.4		1.4	2.4	1.7	1.9	N^*
NO_3^-	O	0.7	0.8	0.6	0.8	P^*	0.8	1.1	0.9	1.2	
	A	0.8	0.7	0.6	0.7		0.6	0.6	0.6	0.6	
PO_4^{3-}	O	49.9	19.6	116.3	63.2	P^{***}	62.3	49.5	171.0	189.2	P^{***}
	A	6.4	4.5	6.8	6.5		5.9	5.0	7.9	9.5	
N_t	O	11609	10631	14067	10805	$\text{N}^* \text{N} \times \text{P}^*$	15783	16119	13614	14535	P^*
	A	820	1055	970	1086		1085	779	1023	1010	$\text{N}^* \text{N} \times \text{P}^*$
P_t	O	396	339	452	505	P^*	416	614	733	820	P^*
	A	111	89	90	105		96	94	104	90	
N:P	O	24.1	31.3	26.8	21.4	P^{**}	28.0	26.3	17.7	19.5	P^{**}
	A	7.4	11.8	9.1	10.6		11.3	8.3	9.8	12.8	$\text{N} \times \text{P}^*$
C:N	O	24.5	22.8	24.1	23.5	N^*	24.2	23.4	23.8	23.5	
	A	31.6	31.1	33.5	32.2		32.5	33.8	32.7	34.0	

Results of GLM analyses with nitrogen (N), phosphorus (P) and $\text{N} \times \text{P}$ interactions given where significant: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

treated plots, although the effect was only marginally significant ($P = 0.05$ in May and $P = 0.06$ in October).

Neither total N and P nor the N:P ratios of either horizon varied significantly between treatments in May 2005 (Table 1). In October 2005, however, P fertilization significantly decreased total N in the O-horizon, and the addition of N and N + P resulted in a significant reduction in total N concentrations in the A-horizon. Phosphorus addition significantly increased total P of the O-horizon in October 2005; N:P ratios of this horizon were lowest in the P and N + P plots, reflecting the effect of P treatments. By contrast, in the A-horizon there was a significant effect of N + P treatments on N:P ratios. The C:N ratio of the O-horizon was significantly decreased by N addition in May 2005, whereas no treatment effects were observed for the A-horizon or for either horizon in October 2005.

In the greenhouse experiment, the mean above-ground biomass per pot was significantly higher in the N and N + P treatments than in control and P treatments ($F = 59.7$; $P < 0.001$). Tissue N concentrations were significantly increased in the N and N + P treatment ($F = 120.9$; $P < 0.001$), whereas P addition significantly increased tissue P concentrations ($F = 26.3$; $P < 0.001$). Figure 4A shows that N accumulation was associated with increased above-ground biomass indicating N limitation already in the first year's growth. The response was, however, stronger when N and P were applied simultaneously. The main effect of P addi-

tion was increased P accumulation without gain in above-ground biomass suggesting luxury consumption of the nutrient (Figure 4B).

DISCUSSION

Growth Limitation of *Calluna*

Contrary to our hypothesis, a highly significant N effect on shoot length was found in the field experiment over the whole study period, which is indicative of N limitation. This positive response of *Calluna* to N addition mirrors that seen in some other N addition experiments (Uren and others 1997; Carroll and others 1999). This is also consistent with the results of a short-term N fertilization experiment in the Lüneburger Heide 18 years ago (Mück 1998). In autumn 1989, NH_4NO_3 was added in solid form in a single dose of either 40 or 100 kg ha^{-1} , to pioneer stands of *Calluna*. One year later, shoot length was higher in the fertilized plots (72 and 75 mm, respectively) than in the controls (66 mm). The results of the current study suggest that growth of *Calluna* remains predominantly N-limited, despite a prolonged period of N deposition (Matzner 1980; Engel 1988; Härdtle and others 2007) in excess of the critical load for lowland heathlands (Achermann and Bobbink 2003).

For several reasons, the general conditions in the Lüneburger Heide are suitable for a shift of the heathland community toward N-P co-limitation. (i) In contrast to the high N input rates, P deposi-

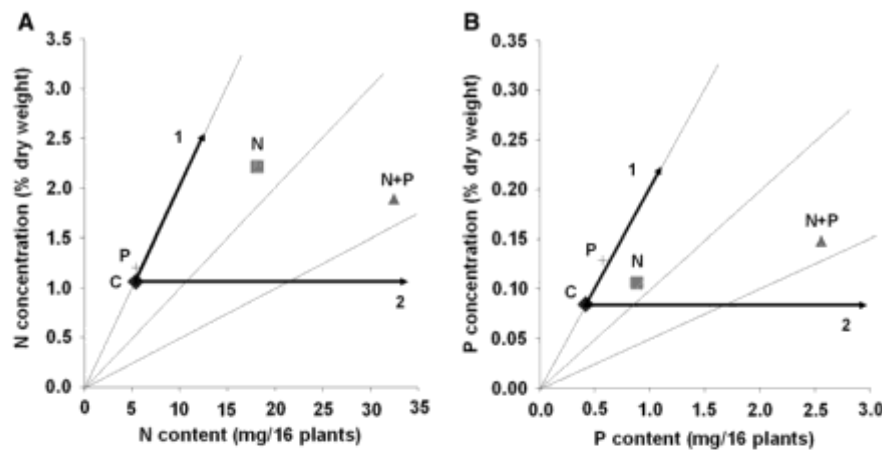


Figure 4. The relationship between tissue nutrient concentration and nutrient content (**A**: N, **B**: P) of the aboveground biomass of 16 *Calluna vulgaris* plants in the greenhouse experiment (data plotted according to the method of Timmer and Stone 1978). Each point represents the mean value of 10 pots. A shift along *arrow 1* denotes increased nutrient accumulation without gain in biomass (luxury consumption), whereas a shift along *arrow 2* indicates increased nutrient content and biomass without change in concentration. A shift into the sector between 1 and 2 denotes increases in both nutrient concentrations and biomass, indicating that the initial level of the nutrient was limiting growth. A shift into the sector below *arrow 2* signifies that the nutrient concentration has been diluted by additional growth.

tion rates have been very low over the last decades (Matzner 1980; Engel 1988; Härdtle and others 2007). (ii) The sandy podzols are a P-poor substrate and have low P fluxes related to mineral weathering, but exhibit high immobilization rates for P (van Meeteren and others 2007). (iii) At all sites where *Calluna* is monodominant, grazing and mowing are applied as regular management measures. Both measures only affect the aboveground biomass, but not the humus layers. Because the aboveground biomass has comparatively high P stores, the overall P budgets of managed heaths are negative (Härdtle and others 2006; Fottner and others 2007).

The question remains in which compartments of a heathland atmospheric N has been accumulated over the years. According to Aber and others (1998) an accumulation of N in the humus layers occurs during early stages of N saturation. In the Lüneburger Heide high-intensity management measures such as sod-cutting were applied only very infrequently during the 1990s. As a result, the thickness of organic layers increased during that time, in line with management plans of the land owner (Verein Naturschutzpark). Concurrently with an accumulation of organic matter in the humus layers, the N:P ratio of the O-horizon has increased from 20.6 to 24.5–31.3 and the C:N ratio decreased from 27.7 to 22.1–24.2 in the Lüneburger Heide during the last 25 years (Matzner 1980; Härdtle and others 2007; Table 1). In addition, leaching rates have in-

creased over the last decades (Matzner 1980; Härdtle and others 2007), and currently about 15% of the total N deposition is lost from the system through seepage water. However, Härdtle and others (2007) found that leaching rates were strongly controlled by internal turnover processes such as mineralization rates within the Lüneburger Heide. An important role in immobilizing atmospherically deposited N may be played by the soil microbial biomass. In a meta-analysis of a wide range of field studies on the effects of N fertilization on microbial biomass Treseder (2008) found an overall decline of microbial biomass under N fertilization. However, Power and others (2006) measured higher microbial biomass and activity in former N-treated plots, 6 years after experimental N additions ceased in a British lowland heath. Nielsen and others (2009) showed that N and P additions had little direct effect on microbial biomass in the soil below a *Calluna* canopy. However, microbial N and P pools were much higher than the pool of inorganic N and P, and the authors concluded that microbes may play an important role in regulating plant nutrient supply. Altogether, the growth response patterns of *Calluna* still indicate N limitation, although the accumulation of N in the humus layers and the N leaching patterns may indicate that the Lüneburger Heide is currently approaching an early stage of N saturation.

In the first year of the field experiment, shoot-length response to fertilization showed a different

pattern than in the following years (Figure 1). Shoot length was negatively affected when only N was added and was positively affected when N and P were applied simultaneously. However, the first year results are not unambiguous, because fertilizer applications started later than in the following years (in July, that is, in the middle of the growing season, when *Calluna* had already achieved 75% of its whole season's growth). In a long-term N addition experiment at an upland moorland in Wales, Carroll and others (1999) found that shoot length of *Calluna* was significantly increased by regular N additions, but not until the second season of the investigation. Carroll and others (1999) attributed the lack of initial response to the late start of N additions, relative to the growing season. In both the field and the greenhouse experiment, N additions considerably increased tissue N concentrations in the first year, indicating that N was readily taken up by *Calluna* plants. Because, significant growth stimulation was observed in the greenhouse experiment in response to N (only) addition, the lack of a growth response in the field experiment is likely to be a reflection of the relatively late-season start of nutrient additions in 2004. However, we cannot explain the positive effect of the combined addition of N and P in the first year of the field experiment.

Enhanced growth associated with elevated rates of N deposition may have increased the standing plant biomass in the Lüneburger Heide during recent decades. However, *Calluna* tissue N concentrations have changed little during this time. End of season (October) foliar concentrations of 13.8 and 13.9 mg N g⁻¹ reported for the late 1970s (Matzner 1980) and mid 1980s (Mück 1998), respectively, are remarkably similar to current concentrations of 10.8–13.7 mg N g⁻¹ (Figure 2; Mohamed and others 2007). N addition experiments conducted by Power and others (1998a) and Pilkington and others (2005) demonstrate the ability of *Calluna* to assimilate N additions for increased growth rates and cover with relatively small increases in tissue N concentration. In our experiment, we observed a significant N treatment effect on tissue N with an increase of about 1–2 mg N g⁻¹ tissue dry weight when compared to the control (Figure 2). However, over long timescales, N concentrations of *Calluna* foliage do not seem to be a good indicator of the ecosystem's N status.

Addition of N and P enhanced plant-available NH₄⁺ and PO₄³⁻ concentrations in the soil in October 2005, which confirms the findings of Nielsen and others (2009). The first year's nutrient additions had no significant effect on the total N

and P concentrations in the beginning of the second year (Table 1). Phosphorus addition in the second year, however, significantly decreased total N in the O-horizon. In this P-poor substrate, P fertilization might increase mineralization rates, therefore releasing more N for plant uptake or leaching and thus reducing total N levels in this horizon. Although not statistically significant, consistent small increases in tissue N concentrations in the P treatments have been found which may be an indication for increased N uptake from the O-horizon (Figure 2).

Interpretation of the results from fertilization experiments must take into account not only the effects of the duration, but also the dose and frequency of N and P additions, sampling dates, and meteorological conditions when evaluating observed plant responses (Aerts and Chapin 2000; Davidson and Howarth 2007). In our field experiment, N additions were twice the annual deposition rates, and P was applied in relatively high doses to take account of high immobilization rates for P in heathland soils (van Meeteren and others 2007). As shoot nutrient contents change during the course of the year, it is important that comparative studies use data from samples collected at the same time of year. The climatic conditions may be the main factor explaining the differences of shoot length in control plots between the study years (Figure 1). In June and July 2006 as well as in May 2008, there were exceptionally dry periods (precipitation was approximately 65% and approximately 80% below the long term average, respectively). Despite its xeromorphic characteristics, *Calluna* is sensitive to spring and summer drought (Gimingham 1960; Gordon and others 1999). As *Calluna* shoot extension takes place predominantly during these months (Gordon and others 1999), the relatively low rainfall during this time is likely to explain the comparatively low *Calluna* shoot lengths in these years (Figure 1).

Tissue N:P Ratio as Indicator of Nutrient Limitation

We found considerable between-year variation in the mean tissue N:P ratios in the control plots (Figure 2). According to the threshold values proposed by Koerselman and Meuleman (1996), this would suggest that the control plots moved from N limitation in the first 2 years to P limitation in 2006 and then back to N limitation in 2008. In ecosystems with highly weathered, acid soils and low P availability in the mineral soil, mineralization through organic matter decomposition is the main

P source for plants and microorganisms (Turner and others 2003). Under desiccated conditions, such as occurred during summer 2006, P mineralization is likely to have been reduced due to decreased soil microbial activity (van Meeteren and others 2007). In addition, P diffusion to the roots is reduced; both these factors result in decreased plant P uptake (Sardans and Peñuelas 2007). However, *Calluna* is adapted to low P availability, and plant P demand may be met partly by P resorption from senescing leaves in this situation (Aerts 1996).

Although a considerable range of tissue N:P ratios was found in the control plots (Figure 3), there was no relationship between foliar N:P ratios and the shoot length response of *Calluna* to nutrient addition in this study. This, together with the high temporal variability in N:P ratios found in both our study and others (Gress and others 2007) strongly implies that the N:P tool should only be used as a rough indicator for the prediction of the type of nutrient limitation in lowland heathland on a larger geographical scale with a broader interval of N:P ratios. Our results reinforce the hypothesis of Güsewell (2004) that the nature of limitation is difficult to predict from N:P ratios unless values are particularly low (<10) or particularly high (>20).

ACKNOWLEDGMENTS

We would like to thank the Verein Naturschutzpark e.V. for collaboration and for permission to conduct this study in the nature reserve. We thank Claudia Drees, Thomas Huk and Walter Seidling for statistical advice.

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