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Source and sink activity of *Holcus lanatus* in response to absolute and relative supply of nitrogen and phosphorus

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Abstract. Mineral nutrients influence photosynthesis and tissue formation; a shift from nitrogen (N)-limited to phosphorus (P)-limited growth induced by high N deposition may change plant growth in terms of physiology and morphology. This experiment showed that absolute and relative N and P supply affected net photosynthesis (source activity) and biomass formation (sink activity), and the relationship between source and sink activities of *Holcus lanatus* L. under various nutrient treatments. *H. lanatus* was grown at three N:P ratios (5, 15, 45) with two absolute supply levels of N and P. Between N:P 5 at low level and N:P 45 at high level, and between N:P 45 at low level and N:P 5 at high level, there was a nine-fold difference in N and P supply. Maximum light-saturated net photosynthesis rate (A_{max}), specific leaf area (SLA), leaf area, and shoot and root biomass were determined during and after the growth process. A_{max} was minimal at N:P 5 and increased only with increasing absolute N supply. Neither SLA nor leaf area were affected by N:P; increasing absolute P supply significantly increased leaf area. Shoot and root biomass were minimal at N:P 45 and increased dramatically with increasing absolute P supply. Plant biomass was not correlated with A_{max}. Our results highlight that *H. lanatus* growth is predominantly controlled by P supply and to a lesser extent by N, whereas net photosynthesis exerted no apparent control on growth under these sink-limited growth conditions. Our findings contribute to understanding of plant growth under sink-limited conditions.

Keywords: biomass, grassland, greenhouse experiment, *Holcus lanatus* L., low relative nutrient supply, net photosynthesis, N:P supply ratio.

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Introduction

Anthropogenic disturbance has been changing nutrient cycles across various ecosystems globally (Vitousek *et al*. 1997; Sims *et al*. 1998) leading to serious environmental issues; for example, greater primary productivity (Smith *et al*. 1999), shifts in species composition and lower diversity (Vermeer and Berendse 1983; Wassen *et al*. 2005). As nitrogen (N) and phosphorus (P) are the two elements most frequently limiting plant growth in natural vegetation (Wielgolaski *et al*. 1981; Marion *et al*. 1989; Shaver and Kummerow 1992), their increased availability due to anthropogenic activities has impacted plant communities in natural and semi-natural ecosystems (Elser *et al*. 2000; Fujita *et al*. 2014). Numerous field surveys and fertilisation experiments carried out in grasslands have revealed that N and P enrichment could drive changes in plant species diversity (Bobbink *et al*. 2003, 2010; Stevens *et al*. 2004; Ceulemans *et al*. 2013; Avolio *et al*. 2014) due to increased competition for light in response to productivity increases (Hautier *et al*. 2009) and to other factors such as low investment in sexual reproduction due to P limitation (Fujita *et al*. 2014; Wang *et al*. 2019).

The common perception is that N rather than P limits primary productivity in most terrestrial ecosystems in the temperate zone (Smith *et al*. 1999) leading to low N:P ratios in aboveground vegetation of N-limited ecosystems (Vitousek and Howarth 1991; Stevens *et al*. 2004; Fujita *et al*. 2014). However, continued enrichment with N may induce or reinforce P deficiency, which may gradually increase the N:P ratio not only in vegetation but also in litter and organic matter, especially in ecosystems with a low soil P content (Flückiger and Braun 1998). The physiological responses of plants to atmospheric N deposition have been investigated extensively (Aerts and Bobbink 1999; Güsewell 2005). However, there is little information on the specific effects of P limitation as opposed to N limitation on grassland plant growth (Keddy *et al*. 2001; Güsewell 2005; Olde Venterink 2011). Moreover, the relative effects of N and P on plant growth are complex because N and P are needed in various amounts for different organs and plant processes (Güsewell 2004). A large-scale field survey by Ceulemans *et al*. (2011) showed that the relative contribution of P enrichment to species loss through increased productivity was actually higher than that of N; however, the ecological mechanisms underlying productivity increases in response to increased N or P availability are still unclear.

Plant growth is the process of accumulating dry matter. This occurs via two main nutrient pathways: photosynthesis in leaves in which carbon (C) is assimilated, and the acquisition of mineral elements (e.g. N, P, K, Fe, Cu) mainly via roots. The maximum light-saturated photosynthesis rate (A_{max}) has traditionally been assumed to be the factor controlling the rate of plant growth (Gifford 1974; Wardlaw 1990). Foliar N and P are the main mineral nutrient elements of critical compounds in the photosynthesis process such as Rubisco (rich in N) and ATP (rich in P) and are generally found to be correlated with A_{max} (Herold 1980; Johnson 1984; Reich *et al.* 1992). Aerts and Chapin (2000) found that in most cases, A_{max} correlates more closely with foliar N than with foliar P, with A_{max} decreasing significantly when N deficiency in soil increases. The explanation for this is that N deficiency decreases chlorophyll content, whereas low P either increases or has no effect on the concentration of chlorophyll in leaf tissue (Terry and Ulrich 1973; Chrysargyris *et al*. 2016). Nonetheless, increased foliar P stimulates the A_{max}–foliar N relationship, as indicated by Reich *et al*. (2009). However, because the function of P in photosynthesis is much less clear than that of N and it is difficult to separate N and P when determining their effect on A_{max} (Reich *et al*. 2009), and clear insight into the effect of N and P availability on A_{max} is still lacking.

Although plant growth depends on photosynthesis, Poorter and van der Werf (1998) found no direct link between the capacity for C uptake per unit leaf area and growth rate or yield. Moreover, Liebig (1842) had already shown that crop yield was commonly limited by one or more mineral elements that were in short supply. Following this line of research, Körner (2015) made a distinction between A_{max}, which is referred to as a source activity, and the formation of tissue, which is referred to as a sink activity. Körner (2015) suggests the latter is a critical controller of plant growth and states that net primary production (NPP) of plants is constrained not by the photosynthesis ability in leaves (source activity) but by the tissue growth itself (sink activity). This also implies it is not the CO_2 concentration (directly related to source activity) that is the ultimate limiting resource. Supportive evidence for this view comes from Herold (1980) who concluded that artificial manipulation of sinks for carbohydrates can result in changes in photosynthetic rate, but photosynthesis must be in balance with the acquisition of the other resources and substances needed to form tissues, which are moisture, mineral nutrients and chemical transmitters such as various hormones. Multiple studies have shown that C uptake by photosynthesis continued long into winter before the peak of tissue formation in spring and stopped long after tissue formation ceased under water shortage (Körner 2003; Muller *et al*. 2011). This indicates that the function of photosynthesis for plant growth is influenced by other factors and has an impact on multiple plant functional processes. In grasslands, mineral nutrient availabilities have been identified as the main resources competed for by plants, apart from water (Veresoglou and Fitter 1984). The constraints of mineral nutrient availability on plant growth have been studied extensively but little research has considered the influence of mineral nutrients on plant growth via their influence on photosynthesis (Herold 1980).

In this paper, we tested rigorously how various physiological and morphological plant vegetative growth traits are influenced by absolute and relative supplies of N and P in a factorial greenhouse fertilisation experiment with *Holcus lanatus* L., a common fast-growing grass species in European grasslands. The growth traits were divided into two groups: (1) maximum light-saturated net photosynthesis rate (A_{max}), SLA and leaf area as three indicators of 'source activity' of C absorption; and (2) shoot and root biomass as two direct growth trait indicators of sink activity. In contrast with most experiments where absolute levels of one nutrient are varied while keeping the other one constant, we aimed to identify the effect of both absolute and relative N and P supply on plant vegetative growth (i.e. nutrient supply level versus N:P supply ratio). Moreover, by comparing different responses of A_{max} (as well as SLA and leaf area) and biomass between specific nutrient treatments, we aimed to identify the main drivers of plant growth as proposed by Körner (2003, 2015). Two hypotheses were tested: (1) that A_{max} is primarily determined by absolute and relative N supply rather than absolute and relative P supply; (2) that plant growth is restricted more by low absolute and relative N supply than by low absolute and relative P supply. Furthermore, our factorial setup allowed us to test whether the degree of conversion of C uptake by photosynthesis to plant tissue depends on sufficient nutrient supply or on a balanced N:P supply ratio.

Materials and methods

Seed collection

Seeds of *Holcus lanatus* L. were collected on 16–17 August 2014 from the Middenduin nature reserve (52°24′N, 4°35′E) located in the western Netherlands and owned by the State Forestry Service.

Seedling preparation

After germination in June 2015, seedlings were transplanted into separate pots (length × width × height: 22 × 22 × 26 cm, volume 11 L) (four plants per pot, 48 pots in total). Pots contained a mixture of quartz sand (0.1–0.7 mm; Carlo Bernasconi, Zürich, Switzerland) and dune sand (passed through a 1-mm sieve) in a ratio of 11:1. The total volume of the mixed sand was 8 L. To mimic natural conditions for plant growth, dune sand was collected from the Kennemerland National Park close to Middenduin; it has low concentrations of N and P but contains a natural microbial community. Total N and P concentrations in the water extraction of the pure and dune sand (which we treated as the plant-available N and P concentrations (Kachi and Hirose 1983)) were determined using HACH-LANGE colorimetry test cuvettes (LCK 138, LCK 349, Hach-Lange, Germany). We used the measured N and P concentrations in the sand mixture to fine-tune our nutrient recipe, following Güsewell (2005).

Plant cultivation

The nutrient treatments, which lasted from July 2015 to June 2016, were given in a greenhouse with 400 W m^{-2} light from 0900 to 1600 hours. Temperature range was 14–30°C for most of the time and from October 2015 to March 2016, it was manipulated to match Dutch winter conditions (–1°C to 14°C). Each pot was placed on a tray to prevent loss of water and nutrients. The pots were switched around regularly to randomise possible differences in light, temperature and moisture conditions in the greenhouse. A full factorial combination with six nutrient treatments was applied with three N:P supply ratios (5, low relative N supply; 15, co-limitation and 45, low relative P supply), and two nutrient supply levels at low and high. The treatments were defined by the total amounts of N and P applied per plant, as well as per unit area (cm^2) and per unit volume (cm^3) throughout the cultivation process (in mg), and were calculated as:

$$N = L \cdot \sqrt{N : P} \ \text{ and } \ P = \frac{1}{\sqrt{N : P}}, \ (\text{where } L = \sqrt{N : P})$$

in which L is the overall supply level (geometric mean of N and P supply). L was 13.4 mg for the low supply level and 40.3 mg for the high supply level, following the method developed by Güsewell (2005) in the first year of the two-year fertilisation experiment (Table 1).

The experiment was carried out with seedlings grown in separate square pots; each pot contained four seedlings planted in its corners. Nutrient solutions were applied weekly. The nutrient supply was increased gradually during the growth process (Güsewell 2005) such that by weeks 4–6, the weekly supply of nutrients was double the dose in weeks 1–3; in weeks 7–9, it was 1.5-times the dose in weeks 4–6. After week 9, when growth stopped, nutrient doses were kept at those of weeks 4–6 to ensure that plants remained alive and healthy until the day senescence started. Apart from receiving nutrient solutions, plants were watered frequently with demineralised water to prevent drying out; normally this was done once every 1–3 days, but in summer, plants were watered daily.

N was supplied as KNO_3 and $Ca(NO_3)_2$, P was provided as KH_2PO_4. Both KNO_3 and KH_2PO_4 supplied part of the potassium (K) with the rest of K supplied as KCl (Güsewell 2005). All other essential macronutrients such as calcium (Ca) and micronutrients such as iron (Fe) and copper (Cu) were applied in non-limiting concentrations and were supplied in the same amounts to all treatments weekly. Details of the nutrient treatments of N and P are in Table 1. For details of the nutrient treatments of the other elements, see Table S1 available as Supplementary material to this paper. Cl was the only element that was not supplied in fixed amounts to all treatments (see Table S1).

To prevent nutrient toxicity (Fujita *et al*. 2010), each pot was leached every six weeks with demineralised water.

Measured traits

A_{max}, specific leaf area and leaf area

After 5 months of growth, the rate of maximum light-saturated net photosynthesis gas exchange (A_{max}) was determined from 8 to 19 December 2016 between 0900 h and 1500 hours using an open portable gas exchange chamber (LI 6400 portable, Li-Cor Inc., Lincoln, NE, USA) equipped with a light source (6400–02B LED, Li-Cor). We used an internal CO_2 concentration of 400 ppm and a flow of 350 μmol s^{-1}. The measurements were carried out in full sunshine on a young, healthy, fully expanded leaf on the longest shoot per plant.

Two leaves per pot were cut off at their nodes. The freshly cut leaves were placed on a flat sheet of white paper next to a ruler and photographed immediately. Leaf area (cm^2) was measured from the photos, using ImageJ software. Leaf DW (g) was measured after drying in an oven for 48 h at 70°C. The data were used to calculate specific leaf area (SLA, m^2 kg^{-1}); i.e. the ratio of leaf area to leaf DW.

Shoot and root biomass

Biomass production of *H. lanatus* was measured for both shoot and root biomass. The shoots in each pot were severed at soil level, rinsed with demineralised water, dried for at least 48 h at 70°C, and weighed. The roots were carefully washed with demineralised water. Subsequently, roots were dried at 70°C for at least 48 h and weighed. Total biomass and shoot: root biomass ratio were calculated.

Hypotheses testing and statistical analysis

The first hypothesis (A_{max} is primarily determined by absolute and relative N supply rather than absolute and relative P supply) was tested by *t*-test: at low and high nutrient supply level, A_{max},

Table 1. Total amounts of nitrogen (N) and phosphorus (P) supplied per plant, per unit area (cm^2), and per unit volume (cm^3) during cultivation of *Holcus lanatus* L. with different treatments of N and P

N and P supplied per unit area (cm^2) and per unit volume (cm^3) sand were calculated from N and P supplied per plant, and were not as reliable as N and P supplied per plant, given the error in measuring sand volume and density

Nutrient supply level	Treatment no.	N:P supply ratio	N supplied (mg per plant)	P supplied (mg per plant)	N supplied (mg cm^{-2})	P supplied (mg cm^{-2})	N supplied (mg cm^{-3})	P supplied (mg cm^{-3})
Low	1	5	30	6	0.26	0.05	0.02	0.003
	2	15	52	3.5	0.46	0.03	0.03	0.002
	3	45	90	2	0.79	0.02	0.05	0.001
High	4	5	90	18	0.79	0.16	0.05	0.009
	5	15	156	10.5	1.38	0.09	0.08	0.005
	6	45	270	6	2.38	0.05	0.14	0.003

SLA and leaf area were compared respectively between treatment N:P 5 and N:P 15, and N:P 45 and N:P 15. In addition, the aforementioned traits were also tested (*t*-test) by means of the following comparisons:

1. treatments 5L (1) and 45H (6) (refer to Table 1 for treatment numbers): 45H had the same absolute P supply as 5L but its absolute N supply was nine-times higher; and
2. treatments 45L (3) and 5H (4) (refer to Table 1 for treatment numbers): both treatments had the same absolute N supply but 5H had a nine-fold higher absolute P supply than 45L.

In this way, we tested both the effects of low relative N supply compared with low relative P supply and the effects of absolute N and P supply on A_{max}, SLA and leaf area.

The second hypothesis (plant growth is restricted more by low absolute and relative N supply than by low absolute and relative P supply) was also tested by *t*-test: at low and high nutrient supply level, shoot biomass, root biomass, total biomass and shoot:root biomass ratio were compared respectively between treatment N:P 5 and N:P 15, and N:P 45 and N:P 15. Again, the aforementioned traits were also tested (*t*-test) by means of the following comparisons: treatments 5L (1) and 45H (6) (with the same amount of absolute P supply but a nine-fold higher absolute N supply of 45H than 5L), and between treatments 45L (3) and 5H (4) (with the same amount of absolute N supply but with 5H having a nine-fold higher absolute P supply than 45L). These comparisons tested for the effects of raised N and P supply separately under the same supply of the other nutrient.

In addition, we used the full factorial set-up to test whether the degree of conversion of C uptake by photosynthesis to plant tissue depends on sufficient nutrient supply or on a co-limited N:P supply ratio. To do so, we compared the differences in A_{max} and biomass between the co-limited supply ratio (N:P 15) with specific nutrient treatments N:P 5 (low relative N supply) and N:P 45 (low relative P supply) at both low and high nutrient supply. The relationships between A_{max}, and shoot biomass, root biomass and total biomass were analysed by Pearson correlation.

The effects of N:P supply ratio and supply level and their interactions were analysed with two-way ANOVA.

All statistical analyses were performed with SPSS 23.0 (SPSS, Chicago, USA); figures were also created in SPSS.

Results

Effect of N supply on A_{max}

N:P supply ratio and supply level separately as well as their interaction had significant effects on A_{max} (Table 2). However, neither SLA nor leaf area responded to nutrient treatments (Fig. 1*b, c*) apart from a significant increase of leaf area at increased nutrient supply (Table 2).

Table 2. ANOVA results (*P* values) for the effects of the nitrogen (N) and phosphorus (P) supply ratio and overall supply level on various plant growth traits of *Holcus lanatus* L.

Effect	Maximum light-saturated net photosynthesis rate (A_{max}, µmol CO_2 m^{-2} s^{-1})	Specific leaf area (m^2 kg^{-1})	Leaf area (cm^2)	Shoot biomass per plant (g)	Root biomass per plant (g)	Total biomass per plant (g)	Shoot:root biomass ratio
N:P supply ratio	0.008	0.533	0.514	0	0	0	0
Overall supply level	0	0.255	0.011	0	0	0	0
N:P supply ratio × overall supply level	0.021	0.32	0.467	0	0	0	0.02

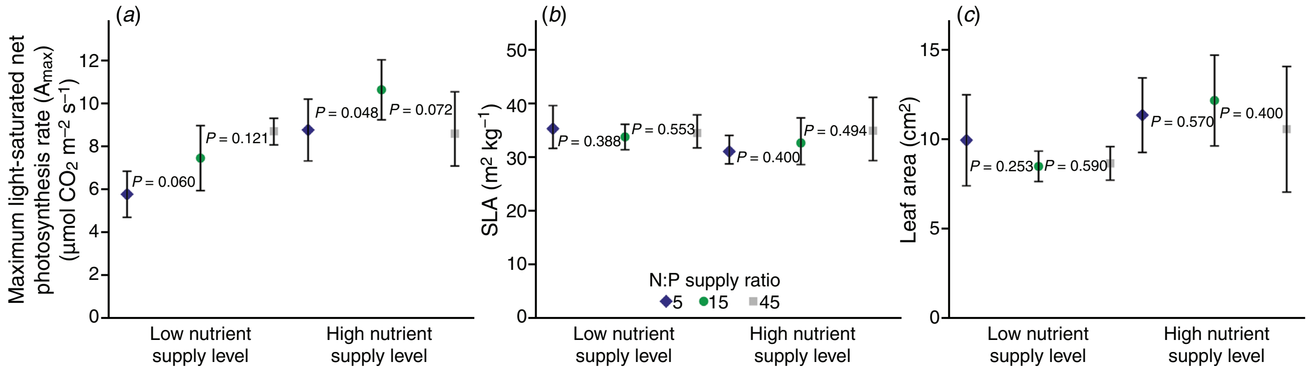

Fig. 1. (*a*) Maximum light-saturated net photosynthesis rate (A_{max}), (*b*) specific leaf area (SLA) and (*c*) leaf area of *Holcus lanatus* L. grown with different treatments of N and P. *P* values were tested by *t*-tests between treatments 45L–15L, treatments 15L–5L, treatments 45H–15H and treatments 15H–5H for each trait.

In general, A_{max} responded to changes in N supply rather than in P supply (Fig. 1*a*; Table 3). At high nutrient supply and an N:P supply ratio of 5 (low relative N supply), A_{max} was significantly lower than at N:P 15 (co-limited). However, A_{max} at N:P 45 (low relative P supply) at high nutrient supply was not significantly different from A_{max} at the co-limited supply (N:P 15). Moreover, at low supply, no significant difference was found for A_{max} among the various N:P supply ratios, although the negative influence of supply ratio 5 (low relative N supply) compared with supply ratio 15 (co-limited) was near significance (P = 0.060) (Fig. 1*a*).

Because N and P have different roles in source activity, we also looked at the influence of the absolute supply of N and of P. The results indicated that absolute N supply positively influenced A_{max} (P = 0.004) but no effect was found for absolute P supply (P = 0.920) (Table 3). P had a significantly positive effect on leaf area (P = 0.024) but no other effect of absolute N and P supply was found on leaf traits apart from the positive effect of P on leaf area (Table 3). In detail, by comparing treatments 5L–45H and treatments 45L–5H, we found that a nine-fold increase in absolute N supply (from 30 mg to 270 mg) significantly increased A_{max} with 52.9% (increase from 5.76 to 8.80 μmol CO_2 m^{-2} s^{-1}), whereas there was no significant change in A_{max} in response to a nine-fold increase of absolute P supply (Table 3). In addition, absolute P supply influenced leaf area positively. This was indicated by the differences in leaf area between treatments 45L–5H (P = 0.024; Table 3) (i.e. enhanced P supply increased leaf area). Absolute N supply had no effect on leaf area (P = 0.746). Neither absolute N supply nor absolute P supply had a significant effect on SLA (P = 0.904 and P = 0.058, respectively; Table 3).

Effect of N:P supply ratio on plant growth

N:P supply ratio and supply level had a separate and interactive significant effect on shoot biomass, root biomass, total biomass, and shoot:root biomass ratio (Table 2).

Plant biomass was restricted by low relative P supply rather than N for both the low and the high nutrient supply (Fig. 2). At low nutrient supply, shoot and root biomass were both significantly higher at N:P 5 (low relative N supply) and lower at N:P 45 (low relative P supply) than they were at the co-limited supply ratio of N:P 15 (Fig. 2*a, b*). At the high nutrient supply, root biomass showed the same pattern as at low nutrient supply, with the highest biomass at N:P 5 and the lowest at N:P 45 (Fig. 2*b*). However, shoot biomass at the high supply was highest at N:P 15 while at N:P 45, the shoot biomass was much lower than at N:P 5 (Fig. 2*a*). At both low and high nutrient supply, total biomass was lowest at low relative P supply (N:P 45) (Fig. 2*c*). The ratio of shoot:root biomass ratio increased with increasing N:P supply ratio (Fig. 2*d*).

Because N and P have different roles in plant growth, we also examined the influence of the absolute supply of each. Both absolute N and absolute P supply influenced plant biomass (Table 3). However, P supply had a much stronger influence than N supply. As was the case for A_{max}, SLA and leaf area, this was clearly apparent from the comparison between treatments 45L–5H and treatments 5L–45H. A nine-fold increase in absolute P supply (from 2 mg to 18 mg) led to a 460% increase in shoot biomass (26.51 g compared with 4.73 g on average), whereas a nine-fold increase in absolute N supply (from 30 mg to 270 mg) led to a shoot biomass increase of only 102% (18.15 g compared with 9.00 g on average) (Table 3). For root biomass, comparisons between treatments 45L–5H and treatments 5L–45H showed that a nine-fold increase in P supply led to a 1062% higher root biomass (21.91 g compared with 1.89 g on average) whereas a nine-fold increase of absolute N supply reduced root biomass from 9.10 g to 5.55 g on average (–39%) (Table 3). In terms of total plant biomass, the nine-fold increase in absolute P supply led to a total biomass of 50.20 g compared with 6.61 g on average (+659%), whereas a nine-fold increase in absolute N supply led to a total biomass of 23.78 g compared with 18.10 g on average (+31%) (Table 3). Moreover, shoot:root biomass ratio was lower at high absolute P supply than at lower P supply (1.31 vs 2.51 on average (–48%), whereas under higher absolute N supply, the ratio was 3.36 compared with 1.02 on average (+229%) (Table 3).

Relationship between A_{max} and biomass

Pearson correlation analyses revealed no significant relationship between A_{max}, and shoot biomass, root biomass nor total biomass (Fig. 3). On the other hand, A_{max} and biomass (whether shoot and root biomass separately, or

Table 3. *t*-test between treatments 45L–5H (the same N supply but different P supplies), and treatments 5L–45H (the same P supply but different N supplies) for plants of *Holcus lanatus* L.

+ indicates positive change of the growth traits; – indicates negative change of the growth traits; / indicates no significant change; d.f., degrees of freedom

Growth trait	45L–5H (nine-fold increase of P supply)				5L–45H (nine-fold increase of N supply)			
	d.f.	+/–	% increase	*P* value	d.f.	+/–	% increase	*P* value
Maximum light-saturated net photosynthesis rate (A_{max}, μmol CO_2 m^{-2} s^{-1})	23	/	/	0.92	19	+	53	0.004
Specific leaf area (m^2 kg^{-1})	30	–	12	0.058	30	/	/	0.904
Leaf area (cm^2)	30	+	29	0.024	30	/	/	0.746
Shoot biomass per plant (g)	14	+	460	0	12	+	102	0
Root biomass per plant (g)	9	+	1062	0	8	–	39	0.027
Total biomass per plant (g)	9	+	659	0	8	+	31	0.007
Shoot:root biomass ratio	9	–	48	0	8	+	230	0

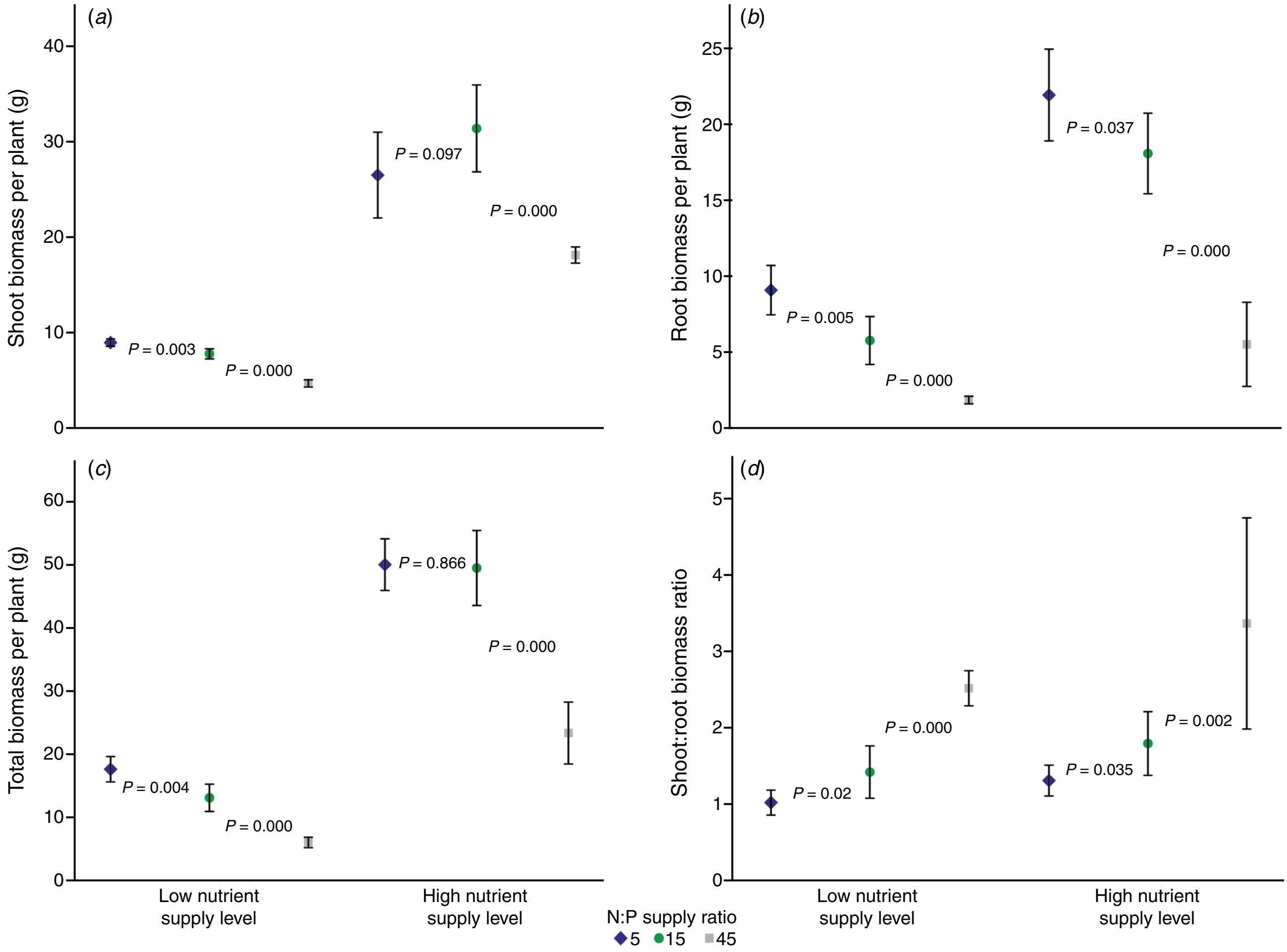

Fig. 2. (*a*) Shoot biomass per plant, (*b*) root biomass per plant, (*c*) total biomass per plant and (*d*) shoot:root biomass ratio of *Holcus lanatus* L. grown with different treatments of N and P. *P* values were tested by *t*-tests between treatments 45L–15L, treatments 15L–5L, treatments 45H–15H and treatments 15H–5H for each trait.

total biomass) were higher at high nutrient supply than at low nutrient supply (Fig. 3).

Discussion

The results indicate that with the exception of SLA, nutrient treatments significantly influenced the set of traits associated with the source function (net photosynthesis and leaf area) and the sink function (biomass formation) of *H. lanatus*.

The impact of N supply on source activity

Our data support our first hypothesis that A_{max} is primarily determined by absolute and relative N supply rather than absolute and relative P supply. In detail, low relative N supply (N:P 5) significantly reduced A_{max} compared with co-limited nutrient supply (N:P 15), especially at high nutrient supply, whereas low relative P supply (N:P 45) had no impact on A_{max}, independent of the total nutrient supply level. Moreover, the comparisons between treatments with increased N supply but constant P supply (treatments 5L vs 45H), as well as between increased P supply but constant N supply (treatments 45L vs 5H) suggest that this constraint of low relative N supply on A_{max} was mainly attributable to low absolute N supply rather than to the increase in absolute P supply. This finding is well supported by previous research that showed a positive relationship between leaf N status and photosynthetic biochemical capacity (e.g. Walker *et al.* 2014). Soil N availability to plants can also strongly influence whether the A_{max} of plants responds positively to elevated CO_2 (Reich and Hobbie 2013). Although the positive relationship between A_{max} and N supply is well established, the effect of P supply on A_{max} is less clear. In our experiment, neither relative nor absolute P supply affected A_{max} but other studies have reported a clear positive relationship between leaf P content and photosynthetic capacity across species (Güsewell 2005; Walker *et al.* 2014). Fay *et al.* (1996) found a significant linear relationship between leaf P content and A_{max} when the leaf P content was at 50–200 mg kg^{-1} but no relationship when leaf P content increased to 200–800 mg kg^{-1}. Ghannoum and Conroy (2007) found that low relative P supply inhibited the A_{max} of both C_3 and C_4 grasses but showed that there was no influence of P on the foliar content of non-structural carbohydrates that supply most of the energy for plant growth (i.e. starch, simple sugars and

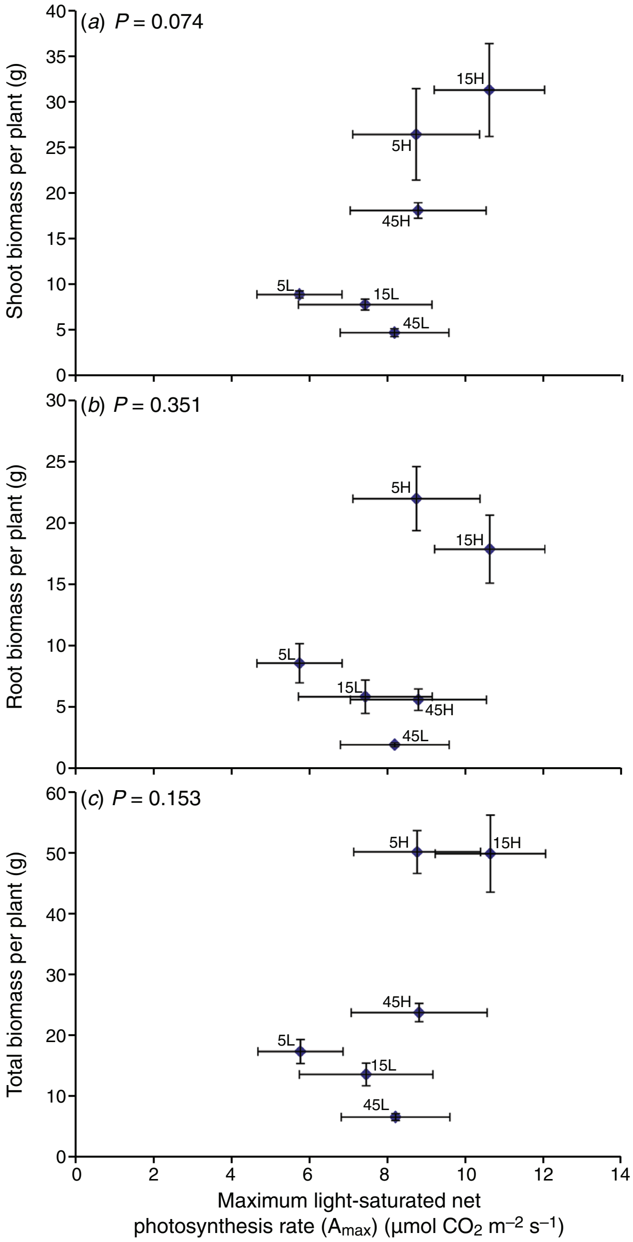

Fig. 3. Pearson correlation analyses between maximum light-saturated net photosynthesis rate (A_{max}) and (*a*) shoot biomass per plant, (*b*) root biomass per plant and (*c*) total biomass per plant of *Holcus lanatus* L. grown with different treatments of N and P.

soluble fibre). Apart from grassland species, the effect of N but not P on A_{max} has also been found in trees, with a clear influence of leaf N content on A_{max} but no correlation between P and CO_2 uptake rate (Tuohy *et al*. 1991). However, our finding that P supply did not affect A_{max} does not mean that P is not important in the photosynthesis process. It is generally known that photosynthetic reactions within the chloroplast consume orthophosphate (Pi) and are therefore likely to be inhibited by low Pi and promoted by increased Pi (Ghannoum *et al*. 2008). The obvious lack of any influence of low relative and absolute P supply on A_{max} could indicate that P is actively reallocated to chloroplast when P supply is low (Herold 1980; Güsewell 2004).

In addition to A_{max}, SLA and leaf area were analysed to provide additional information indicating source activity as these two traits are known to be closely related to A_{max} (Reich *et al*. 1992; Reich and Walters 1994; Evans and Poorter 2001; Cornelissen *et al*. 2003). However, our results revealed no effect of nutrient treatments on SLA and leaf area apart from a significant increase in leaf area with increased nutrient supply. This lack of effect of nutrient treatments on SLA and leaf area suggests that the investment in leaf morphology might not be a good indicator of the influence of nutrient treatments on source activity in fertilisation experiments.

The impact of absolute and relative N and P supply on plant growth

Our results allow us to refute the second hypothesis that plant growth is restricted more by low absolute and relative N supply than by low absolute and relative P supply. In detail, low relative P supply (N:P 45) resulted in the lowest shoot and root biomass per plant, whereas plants grown at low relative N supply (N:P 5) produced the highest biomass, although differences of shoot biomass as well as total biomass between low relative N supply (N:P 5) and co-limited supply (N:P 15) at high nutrient supply were not significant. The preference of *H. lanatus* for N limitation is consistent with the result of the field survey by Fujita *et al*. (2014) showing that the averaged N:P ratio of the aboveground vascular plants at 191 Eurasian sites with the occurrence of *H. lanatus* was 9.2 (N limitation). Besides, the remarkable constraint of low relative P supply on the growth of the grassland species in our experiment agrees with the result of the two-year fertilisation experiment on wetland species by Güsewell (2005) who showed that P limitation reduced shoot growth more than N limitation. Moreover, absolute P supply proved to have a dramatically bigger impact on plant growth (for both shoots and roots) than absolute N supply. Given that roots absorb most mineral elements for plant growth and that plants adjust to an imbalance of exogenous resources by allocating new biomass to the organs involved in acquiring the scarcest resources (Marschner 1995), we also analysed the impact of nutrient treatment on shoot:root biomass ratio in addition to the impact on plant growth. In our experiment, the effects of N:P supply ratios on shoot:root biomass ratio indicated that this ratio was significantly increased by low relative P supply but significantly decreased by low relative N supply. This increased biomass allocation to roots at low relative N supply is consistent with findings from previous studies (Andrews *et al*. 1999; De Groot *et al*. 2003). However, the effect of low relative P supply on biomass allocation to roots varies between different studies. De Groot *et al*. (2003) did not find any relationship between P supply and root biomass, whereas Hermans *et al*. (2006) showed that P and N limitation both led to increased root biomass allocation. Several studies showed that internal reallocation of P probably supports shoot growth at low relative P supply (Usuda 1995; Köhler *et al*. 2001; Shen *et al*. 2003), which may be the reason for the lowest root biomass was observed in treatment N:P 45 in our experiment.

Source–sink relationships

Although photosynthesis is the critical process providing carbohydrates (source activity) in plants, it was not related to plant growth in our experiment, as shown by the lack of correlation between A_{max} and shoot biomass, root biomass and total biomass. Furthermore, biomass responses to nutrient treatments were different from the responses of A_{max} and were sometimes even the opposite; e.g. the treatment of low relative N (N:P 5) led to lower A_{max} but higher biomass compared with treatment N:P 15, while the treatment of low relative P (N:P 45) led to higher A_{max} but lower biomass compared with treatment N:P 15 (Fig. 1*a*, Fig. 2*a*–c). These results underline that in our experiment, biomass formation (sink activity) was regulated by N and P supply rather than by net photosynthesis (source activity). This result is consistent with the statement of Herold (1980) that a plant's requirements for photosynthesis (source activity) are ultimately linked with the sink activity by transmitting the appropriate message of this requirement through the envelope of the chloroplast. Therefore, our results, combined with the findings of previous studies (Kuppers *et al*. 1988; Reich *et al.* 2018), support the view of Körner (2015) that tissue formation and cell growth are regulated largely independent of photosynthesis.

The observed responses to changes in P and N in our experiment indicate that distinct mechanisms influence plant growth, depending on whether P is limiting the sink activity. In detail, under conditions with ample P supply (6–18 mg), increases in N supply enhanced the source activity (see Fig. S1*a* available as Supplementary material to this paper) which in turn should increase the availability of carbohydrates available for growth, as indicated by the increased shoot and root biomass (see Fig. S1*g*, *i*). However, under conditions with low P supply (2–6 mg), increases in N supply can enhance source activity (see Fig. S1*a*) without stimulating sink activity (see Fig. S1*g*, *i*). Hence, this result indicates that under these conditions, P supply is limiting sink activity independent of source activity. Furthermore, our data indicate that influence of P supply on sink activity cannot readily be disentangled from N supply as shoot and root biomass formation were also influenced by increased N supply when P supply was constant (see Fig. S1*h*, *j*).

This critical effect of mineral elements, especially of P supply, on plant growth may extend to community level in natural plant communities. Our results indicate that changes in P supply, which had a large effect on *H. lanatus* shoot and root growth, may significantly influence inter-species competition via increased productivity of fast-growing species in natural plant communities (Wheeler and Shaw 1991; Aerts and Chapin 2000) and thereby influence species composition and diversity (Wassen *et al*. 2005; Roeling *et al*. 2018). Although the influence of P in natural plant communities has been observed in semi-natural grasslands (Wassen *et al.* 2005; Ceulemans *et al*. 2011; Roeling *et al*. 2018), coastal ecosystems (Adam *et al*. 1989) and forests (Beadle 1962; Huston 1980), we need more information about the specific responses in photosynthesis and growth of other species to better understand and predict community responses to N and P enrichment.

Conclusions

In conclusion, this experiment has confirmed the critical effect of P supply on plant growth and N supply on A_{max} in plants of *H. lanatus*, while growth and A_{max} were found to be independent. These distinct plant responses to differences in P and N supply may explain the lack of correlation between plant growth and A_{max}: i.e. N and P supply, rather than carbon assimilation rate, were controlling plant growth.

Conflicts of interest

The authors declare no conflicts of interest.

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