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Contrasting effects of long term versus short-term nitrogen addition on photosynthesis and respiration in the Arctic

Martine J. van de Weg · Gaius R. Shaver · Verity G. Salmon

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Abstract We examined the effects of short (<1–4 years) and long-term (22 years) nitrogen (N) and/or phosphorus (P) addition on the foliar CO_2 exchange parameters of the Arctic species *Betula nana* and *Eriophorum vaginatum* in northern Alaska. Measured variables included: the carboxylation efficiency of Rubisco (V_{cmax}), electron transport capacity (J_{max}), dark respiration (R_d), chlorophyll *a* and *b* content (Chl), and total foliar N (N). For both *B. nana* and *E. vaginatum*, foliar N increased by 20–50 % as a consequence of 1–22 years of fertilisation, respectively, and for *B. nana* foliar N increase was consistent throughout the whole canopy. However, despite this large increase in foliar N, no significant changes in V_{cmax} and J_{max} were observed. In contrast, R_d was significantly higher (>25 %) in both species after 22 years of N addition, but not in the shorter-term treatments. Surprisingly, Chl only increased in both species the first year of fertilisation (i.e. the first season of nutrients applied), but not in the longer-term treatments. These results imply that: (1) under current (low) N availability, these Arctic species either already optimize their photosynthetic capacity per leaf area, or are limited by other nutrients; (2) observed increases in Arctic NEE and GPP with increased nutrient availability are caused by structural changes like increased leaf area index, rather than increased foliar photosynthetic capacity and (3) short-term effects (1–4 years) of nutrient addition cannot always be extrapolated to a larger time scale, which emphasizes the importance of long-term ecological experiments.

Keywords Nitrogen use efficiency · Fertilisation · LTER · Alaska · Chlorophyll · Canopy · Leaf mass per area

M. J. van de Weg (✉)
Amsterdam Global Change Institute, Vrije Universiteit Amsterdam, De Boelenlaan 1085, 1081 HV Amsterdam, The Netherlands
e-mail: m.j.vande.weg@vu.nl

M. J. van de Weg · G. R. Shaver
The Ecosystem Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA, USA

V. G. Salmon
Department of Biology, University of Florida, Gainesville, FL, USA

Introduction

The productivity of Arctic tundra ecosystems is limited by cold temperatures, short growing seasons and low nutrient (nitrogen (N) and phosphorus (P)) supply (Shaver and Chapin 1980, 1986; Chapin et al. 1995). Although the N deposition rates in the Arctic are relatively low compared to industrialized and temperate regions in the northern hemisphere, N deposition has increased with the global rise in

anthropogenic N emissions the past century (Bobbink et al. 2010). Furthermore, warming of the Arctic is expected to increase N availability, as warming experiments in Alaska have shown increased available N though increased mineralization rates, or through permafrost thawing (e.g. Johnson et al. 2000; Shaver et al. 2001; Keuper et al. 2012). Additionally, studies on Arctic watersheds already have observed higher export rates of different N forms (i.e. nitrate, ammonium, dissolved organic nitrogen) most likely as a consequence of warming of tundra and increased thawing of permafrost (Frey et al. 2007; McClelland et al. 2007). Given the anticipated environmental change, N availability in the Arctic is expected to increase in the coming century.

On a plot stand scale, it has been well established that long-term N (together with P) addition in Arctic tundra increases the biomass, leaf area index (LAI), gross ecosystem production and ecosystem respiration (Shaver et al. 1998; Boelman et al. 2003; Mack et al. 2004). Furthermore, long-term N and P addition causes a shift in species composition, with an increase in shrub cover, while bryophytes and forbs are reduced (Shaver and Chapin 1991, 1995; Bret-Harte et al. 2002; Hobbie et al. 2005, Zamin and Grogan 2012). Less is known, however, about the long-term effects of N and P addition on dark respiration (R_d) and net photosynthesis (A_{net}) at the leaf level. Moreover, the effects of increased N on the more fundamental determinants of photosynthetic capacity, the maximum carboxylation velocity (V_{cmax}) of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the maximum rate of electron transport (J_{max}), remain minimally explored for tundra vegetation. Maximum A_{net}, V_{cmax}, J_{max} and R_d are tightly related to N content, mainly because of the high investment of N in the C_3 photosynthetic apparatus (Field and Mooney 1986; Evans 1989), and high involvement of N-rich proteins in maintenance respiration for protein turnover (De Vries 1975). Previous N and/or P addition experiments in Arctic tundra (lasting 1–4 years) showed a diverse array of effects on (maximum) A_{net} and R_d for different species. For example, Matthes-Sears et al. (1988) found an increase of total foliar N after 4 years of nutrient addition with N and P, but this had no consequence for A_{net} in *Betula nana* and *Salix pulchra*. This is similar to a recent finding of Heskel et al. (2012) who found no effect of 4 years of N and P addition on maximum A_{net} for *B. nana* and *Eriophorum vaginatum*, but increased levels of R_d in the Alaskan tundra. Contrastingly, Oberbauer et al. (1989) found higher rates of maximum A_{net} after one and 2 years of NPK fertilizer in *B. nana* and *Ledum palustre* (but not in *Carex bigelowii*), while Chapin and Shaver (1996) similarly found higher foliar N and maximum A_{net} values in *B. nana* and *E. vaginatum* after 4 years of N and P addition, but not in *L. palustre* and *Vaccinium vitis-ideae*.

Overall, the effects of N and/or P addition to tundra vegetation on foliar gas exchange parameters are not straightforward. Furthermore, field nutrient addition experiments that exceed 5 or 10 years of nutrient addition are rare, not only in Arctic tundra. It is questionable whether results regarding maximum A_{net} and R_d, or the proportional investment of N in the photosynthetic apparatus, can extrapolated from a relatively small number of years to a prolonged period of elevated nutrient supply (>20 years). Whether the photosynthetic nitrogen use efficiency (PNUE) changes after long periods of increased N supply is of particular interest, since in increasingly more vegetation models or up-scaling exercises the parameters V_{cmax} and J_{max}, as well as R_d, are scaled by the amount of foliar N (Friend et al. 2009; Zaehle and Dalmonech 2011). Therefore, if the PNUE or R_d–N relationships change with changes in N availability or N, this might have consequences for the accuracy of predictions of future carbon uptake. Finally, most of the nutrient addition studies mentioned above only included fully sunlit leaves, from the top of the tundra canopy. In general, foliar N on an area basis (N_{area}), as well as V_{cmax-} and J_{max} on an area basis decline with decreasing light throughout a canopy, though the pattern of the foliar traits throughout the canopy does not follow the patterns of decrease in irradiance in 1:1 proportion (e.g. Meir et al. 2002; Niinemets 2007). Whether the investment of foliar N in the photosynthetic apparatus throughout the canopy in the Arctic differs under fertilized and an unfertilized condition has received little attention.

In this study we investigated the effects of different durations and rates of N and/or P fertilizer addition on the foliar CO_2 exchange parameters of the two common Arctic tundra species *B. nana* and *E. vaginatum*. More specifically, the aims of this study were: (1) to investigate the effects of short and long term N addition on the foliar CO_2 exchange

parameters V_{cmax}, J_{max}, and R_d, as well as foliar N, the foliar chlorophyll content (Chl) and leaf mass per area (LMA); (2) to investigate whether the relative N investment in V_{cmax}, J_{max}, R_d and chlorophyll changes with different nutrient addition amount and different durations, and (3) to investigate whether the relative N investment in these CO_2 exchange parameters differs at different canopy positions in fertilized and unfertilized tundra.

Methods

Research area and species

For this study we sampled two N and P addition experiments that are located in moist acidic tundra within the Arctic Long Term Ecological Research (LTER) site in the northern foothills of the Brooks Range, Alaska (68°38′N, 49°43′W, elevation 720 m). In both experiments, nutrients are added to plots of 50 m^2 (N as NH_4NO_3 and P as P_2O_5) every spring following snow melt. Site 1 was installed in 1988, and details can be found in Bret-Harte et al. (2001). From Site 1 the control (CT), N addition, P addition and N and P addition (NP) treatments were used, with all treatments replicated in four blocks (Table 1). Site 2 was installed in 2007, approximately 150 m south of Site 1, and includes a range of quantities of N+P addition (up to 10 g N m^{-2} $year^{-1}$). Site 2 consists of 50 m^{-2} plots which are replicated in four blocks as well. From Site 2 the treatments that receive respectively 5 and 10 g N m^{-2} $year^{-1}$ were selected for this study, together with the accompanying control treatment (Table 1). Additionally, we added a very short term N+P fertilisation treatment to Site 2. For this we installed four extra 1 m^2 plots to which 10 g m^{-2} N and 5 g P m^{-2} was added on 30 May 2010 (Table 1).

The two species included in the study are the dwarf shrub *B. nana* L. and the sedge *E. vaginatum* L., which are common throughout the whole Arctic region (Britton 1966). These two species were chosen because they were both abundant enough in the control and nutrient addition plots from both experimental sites to sample for this study, as in particular many evergreen species have decreased in (relative) abundance in the NP plots of Site 1 (Gough et al. 2012).

Experimental design

Gas exchange measurements were conducted on fully sun-lit leaves that were collected from all of the research plots in Table 1 between 5 and 15 July 2010 (i.e. around the peak of the growing season). Per species, treatment, and plot, two A–C_i curves (measuring maximal photosynthesis rates at a range of intercellular CO_2) were performed, leading to eight replicates per treatment and 64 measurements in total for each species. For the *E. vaginatum*, 6–10 leaves were used and measurements took place between 8 and 15 July 2010 for site 1 and between 5 and 15 July 2010 for site 2. For *B. nana*, 3–4 mature leaves attached to the twig were used and the measurements for site 1 took place between 6 and 8 July 2010, and between 5 and 6 July 2010 for site 2. Furthermore, for *B. nana*, the respiration rate of the corresponding twig part that had been in the cuvette was measured afterwards, in order to correct the values from gas exchange measurements. In addition, for both species the gas exchange measurements were spread in a way that CT and nutrient addition samples were alternated throughout the day and per photosynthesis system (see below or description of the gas exchange measurements). Although this resulted in a not-complete random design, it avoided one of the treatments being measured in a cluster in time or per photosynthesis system.

Between 10 and 30 July in 2011, we additionally measured foliar gas exchange on *B. nana* leaves that were growing at different light regimes (i.e. the top, middle and bottom of the canopy) from a CT and NP plot at site 1 (Table 1). The light regime of the three canopy positions were characterized using three quantum sensors (LI-90 Li-Cor Inc, Lincoln, USA) that were placed at different canopy positions in both the CT and NP. Diurnal relative photosynthetic active radiation (PAR) and average PAR was calculated using the average photon flux density logged every 10 min (CR1000X, Campbell Scientific Ltd, Logan, UT, USA) throughout the month long measurement period (Fig. 1). We sampled six replicates of twigs with 3–4 leaves positioned around each of the quantum sensors (i.e. these leaves were experiencing the same light regime) for gas exchange measurements, leading to 36 samples in total.

Table 1 Overview of the nutrient addition treatments and their codes from the two different sites used in this study

Plot code	Treatment	Annual N addition (g m^{-2} $year^{-1}$)	Annual P addition (g m^{-2} $year^{-1}$)	Duration of nutrient addition
Site 1				
CT	Control	0	0	0 years
NP	N+P addition	10	5	22 years
N	N addition	10	0	22 years
P	P addition	0	5	22 years
Site 2				
CT	Control	0	0	0 years
F10	N+P addition	10	5	5 years
F05	N+P addition	5	2.5	5 years
YR1	N+P addition	10	5	6 weeks (first year)

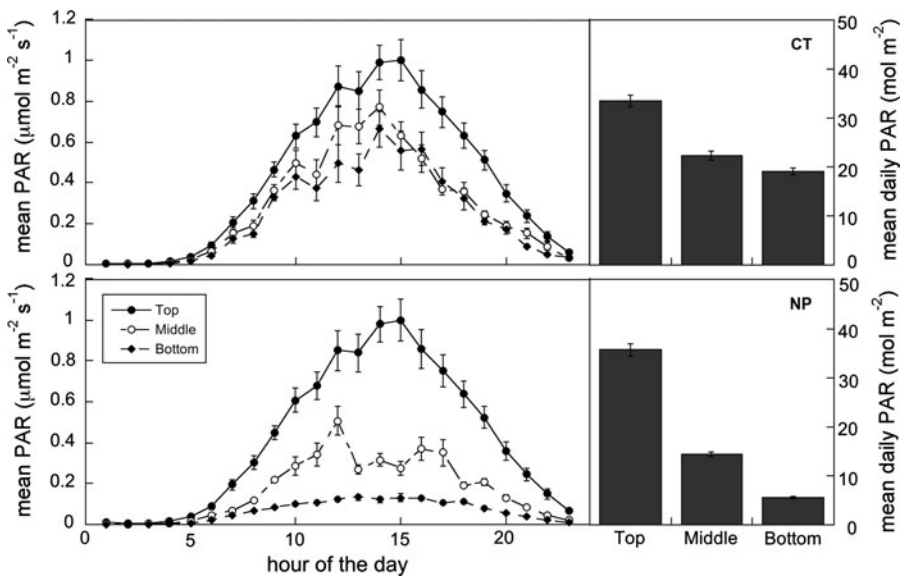

Fig. 1 Diurnal average relative received PAR at three different canopy positions in the CT and N+P plot of site 1 throughout the day from 10 to 30 July 2011 (*left panes*) (± standard error, n = 21), and the average received PAR per day (*right panes*)

Gas exchange measurements

For the measurements in 2010, three open portable photosynthesis system (Li-Cor 6400, Li-Cor Inc, Lincoln, USA), fitted with LED light sources (6400-02B Red/Blue Light Source, Li-Cor, Inc, Lincoln, USA), were used for the A–C_i curves, following the procedural guidelines in Long and Bernaccchi (2003). The CO_2 concentrations inside the chamber ranged from 50 to 2,000 ppm, and leaf temperature was set at 20 °C (average T_{leaf} was 20.1 °C ± 0.05, n = 128). Gas exchange measurements were conducted only between 10:00 and 18:00, to avoid any diurnal artefacts on leaf functioning. In the field, *B. nana* branches and *E. vaginatum* leaves were detached and immediately re-cut under water in the field, in order to reconstitute the water column. The sample was subsequently brought to the Toolik Field Station lab (at ~1 km distance) in order to start the measurements. This method, as opposed to conducting the A–C_i curves in the field on attached leaves and twigs, limited trampling of the vegetation in the long-term nutrient addition plots and it avoided taking the sensitive photosynthesis systems out in unfavourable weather. Most importantly, conducting the gas exchange measurements in the field station lab enabled us to do this at nearly the same temperatures (20 °C), since the Li-Cor 6400 can control the leaf

chamber temperature only within a limited range from ambient temperatures. Tests on non-detached and attached branches and leaves showed the shape or values of the $A–C_i$ curves stayed the same before and after detachment. Following the $A–C_i$ curves, R_d was measured, after keeping the leaf in darkness for a minimum of 20 min to avoid transient changes in CO_2 release associated with post-illumination changes in metabolism (Azcón- Bieto and Osmond 1983). Gas exchange measurements made in 2011 followed the same procedures though the Li-Cor 6400 was fitted with a lighted conifer chamber (6400-22L, Li-Cor, Inc, Lincoln, USA).

After the gas exchange measurements, leaf area was measured using a desktop scanner and Winfolia software (Regent Instruments Inc, Canada). The leaves were then dried to a constant weight at 60 °C and weighed. Subsequently, the leaves were ground and analysed individually for total C and N content with a Perkin-Elmer Series II 2400 CHNS/O Analyzer (LECO Corporation, USA). The LMA was calculated by combining the leaf area and leaf dry weight measurement and the LMA values were used to convert mass-based leaf parameters area-based ones.

$A–C_i$ response curve analysis

We used a curve fitting routine (Sharkey et al. 2007) to analyse the $A–C_i$ curves to calculate V_{cmax} and J_{max} on a leaf area basis. The curve fitting is based on minimum least-squares was used in "R" (R Development Core Team 2008). The fits were obtained using the Farquhar biochemical model of leaf photosynthesis (Farquhar et al. 1980; von Caemmerer 2000). The enzymatic kinetic constants were taken from Table 1 in Sharkey et al. (2007), while the parameters for the curve fitting to 20 °C were scaled using temperature dependencies provided by Bernacchi et al. (2001, 2002, 2003).

Chlorophyll analyses

Chlorophyll content of the *B. nana* leaves was determined using leaf level reflectance measurements, as the leaf tissue from the gas exchange measurements was not enough to measure both foliar N and Chl from. Therefore, directly after the gas exchange measurements and before drying, leaf level reflectance for each wavelength between 350 and 1,100 ($R_{350}–R_{1000}$) was measured with a field portable spectrometer (Unispec, PP Systems, Amherst MA, USA) and its accompanying bifurcated fibre optic cable and leaf clip. A calibration curve for leaf level reflectance and chlorophyll content (*a* and *b*) was made with a subset of 50 *B. nana* leaves from the research site. From these leaves, chlorophyll was extracted with *N*,*N*-dimethylformamide (DMF) and determined photospectrometrically as described in Porra et al. (1989), after they were freeze dried and stored temporarily at −80 °C. The mSR705 index ($(R_{750}–R_{445})/(R_{705}–R_{445})$) and chlorophyll content were then used to create a calibration curve ($P < 0.0001$, $R^2 = 0.67$). With this calibration curve we determined the chlorophyll content of the *B. nana* leaves from the gas exchange measurements based on leaf level reflectance. For the *E. vaginatum* samples, the leaves were not suitable for leaf level reflectance measurements (i.e. the leaf area did not fit in the leaf clip). Therefore, we collected a representative subset of sunlit leaves from each plot ($n = 4$) and their chlorophyll content was determined directly at the research station using a Tris/acetone solution for extraction agent, as described by Sims and Gamon (2002).

Statistics

Statistical analyses were performed in R (R Development Core Team, 2008). There were no interactions of the blocks with the treatments throughout the suite of measured parameters, therefore, the replicates per treatment were grouped together. To test for significant differences between the treatments and their controls, we performed ANOVA's with post hoc Dunnett's tests. This form of post hoc test compares between the control treatment and all other treatments, as these were the contrasts of interest in this study.

Results

Foliar nitrogen content

Nutrient addition effects on foliar N were not consistent through time or across species. Addition of both N and N+P increased N_{area} in the leaves of shrub *B. nana* with 42 % after 4 and ~50 % after >20 years of fertilisation ($P < 0.001$ and $P = 0.002$, respectively), while no increase was observed after the first year of

N+P addition (Fig. 2). However, for the *B. nana* that had received only 4 years of nutrient addition, only the treatment that had received the highest dosage of N+P for 4 years (F10) had a significantly higher N_{area} values ($P < 0.05$), while N_{area} was not significantly higher than the control in the treatment that received half the dosage of fertiliser (F05). Additionally, N_{mass} of *B.nana* was 20 % lower than the control in the P only treatment ($P < 0.05$) for this species. For the sedge *E. vaginatum* there was no effect of N and/or P addition when expressed on an area basis. However, N_{mass} for this species was 6 % higher ($P < 0.05$) with N only and 15 % higher with N+P after >20 years ($P < 0.01$; Fig. 2). For *E. vaginatum*, no significant influence of the short-term fertilisation on N_{area} or N_{mass} was found.

Foliar CO_2 exchange parameters

Despite the increase in N_{area} as a result of N and P addition, there was no significant increase in V_{cmax} in *B. nana*, for any length of nutrient addition (Fig. 3a, b). In contrast, *B. nana* V_{cmax} in the P-only treatment was 41 % lower than in the CT treatment ($P < 0.05$). For *E. vaginatum*, there was no significant difference observed in V_{cmax} among the different treatments in site 1 or 2. A similar pattern was found for J_{max}; for both species there was no significant influence of

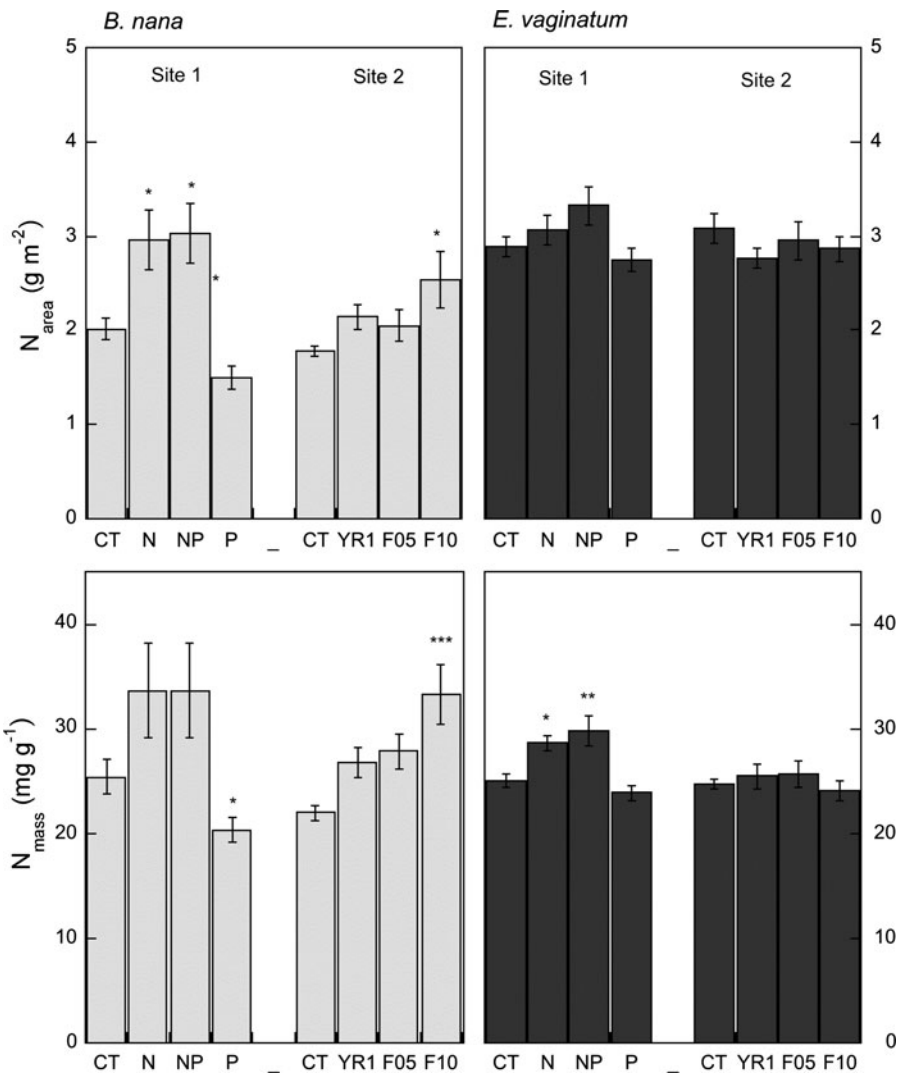

Fig. 2 Foliar N on a mass and area basis per species and per site ± standard error (n = 8). *Asterisks* indicate a significant difference of a treatment from the control for that site and species (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Abbreviations of the treatments are as in Table 1 (*CT* control, *P* phosphorus addition only, *N* nitrogen addition only, *NP* phosphorus and nitrogen addition, *YR1* first year of nutrient addition, *F10* 10 g N m^{-2} year^{-1}, *F05* 5 g N m^{-2} year^{-1})

nutrient addition on this parameter in either of the sites (Fig. 3c, d). In contrast to the photosynthetic parameters, R_d was 50 % higher for *B. nana* and 27 % for *E. vaginatum* in the N+P treatment in site 1 ($P < 0.05$), but not for the N-only treatment (Fig. 3e, f). Finally, no significant differences in R_d between treatments and the controls were found for *E. vaginatum* or *B. nana* in site 2. ($P = 0.43$ and $P = 0.27$, respectively).

Chlorophyll and LMA

The chlorophyll content on an area basis (Chl_{area}) was significantly higher for both species in the YR1 treatment compared with their controls ($P < 0.05$). In contrast, leaves from both species that had experienced nutrient addition for more than one season showed no significant increases or decreases in Chl_{area}

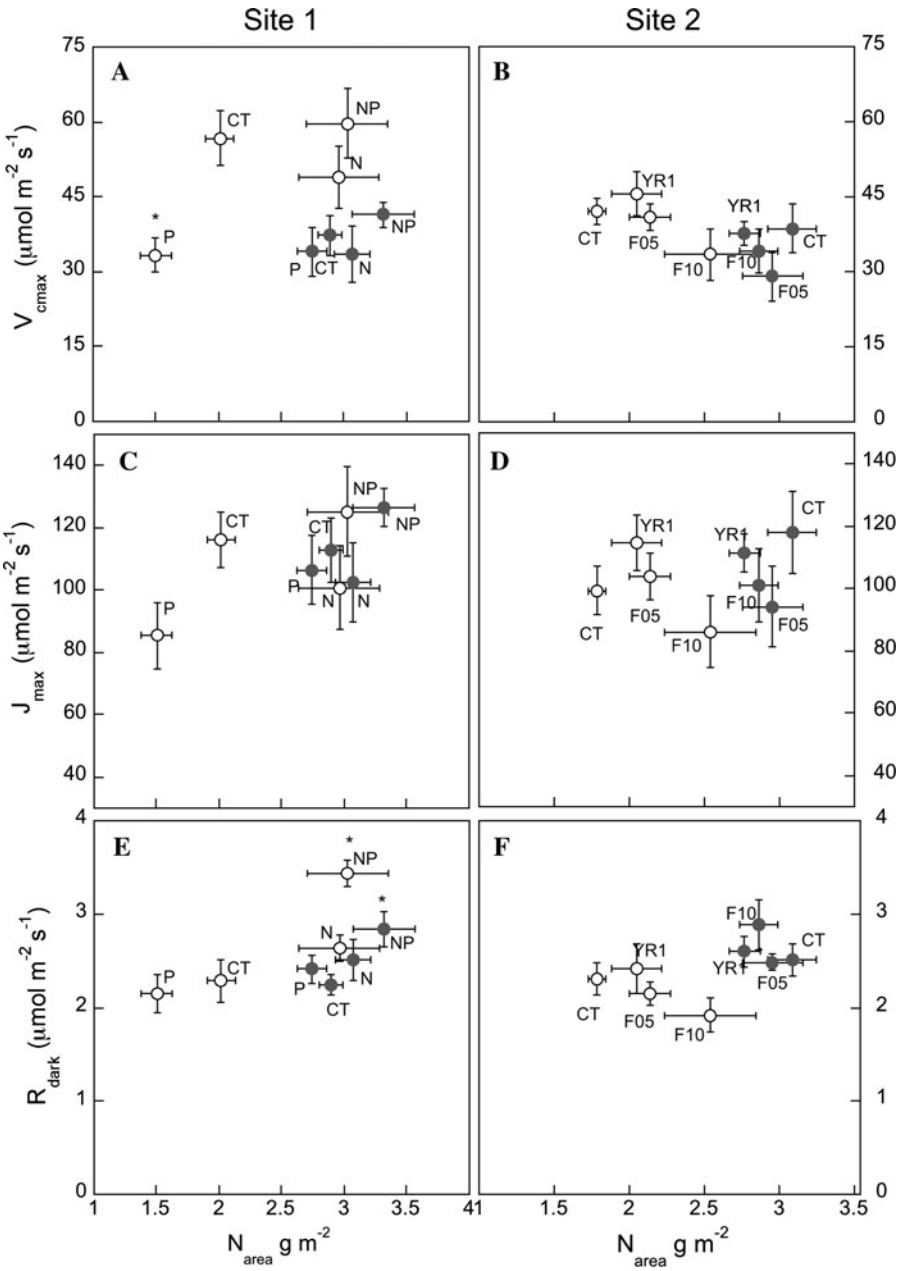

Fig. 3 Foliar CO_2 exchange parameters (V_{cmax}, J_{max} and R_d) and nitrogen content (N_{area}) after long term nutrient addition (site 1) or short term nutrient addition (site 2) for *E. vaginatum* (*closed circles*) and *B. nana* (*open circles*) ± standard error (n = 8). Abbreviations of the treatments are as in Table 1. *Asterisks* indicate that for that treatment the Y axis parameters is significantly different ($P < 0.05$, Dunnet's post hoc test) from the CT treatment of that site and species

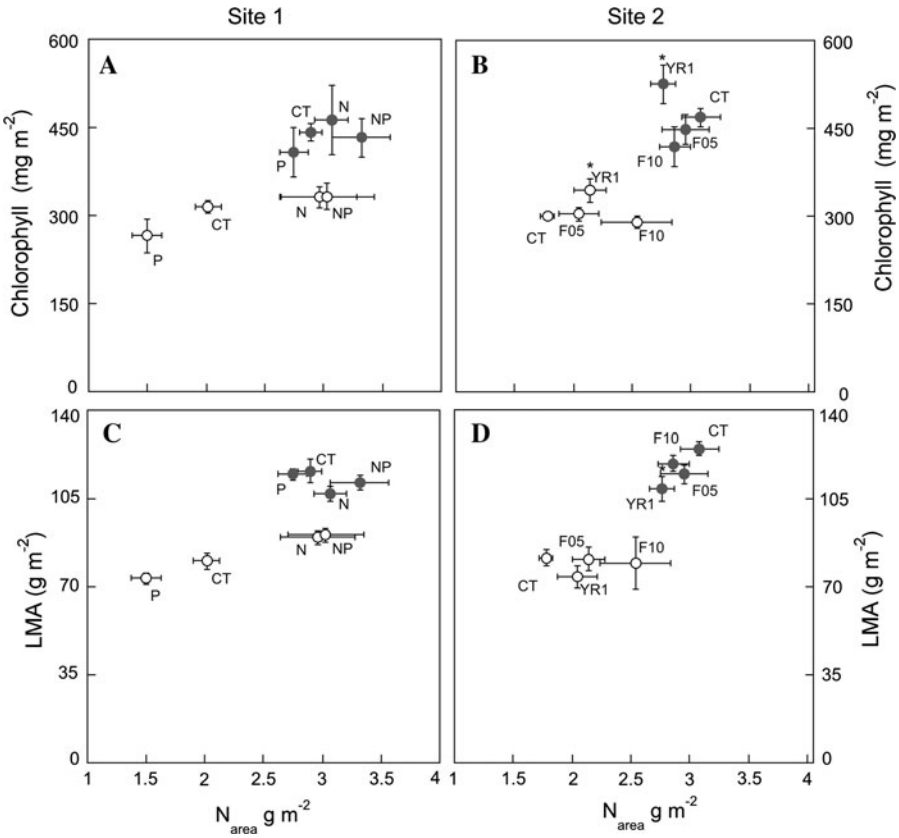

Fig. 4 Foliar chlorophyll (*a*+*b*) and leaf mass per area (LMA) and nitrogen content (N_{area}) after long term nutrient addition (site 1) or short term nutrient addition (site 2) for *E. vaginatum* (*closed circles*) and *B. nana* (*open circles*) ± standard error (n = 8). Abbreviations of the treatments are as in Table 1 and Fig. 2. *Asterisks* indicate that for that treatment the Y-axis parameters is significantly different ($P < 0.05$, Dunnet's post hoc test) from the CT treatment of that site and species

(Fig. 4a, b). Similarly, for LMA only the YR1 treatment in *E. vaginatum* was significantly lower than the control treatment ($P < 0.05$) (Fig. 4d), but no differences in LMA were found in other treatments for either of the investigated species.

Different canopy positions

Because of the lower LAI at the CT plot, PAR levels at the bottom of the canopy was higher than at the bottom of the canopy in the N+P plot (Fig. 1), and it was not possible to collect leaves in the CT treatment that had been grown at the same low light levels as in the NP treatment). Therefore, 'bottom canopy' leaves from the CT treatment cannot be compared with 'bottom canopy' leaves from the N+P treatment directly. Taking all canopy positions together, however, leaves from the N+P treatment had a higher foliar N than those from the control treatment both on a mass and area basis for the whole dataset ($P < 0.001$ and $P = 0.02$, respectively, Fig. 5a, b Student's *t* test). For the N+P treatment, the fully sunlit leaves had similar N_{mass} values to those growing at lower light levels in the canopy, but significantly higher LMA values ($P < 0.001$; Fig. 5f). In contrast, no significant differences in LMA were found in the CT treatment for the leaves from the different light levels. Chl_{area} did not differ significantly between the treatments or between canopy positions (data not shown), but when expressed on a mass basis (Chl_{mass}), the leaves from the NP treatment that received the least PAR, had higher values than the top canopy leaves ($P = 0.039$; Fig. 5e, Student's *t* test). V_{cmax} did not differ between the N+P or CT treatment for top canopy leaves, and the relationship between irradiance and V_{cmax} was similar for the CT and N+P treatment. The low-light leaves in the NP treatment had significantly lower V_{cmax} values than the top canopy leaves ($P = 0.008$; Fig. 5c).

Discussion

This study shows that leaf level photosynthetic capacity of *B. nana* and *E. vaginatum* is insensitive to increases in leaf level N in the Arctic tundra. This suggests that some Arctic species already maximize their photosynthetic

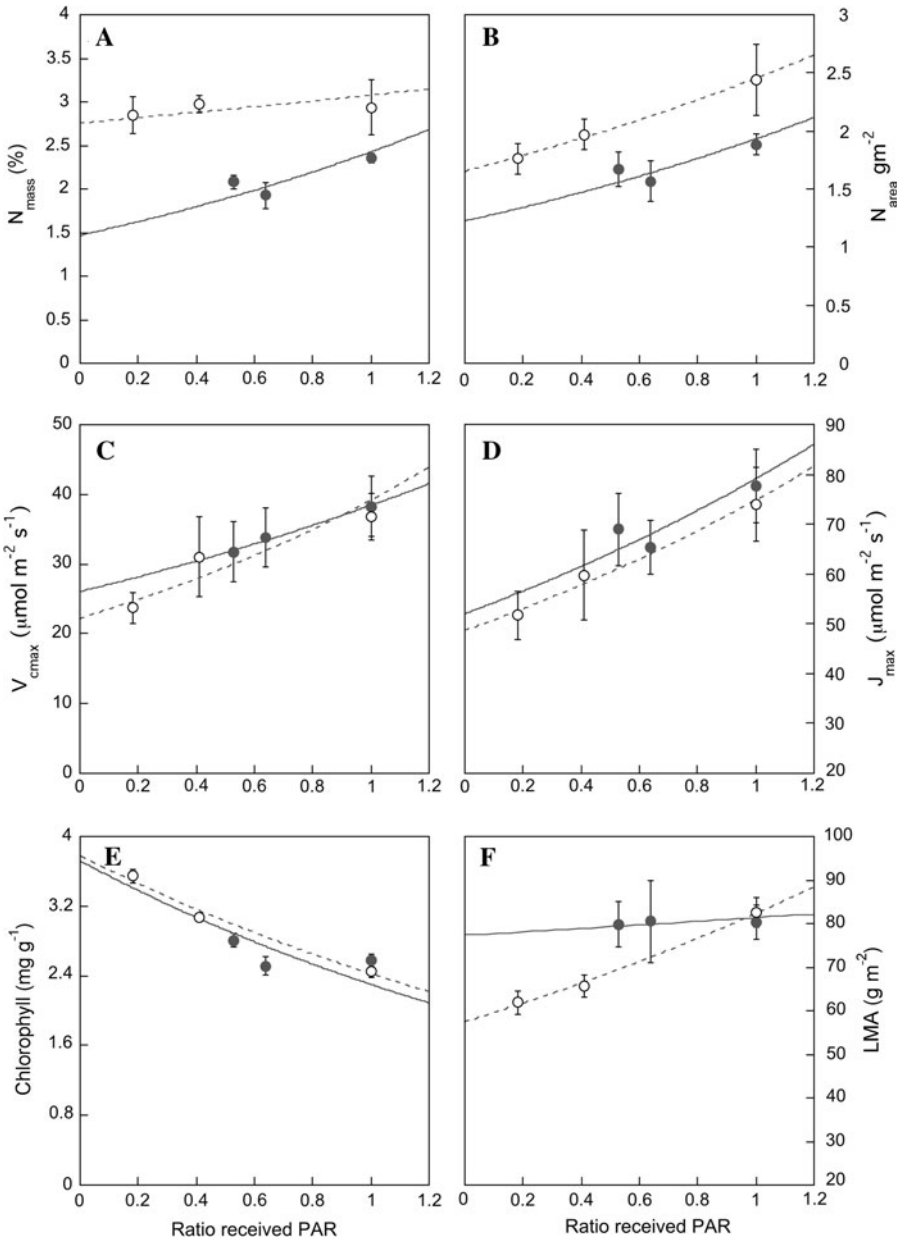

Fig. 5 Foliar N on a mass (**a**) and area basis (**b**), CO_2 exchange parameters (V_{cmax}, J_{max}) on an area basis (**c**, **d**), chlorophyll content (**e**), and leaf mass per area (LMA, **f**) for *B. nana* in the NP treatment of site 1 (>20 years of N+P addition, *open circles* and *dashed line* for trend line) and the CT treatment (*closed circles*, *solid line* as the trend line). *X axes* represent the standardized level of photosynthetic active radiation (PAR) received (1 = top canopy)

capacity per leaf area under ambient nutrient availability, or that they get limited by other nutrients when foliar N increases. Consequently, the relationship between N and V_{cmax} or J_{max} is not linear for the two common Arctic species studied here.

Long term nutrient addition and foliar N

With both a relatively short (4 years) and long duration of the N and P addition, N_{area} and N_{mass} increased in *B. nana*, and for *E. vaginatum* only on a mass basis. This is consistent with other short-term (1–4 year) Arctic nutrient addition studies that showed increases of foliar N (on either a mass or area basis) after N addition (Oberbauer et al. 1989; Chapin and Shaver 1996; Shaver et al. 2001; Heskel et al. 2012), though some did not find this trend (e.g. Van Heerwaarden et al. 2003). One difficulty with comparing nutrient addition studies is that some studies only include fully sun-lit leaves, while in others the

N_{area} and N_{mass} represent an average of leaves from the whole canopy. The LAI and the presence of *B. nana* increase substantially in moist acidic tussock tundra after several years of N (and) P addition (Shaver et al. 2001; Street et al. 2007) and consequently, the bottom leaves in the canopy receive less PAR (Fig. 1). These lower PAR levels cause the lowest leaves in the canopy to have a lower LMA (Ellsworth and Reich 1993; Evans and Poorter 2001; Niinemets 2007; and Fig. 6d this study), and can consequently decrease the bottom canopy N_{area} values, although N_{area} was not significantly lower than the top leaves in the N+P treatment in our observations (Fig. 5b). Nevertheless, if leaves of the whole N+P canopy are included in an average N_{area} value, the leaves from the bottom of the canopy (with lower N_{area} values) could skew canopy averages of N_{area} to lower average numbers because they contain proportionally less canopy leaves than the average of a CT canopy. Indeed, for Alaskan tundra shrub communities, the amount of total N in a canopy does not increase linearly, but asymptotic with increasing LAI (van Wijk et al. 2005), which would result in lower average foliar N values at high LAI. Therefore, comparing only the top canopy leaves (which will have received the same PAR regime) between two treatments can be expected to show differences in foliar nutrients more obvious then when canopy averages are compared.

Foliar N and CO_2 exchange parameters

Firstly, given the generally conservative ratio between V_{cmax} and J_{max} (Wullschleger 1993) it is not unexpected that both photosynthetic parameters show similar patterns with N_{area}. It is more surprising that for both *B. nana and E vaginatum*, no increase in V_{cmax} or J_{max} was observed in the leaves with higher foliar N values from the N addition plots (Fig. 3a–d). In contrast, when foliar N is lower than under control conditions (in the P only treatment, Fig. 2), the photosynthetic parameters V_{cmax} and J_{max} are also lower (Fig. 3a, c). In other words, the relationship with N and photosynthetic parameters holds when N is decreased from ambient conditions, but when it increases the relationship becomes non-linear until it de-couples. In addition, the patterns of V_{cmax} and J_{max} throughout the N+P and CT canopy overlap in an almost continuous pattern (Fig. 5c, d), even though the leaves in the N+P treatment that received the least radiation had high N_{area} and N_{mass} values compared with the leaves in the CT treatment (Fig. 5a, b). This shows how radiation levels are an important determinant for the patterns of photosynthetic capacity throughout a canopy (Meir et al. 2001, 2002; Niinemets 2007), since even when foliar N is high, this N is not used for the photosynthetic capacity when the average received levels of PAR are low.

Similarly, for both species no increase in Chl (Fig. 4a, b) was observed with N_{area} increase, except for the first year of when the N+P addition. This first-year increase in Chl did not coincide with an increase in V_{cmax} or J_{max}, which could be a consequence of the first year N and P addition (i.e. a transient short term effect where in the absence of previous nutrient addition extra N first is invested in Chl). Like V_{cmax} and J_{max}, the pattern of Chl throughout the canopy (Fig. 5e) was overlapping between the CT and N+P treatment, and the increased Chl in the bottom canopy N+P leaves can be attributed to lower irradiance levels, rather than higher foliar N (Evans and Poorter 2001; Niinemets 2003).

The de-coupling of foliar with photosynthetic capacity contrasts with the studies 2–3 year N-addition studies from Oberbauer et al. (1989) and Chapin and Shaver (1996) who found higher levels of maximum A_{net} after fertilisation in *B. nana*. However, both these studies did not include measurements of the photosynthetic parameters V_{cmax} an J_{max}, so there is a chance their increase in maximum A_{net} is a consequence of changes in the stomatal behaviour for example. Furthermore, de-coupling of foliar N and V_{cmax} has also been observed with long-term (9 years) NPK addition in a nutrient poor bog in Canada and after 15 years of nutrient addition to a temperate forest (Bauer et al. 2004), which make our results not unlike those from other ecosystems. The decrease in PNUE suggest that under ambient, low- nutrient conditions of the Arctic, the N investment in foliar C-uptake is already optimal on a leaf level scale, perhaps because these species (*B. nana* and *E. vaginatum*) have evolved under low N availability. Alternatively, scarcity of other nutrients such as Mg^{2+}, could be limiting the photosynthetic capacity in the leaves with high foliar N. For example, Manter et al. (2005) observed an increase in Rubisco (the enzyme involved in the first major step of carbon fixation) in fertilised *Pseudotsuga menziesii* seedlings, but similar to our findings, the activity of this Rubisco decreased with increasing

foliar N. This Rubisco inactivation was linked to a decreased relative availability of Mg^{2+}, which led to Mn-induced Rubisco deactivation. Additionally, Heskel et al. (2012) observed an increase in chloroplast area in *E. vaginatum* and *B. nana* after N+P addition. Larger chloroplasts as a consequence of high N supply has been correlated with decreased Rubisco specific activity and PNUE in other species as well (Li et al. 2013), and is explained by a decreased ratio of mesophyll conductance to Rubisco content and a lower Rubisco specific activity. It is likely that in our study the increased foliar N lead to a similar pattern of increased Rubisco content with a reduced activity, with no increase in V_{cmax} as a consequence.

It could be argued that the high dosages of N and P addition in our study (up to 10 g m^{-2} $year^{-1}$ for N) do not resemble realistic magnitudes of increased N availability due to Arctic warming. Indeed, Keuper et al. (2012), reported an increase of $\sim$240 mg N m^{-2} in the rooting zone of an Arctic bog following thawing permafrost, which is an order of magnitude lower than our largest annual N application. Nonetheless, the decoupling of foliar N and photosynthetic capacity itself is an important observation, since foliar N (or foliar N modelled after N availability) is often used as a (linear) scalar for gross or net CO_2 uptake (Thornton et al. 2007; Kattge et al. 2009; Zaehle and Friend 2010). If this decoupling happened at relatively high foliar N values like in our study, we expect this decoupling to happen also at more moderate increases of foliar N. Therefore, we think that not taking into account this N-photosynthesis de-coupling at increased N availability could lead to overestimations of the photosynthetic parameters V_{cmax} and J_{max} in CN-dynamic models.

As for Arctic stand-scale CO_2 exchange, the decoupled photosynthesis–N relationship in the two Arctic species also implies that the observed increases in gross (and net) CO_2 uptake on a ground area basis (such as measured with 1 m^2 chambers) after N and/or P addition in Arctic tundra are a consequence of increases in LAI, and not a consequence of increased photosynthetic capacity per leaf area (Boelman et al. 2003, Street et al. 2007). Indeed, 75 % of the variability in plot level CO_2 uptake amongst Pan-Arctic vegetation types could be explained by radiation levels and LAI alone, without having to consider foliar N levels (Shaver et al. 2013). In short, adding N (with P) increases ecosystem level CO_2 uptake in the Arctic tundra, which is facilitated through structural changes in the canopy (increased overall leaf area), while on a leaf level, the photosynthetic capacity remains unchanged.

Foliar respiration

In contrast to photosynthetic parameters, 50 % higher N_{area} values corresponded with 50 % higher R_d rates for the N+P treatment in *B. nana* while for *E. vaginatum* respiration was 27 % higher with a 15 % increase in N_{area} or the N+P treatment (Fig. 3e). The lack of increased respiration in the N-only treatment (compared with the N+P leaves) of site 1 could be explained by a lower P availability in this treatment, which for example in tropical forest reduces R_d (Meir et al. 2001), although we do not have foliar P data to confirm this. Increased R_d after nutrient addition has been observed in other species as well (Manter et al. 2005), and for *B. nana* this is a similar observation to Heskel et al. (2012). However, different from the latter study, we only observed a significant increase in respiration after <20 years of N and P addition, and not after a shorter duration of the experiment. Heskel et al. (2012) also observed increased numbers of mitochondrial area (density and size) in *E. vaginatum* and *B. nana* after N+P addition. Since investments in mitochondria require more N, this could partially explain why leaves with a higher N_{area} have higher R_d values. Additionally, if the excess foliar N is invested in more non-mitochondrial proteins, this could cause higher maintenance respiration due to higher protein turnover rates (De Vries 1975).

Overall, the results for V_{cmax}, J_{max} and R_d show that for the investigated species the different gas exchange parameters cannot be scaled with foliar N in a similar way. One implication of higher foliar respiration with no increase in C-uptake in the fertilised leaves is that less photosynthate is available for the metabolism in other parts of the plant and ecosystem. Measuring the effects of long and short-term nutrient addition on whole plant respiration rates (or ecosystem respiration) was beyond the scope of this study. However, long term nutrient addition in the Arctic increases the aboveground biomass more than the belowground (Mack et al. 2004; Sullivan et al. 2007; Gough et al. 2012), which can result in relatively less belowground autotrophic respiration than aboveground (on the premise that the respiration of belowground tissue

would remain the same). Furthermore, N+P fertilisation and N deposition can reduce microbial respiration, especially in the rhizosphere in temperate ecosystems, which is a caused by decreased excretion of root exudates and/or decreases in fine microbial biomass (Phillips and Fahey 2007; Janssens et al. 2010; Jia et al. 2010). It is therefore plausible that the increases in foliar respiration because of higher foliar N are accompanied by decreases in respiration of other ecosystem compartments. We did not measure the respiration rates of the other plant parts so cannot confirm this, but we suggest that future studies on the influence of nutrient supply on Arctic C-budgets and C-fluxes should include gas exchange measurements of all different ecosystem compartments.

Conclusion

Comparing two sites of different durations in N and P addition showed that the PNUE decreases in both *B. nana* and *E. vaginatum* with increased N availability, while R_d increased after long-term (>20 years) and high dosage N addition. This either shows that for these two species photosynthesis is either already highly efficient on a lea level scale, or that they become limited for other nutrients with increasing N and P availability. This should be taken into account when scaling photosynthetic parameters with foliar N data (though is probably of less importance when scaling productivity for the Arctic with only LAI). Additionally, the different results for photosynthetic parameters and foliar respiration show that both parameters cannot be scaled with nutrient concentrations in a similar way, urging for modelling both processes separately. Finally, this study showed that short-term effects (1–4 years) of nutrient addition on eco-physiological parameters cannot by default be extrapolated to a decadal time scale. This underlines the importance and value of long-term ecological experiments when we investigate the effects of environmental change on ecological processes.

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