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TELLUS

The photosynthetic response of a high-altitude spruce forest to nitrogen amendments with implications for gross primary productivity

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ABSTRACT

Characterizing the capacity of the terrestrial biome to absorb CO_2 is one of the most pressing topics in climate science. One of the key players in this arena is also one of the most poorly understood: the role of nitrogen deposition. While N deposition seems to fertilize some forests, in others it has been blamed for forest decline and tree mortality. In order to further understand the role of N deposition in forest primary productivity, an in situ N-amendment study was conducted in a relatively high N deposition region of the CO Rocky Mountains over 3 yr.

Chlorophyll fluorometry is a non-invasive technique that allows for the characterization of photosynthetic efficiency and photoinhibition in plants. Analysis of chlorophyll fluorometry data showed that in this tree line forest, moderate amounts of additional N significantly lower the photosynthetic efficiency of individual branches when compared to control branches, while significantly improving the non-photochemical dissipation of excess absorbed energy. This suggests that in high-altitude spruce forests receiving relatively high N inputs, GPP may be impaired by continued chronic additions of N. One potential cause of this is an increase in the light harvesting capacity of N amended branches without a concurrent increase in photosynthesis.

1. Introduction

With the growing recognition that humans have fundamentally altered the atmosphere and climate, there has been an upsurge of interest in the capacity of forest systems to absorb increasing amounts of anthropogenic carbon dioxide (CO_2) releases. The boreal and temperate forests of North America are considered excellent candidates for carbon sequestration due to their large investments in woody tissue, which contain high amounts of carbon, and their long-lived coniferous population (Luyssaert et al., 2008). In forests, carbon sequestration depends not only on photosynthetic performance, but also on respiration, decomposition, tissue allocation and other processes (e.g. harvesting). Gross primary production (GPP) is a measure of the initial carbon uptake through photosynthesis, but GPP only captures a portion of the carbon sequestration potential of a forest. Net ecosystem production (NEP) accounts for the loss of absorbed carbon through respiratory processes and decomposition, but again only captures part of forest carbon sequestration. A better measure of the carbon sequestration taking place in a forest is net biome production (NBP), which accounts for the reductions in GPP caused by many processes and reflects the sum of these activities over long-term time scales (Luyssaert et al., 2007). The NBP of a forest may be positive, indicating that a forest is acting as a carbon sink, or negative, showing that the forest instead operates as a carbon source (Steffen et al., 1998).

Still, carbon sequestration initially depends upon GPP, the capacity of plants to photosynthesize, and nitrogen (N) plays a substantial role in this process (Evans, 1989). In the natural environment, biologically available N is often in short supply and its abundance is dependent upon the N-fixing capacity of microorganisms. As such, N has historically been viewed as one of the primary limiting factors in a plant's ability to photosynthesize (Xia and Wan, 2008). In recent years, however, anthropogenic sources of biologically available N have met or surpassed the level naturally provided by microorganisms (Vitousek et al., 1997). This may be seen as a boon to GPP in some areas, as additional N inputs remove one of the most common nutritional constraints on photosynthesis. On the other hand, in traditionally N-limited environments, an abundance of N may have the opposite effect, leading to a decline in photosynthesis. The response of a forest to N inputs depends on both the

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nutrient state of the forest and the amount of N that is being added (Hyvonen et al., 2008).

It has been suggested that N may enhance the capacity of plant tissue to absorb light (Millard et al., 2007; Posch et al., 2008). Under non-light-stressed conditions, N can encourage photosynthesis by increasing the amount of photons absorbed by the tissue (Grassi et al., 2001). Under conditions of high-light stress, this extra absorption capacity can lead to damage to PSII by increasing the amount of energy in excess of what can be utilized in photosynthesis. That N amendments can lead to an increased capacity to absorb light has been shown or implicated in other conifers including Scots pine (Wang and Kellomaki, 1997), Monterey pine (Posch et al., 2008), Norway spruce (Grassi et al., 2001) and Engelmann spruce (McKinnon and Mitchell, 2003). Additionally, N may drive an increase in the leaf area index (LAI) of forests (Hyvonen et al., 2007), allowing for more light absorption through an increase in light harvesting tissue.

This hypothesis is also borne out by studies analysing the allocation of N to ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) and chlorophyll within the leaf. The synthesis of chlorophyll, the light-harvesting pigment within plant tissues, is heavily dependent upon the availability of N, and increases in available N are often accompanied by increases in the concentration of chlorophyll within leaves and needles (McKinnon and Mitchell, 2003; Makoto and Koike, 2007; Posch et al., 2008). Studies analysing the concentration of Rubisco in plant leaves under differing N regimes have generally concluded that increasing N availability leads to greater levels of the compound within the leaves (Makoto and Koike, 2007; Millard et al., 2007; Sievering et al., 2007; Posch et al., 2008).

Recent meta-analytical and modelling studies suggest that, globally, N deposition is fertilizing the terrestrial biome, with forests worldwide sequestering more carbon due to increasing N availability (e.g. Magnani et al., 2007; Xia and Wan, 2008). However, carbon–N dynamics are complex and this pattern is not universal. Long-term N amendment studies in Eastern forests where N deposition is greater have suggested that forest health, and thus photosynthetic efficiency, have declined as these areas have become N saturated (e.g. Magill et al., 2004). Estimates of total N deposition vary greatly by location. A recent estimate for U.S. deposition found that, while combined wet and dry deposition of NO_3^- and NH_4^+ averaged $\sim$0.5 gN m^{-2} yr^{-1}, some regions exceeded 1.6 gN m^{-2} yr^{-1} (Holland et al., 2005).

Globally, it seems that N amendments do contribute to increases in forest biomass. In a meta-analysis of over 300 studies, N additions increased the biomass production of broadleaved trees 70–75% and 35–40% in conifers (Xia and Wan, 2008). In another analysis of temperate and boreal forests in Europe and North America, NEP was positively and very strongly ($R^2 = 0.97$) correlated with the amount of N available through wet deposition, suggesting that anthropogenic N is increasing the capacity of forests worldwide to operate as carbon sinks (Magnani et al., 2007). Overall, NEP may be a poor indicator of carbon sequestration, as it does not capture changes in carbon storage or release due to non-CO_2 and non-respiratory CO_2 losses (Luyssaert et al., 2007). Additionally, the magnitude of an increase in carbon sequestration due to N deposition reported by Magnani et al. (2007) has been questioned (e.g. Sutton et al., 2008). Although these results have been controversial, most authors tend to agree that N deposition does play an important role in carbon sequestration in North America and Europe (e.g. Solberg et al., 2004; De Schrijver et al., 2008; de Vries et al., 2008; Hyvonen et al., 2008; Sutton et al., 2008). Yet, it is important to note that most studies do not capture the effects of chronic N inputs over a long time series or the possibility of other nutritional limitations (e.g. phosphorus) that forests may encounter under increased growth scenarios.

The effects of N amendments are more likely to be observed over a time span of several years than within a single growing season (Pregitzer et al., 2008). In addition to the storage or lack of storage of N within an ecosystem (e.g. in soils), long-lived plants can accumulate N within their tissues for later use. Conifers, for example, have the capacity to store excess N within proteins such as Rubisco and in amino acids such as arginine (Calanni et al., 1999; Warren et al., 2003). There have been some long-term studies analysing the responses of forested areas to varying levels of N amendments, and these have shown that N's role in carbon sequestration is complex (e.g. Magill et al., 2004; McNulty et al., 2005; Hogberg et al., 2006; Pregitzer et al., 2008). A study analysing the growth over 30 yr of a Scots pine-dominated forest in Sweden under three N regimes (ranging from 3 to 18 gN m^{-2}) plus a control plot showed that the fertilizing effects of N over time do not always match those observed initially. All three N regimes drove rates of biomass accumulation higher than the control, but rather than a linear increase with increasing N applied, the greatest amount of N applied actually resulted in less increase in growth than the lowest and intermediate levels (Hogberg et al., 2006). Other studies have shown that chronic amendments of N can have a negative effect on biomass production over the long term. In a 14-yr study with red spruce in Vermont, both high (3.1 gN m^{-2}) and low (1.6 gN m^{-2}) concentrations of N resulted in lower biomass production (measured as live basal area- 40% lower for high N and 18% lower for low N sites) than control sites receiving only ambient N deposition (McNulty et al., 2005). It should be noted that the amount of N applied in each of these studies was in great excess of the highest estimates for N deposition in the conterminous United States (e.g. Holland et al., 2005).

The main aim of this study is to examine the potential effects of N deposition on GPP through foliar applications of biologically available N to Engelmann spruce, *Picea engelmanni*, at a high-altitude, tree line site. This site has additional N available through atmospheric deposition and snowmelt run off. In order to achieve this aim, photosynthetic efficiency was measured via chlorophyll fluorescence, and N amendments were designed to mimic N deposition amounts that fall within levels predicted in

the near future; for example, below an estimated 50% increase in terrestrial deposition of NO_y and NH_x by 2050 (Galloway et al., 2004). While this study does not address the physiological response of plant tissue to N amendments, fluorometry provides information on the ultimate fate of absorbed energy, including both the photochemical utilization and non-photochemical dissipation of energy. If plant tissues absorb energy in excess of that which can be utilized in photosynthesis, this excess energy has the potential to damage the photosynthetic apparatus of plant tissue, leading to a reduced capacity for GPP and carbon sequestration. Ultimately, GPP depends on both photochemical and non-photochemical energy use.

2. Materials and methods

2.1 Site description

Experimental trees were located adjacent to the Soddie meteorological station of the University of Colorado's Mountain Research Station on the Niwot Ridge (40°02′52″N, 105°34′15″W) (Fig. 1). Mean elevation of the site is 3345 m on a 10° south-facing slope. This subalpine forest is located at tree line and is dominated by Engelmann spruce (hereafter, spruce), the species chosen for this experiment. Precipitation at the site is monitored by the University of Colorado Mountain Research Station and averaged 43.13 cm yr^{-1} for 2005–2007. The NO_3^- and NH_4^+ components of wet deposition are also monitored at the site and amounted to an estimated annual input of 0.4 gN m^{-2} yr^{-1} via precipitation at the site for the period between 2000 and 2006. Using a wet to dry ratio of 2:1 (Sievering, et al., 2007), the dry deposition can be estimated to be 0.2 gN m^{-2} yr^{-1}, for a wet + dry estimate of 0.6 gN m^{-2} yr^{-1}. Another source of NO_3^- and NH_4^+ rather unique to tree line sites is seasonal snowmelt. The Soddie site is usually covered in snow from October to June, with the remaining months considered the growing season. Each spring, the frozen precipitation that has collected over the winter begins to melt, supplying the site with a seasonal plug of N due to runoff over the early portion of the growing season. This additional input of NO_3^- and NH_4^+ averaged 0.2 gN m^{-2} yr^{-1} between 2006 and 2007 (Williams et al., 2009), for a total (wet + dry + snowmelt) annual new N loading of 0.8 gN m^{-2} yr^{-1} at the site.

2.2 Chlorophyll fluorescence measurements and calculations

Chlorophyll fluorescence was measured and recorded with a PAM-2100 portable fluorometer (Heinz Walz GmbH, Effeltrich, Germany) fitted with the manufacturer's leaf-clip holder (2030-B). The leaf-clip holder maintained a 60° angle and consistent distance between any given sample and the fibre optic sensor. Before any treatments were applied, fluorometry measurements were taken and compared across all experimental branches. All branches began with equivalent values for the F_v/F_m and F'_v/F'_m parameters ($p < 0.03$) (Gurung, 2009). Measurements of both light- and dark-adapted samples were conducted in the first 2 h of direct sunlight in the area (09:00–11:00 h) twice a week during the period of June–August. Samples were considered light-adapted when they had been exposed to full sunlight (wavelengths in the range of 400–700 nm > 1000 μmol m^{-2} s^{-1}) as measured at the shoot with a handheld light meter (Field Scout Quantum Light Meter, Spectrum Technologies, Plainfield, Illinois) for a period greater than 15 min. Measurements were then conducted in saturation pulse mode and the parameters F'_m

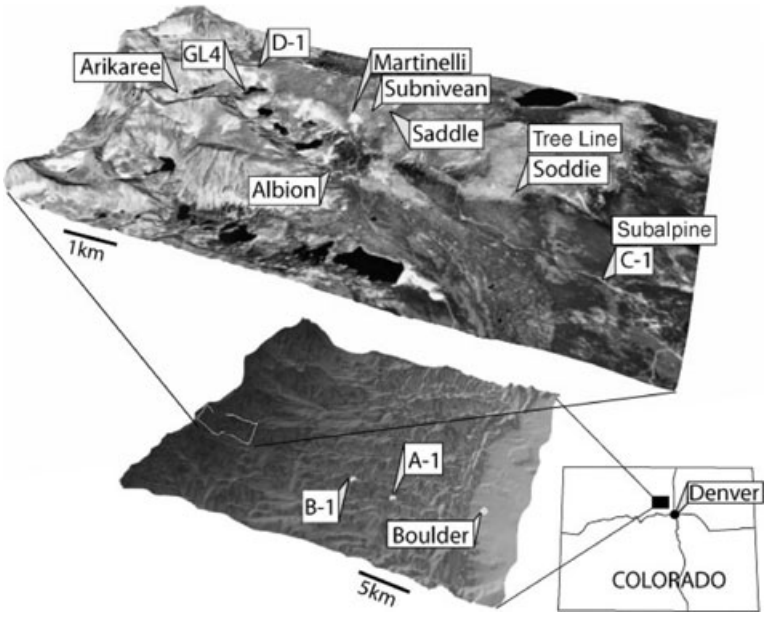

Fig. 1. The Niwot Ridge LTER study sites. The current study was based at Soddie (Tree line) and is compared to C-1 (Subalpine).

and F' were recorded. In this mode, the actinic (650 nm) light was switched on and F' was recorded. Next, a saturating pulse of light (5000 μmol m^{-2} s^{-1}, 710 nm) was applied for 0.8 s and F'_m was determined. Prior to the dark-adapted measurements, samples were covered in opaque cloths for 30 min to fully oxidize the PSII reaction centres (Ritchie, 2006), a common practice in field fluorometry (Maxwell and Johnson, 2000). A comparison conducted at the site before the experiment began showed that dark-adapted measurements taken via this method do not significantly differ from those recorded before dawn ($p < 0.05$) (Gurung, 2009). For all dark-adapted measurements, darkness was maintained throughout the measurements. In this mode, F_m was recorded, following a saturating pulse. F'_o was determined from the measurements of F_o, F_m and F'_m using the following formula:

$$F'_o = \frac{F_o}{(F_v/F_m) + (F_o/F'_m)} \tag{1}$$

(Oxborough and Baker, 1997). Calculating F'_o by this method avoids inaccuracies that may result from the effects of far-red illumination on PSI and rapid changes in the thylakoid proton gradient (Oxborough and Baker, 1997; Baker, 2008; Stefanov and Terashima, 2008). From these basic measurements, all other fluorometry parameters were determined as follows:

$$F_v = F_m - F_o \tag{2}$$

$$F'_v = F'_m - F'_o \tag{3}$$

$$F_v/F_m = \frac{F_m - F_o}{F_m} \tag{4}$$

$$F'_v/F'_m = \frac{F'_m - F'_o}{F'_m} \tag{5}$$

$$\phi_{\mathrm{PSII}} = \frac{F'_m - F'}{F'_m} \tag{6}$$

$$qP = \frac{F'_m - F'}{F'_v} \tag{7}$$

$$qN = \frac{F_v - F'_v}{F_v} \tag{8}$$

$$NPQ = \frac{F_m - F'_m}{F'_m}. \tag{9}$$

As the fluorometry literature is riddled with conflicting symbology (Maxwell and Johnson, 2000), a table summarizing the parameters and their meanings as utilized in this study is included herein (Table 1).

2.3 Nitrogen amendments and applications

Three treatment amendment solutions were applied to treated branches and are referred to as Control (C), Nitrogen One (N1) and Nitrogen Three (N3). The C and N1 treatments were applied in 2005, 2006 and 2008, while the N3 treatment was only added in the 2008 study. The solution used in the C treatment consisted of an array of ions at concentrations found in natural precipitation in the area diluted in deionized water. The N treatments (N1 and N3) included these ions as well as added N in two different concentrations. The ions used in all three treatments were Ca^{2+} (0.46 mg l^{-1}), Mg^{2+} (0.05 mg l^{-1}), Na^{+} (0.54 mg l^{-1}), K^{+} (0.07 mg l^{-1}), Cl^{-} (0.09 mg l^{-1}) and SO_4^{2-} (0.31 mg l^{-1}) (Tomaszewski and Sievering, 2007). The N source in the N treatments was NH_4NO_3 at a concentration of 10 mg l^{-1} in the N1 treatment and of 30 mg l^{-1} in the N3 treatment. This area receives about 0.4 gN m^{-2} yr^{-1} as wet deposition, with an estimated 0.2 gN m^{-2} yr^{-1} as wet deposition and 0.2 gN m^{-2} yr^{-1} through snowmelt. Using an estimate of the total N applied in each treatment (number of applications X concentration of N in solution X volume of solution applied; N1: 0.018 gN branch^{-1}, N3: 0.053 gN branch^{-1}) over the course of a single year and an estimated LAI of 2.5 spruce branches, it is possible to approximate the increase of N applied in each treatment as compared

Table 1. Chlorophyll parameters used in this study

Parameter	Light condition	Represents
F'	Light-adapted	Steady-state fluorescence
F_o	Dark-adapted	Minimal fluorescence
F'_o	Light-adapted	Minimal fluorescence
F_v	Dark-adapted	Variable fluorescence
F'_v	Light-adapted	Variable fluorescence
Φ_{PSII}	Light-adapted	Quantum yield of PSII
F_v/F_m	Dark-adapted	Maximum efficiency of PSII
F'_v/F'_m	Light-adapted	Effective quantum yield of PSII
qP	Light-adapted	Photochemical quenching of fluorescence
qN	Comparative	Non-photochemical quenching of variable fluorescence yield
NPQ	Comparative	Non-photochemical quenching of maximal fluorescence yield

to that available through deposition and snowmelt. Although the amount of N lost in throughfall during the application process was not measured, it is reasonable to estimate that the treatment concentrations increased the N load by 4–13% for the N1 treatment and by 16–33% for the N3 treatment over the N available, with the ranges bounded on the low end by wet + dry + snowmelt deposition estimates and on the high end by wet deposition estimates at the site.

Each treatment solution was applied directly to designated branches with eastward light orientation located in the upper third of the canopy. The trees at the site were approximately 3 m in height. Solutions were applied to each treatment branch by spraying from a point about 10 cm from the trunk to the apex of the branch. During the treatments, each branch was enclosed such that the spray was excluded from the surrounding branches. The treatments were applied during the growing season only, at a rate of one 250 ml application per week. For example, in 2008 treatments were administered for the period June 24–August 22, resulting in a total of seven applications (no spray was administered in the last week, and weather prevented application during another). This corresponds to an application rate of an additional 17.5 mg of N per branch in the N1 branch treatments and an additional 52.5 mg N per branch in the N3 branch treatments over the course of the 2008 growing season.

The trial followed the procedures outlined above and in previous experiments conducted by our group (e.g. Tomaszewski and Sievering, 2007). Three branches on each of three trees were selected that had similar density and eastward light orientation and marked as C, N1 or N3. In 2005 and 2006, C and N1 treatments were applied as described, and the trees and branches used in this study are the same for those two treatments. Additionally, three apical shoots of last year's growth were chosen from each branch and marked to ensure consistent fluorometry measurements throughout the season. Replicating the measurements on three trees ensured that no tree-specific effects could confound the data.

3. Data treatment and statistical analysis

For statistical analysis, raw measurements and calculations, rather than averages or transformed data, were used. For each date, three shoots were recorded from each treatment across three trees. This gave a total of nine replicated measurements for each treatment on each date, which were grouped into a single set of 782 individual sets of measurements. All statistical analysis was conducted with the Statgraphics Plus 5.0, Professional Edition software package (StatPoint Technologies Warrenton, VA). A significance level, α, of 0.05 was decided a priori and utilized in all tests.

The raw data for parameters F_o, F_m, F' and F'_m were recorded in the field and used to calculate F'_o, Φ_{PSII}, F_v/F_m, F'_v/F'_m, qP and qN as described above. After computing the calculated parameters, mathematical and instrumental constraints were applied to all parameters, and both measured and calculated variables that were outside of these constraints were removed. For fluorescence readings, the PAM 2100 has a mechanical upper limit of 2450 mV. Any measurements that exceeded this value were considered unreliable. The light-adapted measurements and calculations that depend on them were more vulnerable to these constraints, with the highest percentage (18.5%) found in the measurement of F'_m.

Z-tests for skewness and kurtosis showed that the fluorometry data were not normally distributed. Although non-parametric analytical methods are not sensitive to outliers, a small number of data points were well outside of the spread of the majority of the data. The outliers were removed on a conservative basis by visually examining the data and removing any data points outside of a threshold set at four standard deviations from the mean. By conservatively choosing an outlier threshold at four standard deviations, the natural skewness exhibited by most of the data is preserved, while anomalous measurements are excluded. The percentage of data points removed as outliers for each calculated parameter was $<2\%$.

In this study, each parameter of interest (e.g. F_v/F_m) is the response variable (Y), while the treatment factor (e.g. control, N2 or N3) is the explanatory variable (X). Homoscedasticity was tested using Levene's Test, which is considered to be robust to data that are not normally distributed as long as there is a large data set (Charway & Bailer, 2007). All of the parameters included in this study passed the Levene's test within Statgraphics, with the exception of *NPQ* (data not shown). For this reason, *NPQ* was not analysed any further than obtaining an estimate of the median value for each treatment.

For data that are non-normally distributed, the median may be a better estimator of the central tendency of the data than the mean (Norman & Streiner, 2008). Box and whisker plots for each parameter are shown in Figure 2. In these plots, the boxes represent the data contained within the upper and lower quartiles. The whiskers extending from each end represent the minimum and maximum end points within the typical range of the data. This typical range was calculated by multiplying 1.5 times the interquartile distance, which is found by subtracting the value of the lower quartile from that for the upper quartile. The points designated outside of this range are technically outliers, but they did not exceed the limits in place for outlier removal. The line drawn between the upper and lower quartile within the box designates the median. These box and whisker plots allow for a visual comparison of both the spread and central tendency of the fluorometry data without relying on distribution assumptions.

In order to assess whether or not each fluorometry parameter expressed differences between treatment groups, a distribution-free test, the Kruskal–Wallis test, was used. The Kruskal–Wallis One-Way ANOVA test is analogous to the ANOVA F-test, but utilizes the median rather than the mean by converting the

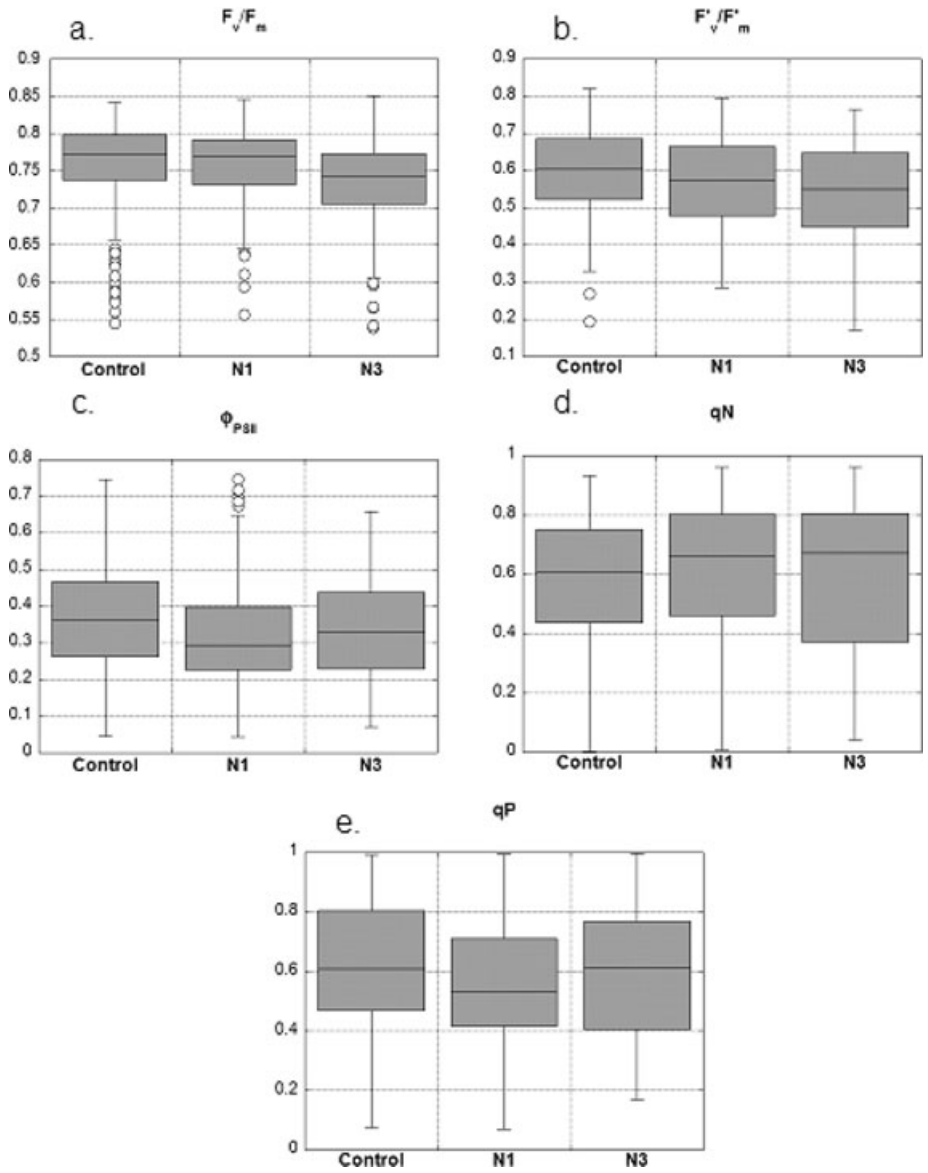

Fig. 2. (a) Values observed for the maximum efficiency of PSII. The median value of the control was higher than those of the nitrogen treatments, indicating that photosynthetic potential decreases with increasing nitrogen ($p = 0.0487$), (b) Values observed for the effective quantum yield of PSII. The median value of the control was higher than those of the nitrogen treatments, indicating that effective photosynthetic efficiency decreases with increasing nitrogen ($p < 0.0005$), (c) Values observed for the quantum yield of PSII. The median value of the control was higher than those of the nitrogen treatments, indicating that control branches were more efficient at carrying out photosynthesis than nitrogen-treated branches ($p < 0.0005$), (d) Values observed for non-photochemical quenching of variable fluorescence yield. The median values of the nitrogen treatments were higher than that of the control, indicating that non-photochemical quenching increases with additional nitrogen ($p = 0.0452$) and (e) Values observed for the photochemical quenching of fluorescence. The median value of the N3 treatment was higher than that of the control and N1. The pattern exhibited by the data is of uncertain meaning. The median values were found to be significantly different ($p = 0.0051$). In (a)–(e), the boxes represent the upper and lower quartiles, while the whiskers represent the range. The line within the box depicts the median, and open circles represent data beyond the typical range. All p-values were determined by Kruskal–Wallis analysis.

values for each data point into an ordered rank and comparing the average ranks for each group by ANOVA (Norman and Streiner, 2008). By this means, it tests the hypothesis that two or more population groups are equal. This test relies on homoscedasticity, but is independent of the normality assumption (Bradley, 1968). The Kruskal–Wallis tests were conducted in Statgraphics with the parameter (e.g. F_v/F_m) as the dependent variable and treatment as the factor. The results of the Kruskal–Wallis tests for each applicable fluorometry parameter are summarized in Table 2.

Table 2. Median values of each parameter and the results of Kruskal–Wallis statistical analysis

Parameter	Treatment	Median value	p-Value
	Control	0.7720	
F_v/F_m	N1	0.7694	0.0487
	N3	0.7420	
	Control	0.6033	
F'_v/F'_m	N1	0.5758	<0.0005
	N3	0.5510	
	Control	0.3617	
Φ_{PSII}	N1	0.2943	<0.0005
	N3	0.3318	
	Control	0.6076	
qN	N1	0.6625	0.0452
	N3	0.6725	
	Control	0.6098	
qP	N1	0.5306	0.0051
	N3	0.6128	
	Control	1.0624	
NPQ	N1	1.2424	NA[a]
	N3	1.2603	

[a]This parameter was not homoscedastic, therefore was not analysed within this study.

4. Results and discussion

The three most common parameters used to report photosynthetic efficiency are Φ_{PSII}, F_v/F_m and F'_v/F'_m, all of which were significantly ($p < 0.0005$, $p = 0.0487$ and $p < 0.0005$, respectively) affected for N treatments in this study (Table 2). Plant tissues with higher values in these three parameters are more capable of efficient photosynthesis than plant tissues with lower values. Higher values for Φ_{PSII}, F_v/F_m and F'_v/F'_m would be expected if N were to increase GPP by improving photosynthetic efficiency at this site. In this case, both the N1 and N3 treatments resulted in tissues that had values of Φ_{PSII}, F_v/F_m and F'_v/F'_m lower than tissues that were not provided with N amendments (Figs. 2a–c). This suggests that the control branches were more capable of photosynthesis than those exposed to N amendment treatments.

Experimentally, Φ_{PSII} has been linearly correlated with rates of CO_2 assimilation and as such, has frequently been used as an assessment of the photosynthetic capacity of plant tissue (Baker, 2008). In this experiment, the two N treatments seemed to depress Φ_{PSII} when compared to the level seen in the control group (Fig. 2c). Although it would be rash to conclude that the N branches were carrying out less photosynthesis than the control branches, it can be said that they were less efficient at utilizing absorbed energy for photosynthesis than the branches that did not receive N amendments. This notion is supported by other fluorometry data within this study.

The parameter F_v/F_m captures the maximum photosynthetic capacity of plant tissue based on the entire suite of reaction centres being in the oxidized state and thus prepared to receive electrons that can be used to carry out photosynthesis. The median values for F_v/F_m were 0.772, 0.769 and 0.742 for the control, N1 and N3 treatments, respectively (Table 2). Clearly, none of the values for F_v/F_m approached the commonly reported maximum value of 0.83, indicating that the trees in this region are under some sort of stress (Rohacek, 2002). This is not surprising as the harsh environmental conditions of a lower elevation site (3050 m) at Niwot Ridge have been well documented and may be responsible for a lower capacity to sequester carbon than predicted by models (Monson et al., 2002). In this study, the F_v/F_m values exhibited a pattern of C > N1 > N3. This trend of decreasing F_v/F_m with increasing N dose suggests that N may suppress the maximum photosynthetic capacity in these trees. Often, lower values of F_v/F_m sustained over a long period of time are indicative of either chronic or dynamic photoinhibition and suggest an increase in non-photochemical processes (Maxwell and Johnson, 2000). It is possible that increasing the amount of biologically available N at this site caused increased photoinhibition. Although the design of the experiment does not allow for differentiating between chronic and dynamic photoinhibition, the sum of the results and the nature of N's effect on light harvesting suggest that the N amended tissues at this site may be chronically inhibited.

F'_v/F'_m captures the actual efficiency of photosynthesis in actinic light, and is typically inversely related to qN (Adams and Demmig-Adams, 2004). In this study, values of F'_v/F'_m followed the same pattern of C > N1 > N3 as seen in the F_v/F_m values (Fig. 2b). The actual capacity for photosynthesis as measured in actinic light was lower in branches receiving N amendments than in control branches. These results also support the conclusion that N amendments negatively affected the photosynthetic capacity of the trees at this site.

The capacity for photosynthesis and, thus, GPP can be greatly influenced by photoinhibition and the non-photochemical processes by which a plant deals with excess absorbed energy. The parameter qN is a sensitive indicator of stress, and tends to vary greatly throughout a growing season as it adjusts rapidly to changing environmental conditions (Ritchie, 2006). qN can be altered by a variety of factors including changes in pH gradient across the thylakoid (ΔpH), photoinhibition of PSII reaction centres and damage to the antennae of PSII (Rohacek and Bartak, 1999). Values for the parameter qN significantly ($p = 0.0452$) varied by treatment, increasing in value with increasing N (N3 > N1 > C) (Fig. 2d). This suggests that N amendments may aid the tissues in dissipating energy in excess of that which can be utilized in photosynthesis. Much like qN, the parameter NPQ also estimates changes in heat dissipation in light-adapted tissue relative to the dark-adapted state but is calculated on the basis of changes in the maximal fluorescence between dark- and light-adapted states (Rohacek, 2002). NPQ data were

heteroscedastic in this study and therefore was excluded from analysis, but it followed the same trend exhibited by *qN* (data not shown).

Interpreting the results of the parameter *qP* based on N effects is more complicated. Although there were significant differences between treatments ($p = 0.0051$), there is no easily discernable trend. The control treatment exhibited *qP* on par with N3, but much greater than N1 (Fig. 2e). It may be that the effects of chronic N applications are not apparent during the first experimental season, and the N3 branches would follow the lower trend seen in the N1 branches if these treatments were to continue over two more seasons, but this is merely speculative.

5. Comparison between two Niwot Ridge sites

The average elevation at which tree line occurs on Niwot ridge is ∼3400 m (Komarkova and Webber, 1978). One of the lower-elevation (3000 m) subalpine forest climate monitoring sites, C-1 (hereafter subalpine), has been continually collecting climatic data since 1952 (Fig. 1). The total annual fixed N deposition at the subalpine site varies in the range of 0.4–0.8 gN m^{-2} yr^{-1} (Sievering et al., 2001) with N wet deposition being twice the N dry deposition (Sievering et al., 2007). In contrast, the Soddie (hereafter tree line) site is located at 3345 m elevation, slightly below tree line (Fig. 1). Given the prior discussion of tree line N loading, ∼0.8 gN m^{-2} yr^{-1} are made available by the sum of wet, dry and snowmelt runoff to the tree line spruce trees.

A similar study to that at tree line was conducted at the Niwot subalpine site over three growing seasons, 2004–2006, with the first season's results discussed in Tomaszewski and Sievering (2007). Engelmann spruce foliage was subjected to three treatments: (1) Background: no spray, (2) Control: non-N ion matrix spray applied to match precipitation in the area (comparable to C used in this study) and (3) Nitrogen: a spray comprised of the same ion matrix used for the control, plus 10 mgN l^{-1} as $NH_4^+NO_3^-$ (comparable to N1 in this study). Chlorophyll fluorometry measurements as well as gas exchange measurements were recorded. In shoots over 1 year old, the N treatment increased F_v'/F_m' by 11.5% ($p < 0.05$), F_v/F_m by 2.8% (not significant) and decreased *NPQ* by 11.5% (not significant) over the control and background treatments. The fluorometry data were supported by gas exchange measurements which showed that V_{max}, the maximum rate of carboxylation by Rubisco, was 14.6% greater in N treated branches ($p < 0.05$) and J_{max}, the maximum rate of light-saturated electron transport, was 11.2% greater in the N treatment (not significant). Additionally, the needles of N treated branches contained an average 8% more N than non-treated branches (Tomaszewski and Sievering, 2007), and the proportion of N allocated to Rubisco was estimates to be 5–9% higher than that of the control (Sievering et al., 2007). These data indicate that N amendments were absorbed by foliage and positively affected photosynthesis at the subalpine site, improving photosynthetic efficiency, quantum yield and the actual CO_2 incorporation rate while reducing the non-photochemical dissipation of energy as heat (Tomaszewski and Sievering, 2007). In the context of GPP, this study indicates that increasing N deposited to the subalpine forest results in increased photosynthesis and biomass production.

Table 3. Comparison between two Niwot Ridge study sites

	Percent difference[a]		
Site	F_v/F_m	F_v'/F_m'	NPQ
C1-subalpine	2.8%	11.5%	−11.5%
Soddie-tree line	−2.1%	−6.9%	15.9%

[a]C1 data from Tomaszweski & Sievering, 2007; Soddie calculated as 100% × [(N1 + N3)/2] – C / [(N1+N3 + C)/3].

Table 3 presents a subset of the subalpine forest results alongside the comparable numbers from this study. The tree line data have been reorganized such that the values for the two N treatments (N1 and N3) are combined and compared to the control treatment as a percent difference. The parameters F_v/F_m, F_v'/F_m' and *NPQ* are shown. Note that *NPQ* was not homoscedastic and therefore not analysed for significant treatment differences in the tree line study. To match the experimental design utilized at tree line, only the active treatment period data for old growth are used from the subalpine study. It is obvious that N exhibited the opposite effects on fluorometry parameters at these two sites despite their close geographic proximity. This further emphasizes that the effects of N deposition are complicated in large part by local ecosystem dynamics, and extrapolations from single study areas should be made with caution. One argument as to why N should affect fluorometric parameters so differently at each site is that the early growing season snowmelt is heavily N-laden at tree line, providing an overall larger amount of N, during the growing season, to tree line spruce versus subalpine spruce. Characterizing the availability and use of snowmelt N by trees at tree line, as well as estimating the dry deposition rates of N at the site would further illuminate this issue.

6. Conclusions

That N often has a fertilizing effect on tree growth is well documented (Evans, 1989), but less clear is whether or not this relationship can be sustained in light of chronic N amendments over the long-term. Many studies attempting to characterize the role of N deposition in GPP are only conducted over a single growing season, severely limiting their applicability to ecosystem-level changes. Using a 3 yr data set, chlorophyll fluorescence parameters indicate that increased N amendments do not stimulate further photosynthesis at this tree line site. In fact, photosynthetic efficiency actually decreases with increasing N at this site, which

implies that at tree line, GPP may decrease with increasing N deposition. Although not explored in this study, the next logical step in this analysis would be to determine what physiological mechanisms are at work in this system.

Chlorophyll parameters related to photosynthetic capacity and efficiency (Φ_{PSII}, F_v/F_m and F'_v/F'_m) were significantly ($p < 0.0005$, $p = 0.0487$ and $p < 0.0005$, respectively) depressed by N treatments, while non-photochemical dissipation processes (qN) were significantly ($p = 0.0452$) elevated when compared to controls and viewed over a 3-yr period. If these parameters accurately reflect the potential for photosynthesis at the site, then these results imply that chronic additions of N could be detrimental to GPP in similar high-altitude sites with relatively large amounts of N deposition. In contrast, a similar study by Tomaszewki and Sievering (2007) at a nearby subalpine site with less N available showed an opposite trend. This further emphasizes that the effects of N deposition are complicated in large part by local ecosystem dynamics, and extrapolations from single study areas should be made with caution.

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