Tellus (2007), 59B, 493–501
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TELLUS

Canopy uptake of atmospheric N deposition at a conifer forest: Part II- response of chlorophyll fluorescence and gas exchange parameters

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(Manuscript received 15 May 2006; in final form 11 December 2006)

ABSTRACT

Spruce foliage was sprayed with $NH_4^+NO_3^-$ and control solutions to investigate the effect of canopy nitrogen (N) uptake on chlorophyll fluorescence and gas exchange at a Rocky Mountain subalpine forest (Niwot Forest) at the Niwot Ridge Long-Term Ecological Research site. N-treated branches received $NH_4^+NO_3^-$ in an ion-matrix solution that was representative of mean precipitation ion concentrations. Branches were sprayed with $NH_4^+NO_3^-$ to increase the wet N deposition to experimental branches 60% above ambient. Control branches received only the ion-matrix solution (no N), while background branches received only natural precipitation. N content of N-treated new growth and old growth shoots was 2 and 8% greater, respectively, than the background and control shoots' N content. N-treatment enhanced photosynthetic efficiency (F_v'/F_m') of old growth spruce shoots ($\geq$1 yr old); N-treated shoots' F_v'/F_m' was 11–12% greater than control and background shoots' F_v'/F_m' ($p < 0.05$). Greater maximum carboxylation rates accompanied this increased photosynthetic efficiency with the N-treated old growth shoots' V_{cmax} 14–15% greater than background and control shoots' V_{cmax} ($p < 0.05$). New growth gas exchange and fluorescence results were similar (although $p > 0.05$). Both inorganic N assimilation and the incorporation of applied N into the photosynthetic apparatus likely account for the above results of this conifer study.

1. Introduction

The light harvesting, electron transport, and carbon metabolism processes of photosynthesis require substantial investment of N within foliar proteins (Evans, 1989). Photosynthetic parameters derived from carbon assimilation versus intercellular CO_2 concentration curves (A/C_i curves) generally display a linear dependence with leaf N. For example, Ripullone et al. (2003) found that leaf N of N-fertilized Douglas-fir trees correlated ($r^2 > 0.5$, $p < 0.001$) with the maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}). Utilizing gas exchange and chlorophyll fluorescence techniques, Grassi et al. (2001) found significant ($p < 0.05$) increases of V_{cmax}, J_{max}, and photosynthetic efficiency (defined as the ratio of variable to maximum fluorescence, F_v/F_m) for Norway spruce receiving high N versus low N treatments. These studies demonstrate the importance of N availability to photosynthesis at conifer forests. To further understand the impacts of N deposition at the Niwot Forest an N fertilizer was applied to the spruce canopy. A major goal of this study is the investigation of potential changes to photosynthetic parameters resulting from wet N deposition to the forest canopy.

Rocky Mountain subalpine forests exhibit increased foliar N levels in response to anthropogenic N deposition and soil N fertilization experiments (Calanni et al., 1999; Baron et al., 2000; Schoettle, 2000; Rueth et al., 2003). At present, in the Colorado Rockies, N-fertilization studies investigating the forest responses to increased N deposition have primarily dealt with deposition loading to the soil. However, studies indicate that forest canopies, especially conifer forest canopies, retain a substantial portion of atmospherically-deposited N before this N reaches the soil in throughfall (Lovett and Lindberg, 1993). Throughfall studies at Rocky Mountain subalpine conifer canopies exhibit similar retention of atmospherically-deposited N (Arthur and Fahey, 1993; Tomaszewski et al., 2003).

Canopy retention of atmospherically-deposited N is thought to, in part, enter the general metabolism of foliage and contribute

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DOI: 10.1111/j.1600-0889.2007.00265.x

to the foliar amino-acid pool where it may then be translocated to other plant tissues or incorporated into N requiring components of the photosynthetic apparatus such as Rubisco and chlorophyll (Kannan, 1986; Raven, 1988). Studies utilizing ^{15}N-labeling methods indicate that wet- and dry-deposited N accumulates in foliage with a portion of the ^{15}N being recovered in the foliar amino acid pool (Vose and Swank, 1990; Nussbaum et al., 1993; Boyce et al., 1996; Garten et al., 1998). Photosynthetic responses to canopy N uptake may be similar to responses observed in soil N-fertilization experiments. Yet, translocation of canopy N uptake to the roots and other plant parts may be substantial (Oren and Sheriff, 1995) and thereby lessen the extent to which canopy N uptake contributes to foliar N levels. Nevertheless, canopy N uptake may measurably alter gas exchange and chlorophyll fluorescence parameters by contributing to leaf N levels and the photosynthetic apparatus.

Increased N availability is known to impact chlorophyll fluorescence parameters by facilitating foliar acclimation to excess light (Verhoeven et al., 1997; Logan et al., 1999). Light is excessive when the rate of electrons generated (by light harvesting and electron transport) exceeds the rate that carbon is made available for reduction. Under these conditions, and assuming limited availability of reducible compounds other than carbon (for example, nitrogen and sulfur compounds), the formation of superoxides will result. In order to avoid photo-damage and function more optimally, plants utilize a mechanism of thermal energy dissipation whereby enzyme pigment complexes dissipate excess absorbed energy harmlessly as heat (Adams and Demmig-Adams, 2004).

N-limited (low %N) spruce foliage in high light exhibits elevated thermal energy dissipation and reduced photosynthetic capacity, whereas N-rich foliage in high light exhibits increased photosynthetic capacity without substantial increases in thermal energy dissipation (Grassi et al., 2001). When N is available, the typical response to excess light is to increase photosynthetic capacity, thereby utilizing light so that it is not damaging (Verhoeven et al., 1997; Cheng, 2003). When N is limited in availability, foliage experiencing excess light typically has reduced chlorophyll pools and increased xanthophyll pigment pools for thermal energy dissipation (Cheng, 2003). Thus, increased N availability, from canopy N uptake, may facilitate foliar acclimation to excess light at the Niwot Forest. Changes in the proportion of photons absorbed by photosystem II that are utilized by electron transport versus those that are dissipated thermally are readily assessed by chlorophyll fluorescence measurements. One aim of the present project was to determine whether the thermal dissipation of spruce foliage at the Niwot Forest is impacted by N-treatment.

In addition to potentially impacting photosynthesis through alteration of foliar N levels and enhancing the photosynthetic apparatus, canopy N uptake is known to directly influence foliar metabolism resulting in a variety of responses ranging from increased rates of photosynthesis (resulting from increased demand for carbon to detoxify NH_4^+) to needle necrosis (Krupa, 2003). Canopy N uptake also has the potential to uncouple photophosphorylation, disrupt needle acid/base regulation, and create foliar cation deficiencies (Skeffington and Wilson, 1988; Raven, 1988; Rennenberg and Gessler, 1999).

NO_3^- and NH_4^+ that enter the apoplast, during canopy N uptake, may ultimately be transformed (in the case of NO_3^- to NO_2^-) and transferred to the cytoplasm and chloroplast where they are assimilated into organic forms (Rennenberg and Gessler, 1999). NO_2^- enters the chloroplast where nitrite reductase reduces NO_2^- to NH_4^+. NH_4^+ is assimilated by various isoforms of glutamine synthetase in either the cytoplasm or chloroplast (Lam et al., 1996; Rennenberg and Gessler, 1999) and NH_4^+ assimilation in foliage is known to increase demand for carbon skeletons and photosynthesis (Krupa, 2003). Thus, the assimilation of NH_4^+ and the reduction of NO_3^- by foliage, especially, to the extent that assimilation and reduction occurs in the chloroplast, may mitigate the need for greater thermal dissipation under excess light conditions and/or increase the rate of electron transport and photosynthetic efficiency since, the assimilation of inorganic N consumes electrons during reduction and increases the demand for carbon skeletons. In summary, increased exposure of foliage to greater NH_4^+ and NO_3^- concentrations has the potential to alter foliar metabolism leading to several potential consequences for photosynthesis.

The potential responses of photosynthetic parameters to canopy N uptake may occur both simultaneously and differentially. It would be premature to attempt a study aimed at discerning the relative contribution of these potential mechanisms, whereby photosynthesis is altered by canopy N uptake, without first investigating whether photosynthetic parameters respond at all to canopy N uptake at the Niwot Forest. Photosynthetic responses to canopy N uptake may differ for old growth and new growth foliage. Little is known about canopy N uptake mechanisms and the ultimate sink of assimilated N. Old growth foliage may take up and assimilate N into amino compounds, yet, this new N may be translocated to new growth foliage that has rapidly developing tissues and greater N demand. For these reasons, both new growth and old growth responses to N treatment were considered. Spruce branches were sprayed with N-solutions to investigate the influence of canopy N uptake on gas exchange and chlorophyll fluorescence parameters. This study represents one of a few chlorophyll fluorescence field studies at conifer forests and, to our knowledge, the first that investigates the influence of direct canopy N uptake on chlorophyll fluorescence. The objectives of this study were: (i) to quantify the influence of N-treatment on chlorophyll fluorescence parameters with an emphasis on photosynthetic efficiency and thermal dissipation estimation and (ii) to determine the influence of N-treatment on shoot level gas exchange parameters derived from A/C_i curves.

Table 1. List of photosynthetic response variables measured at selected spruce branches in the Niwot Forest canopy

Response variable	Definition
Chlorophyll fluorescence	
$F_v/F_m = (F_m - F_o)/F_m$ (Dimensionless)	Potential photosynthetic efficiency of open PSII reaction centres (determined by the chlorophyll fluorescence of a dark adapted sample)
$F_v'/F_m' = (F_m' - F_o')/F_m'$ (Dimensionless)	Maximum observed photosynthetic efficiency of open PSII reaction centres (determined by the chlorophyll fluorescence of a light adapted sample)
$NPQ = (F_m - F_m')/F_m'$ (Dimensionless)	Non-photochemical quenching (NPQ) is an expression that represents the relative magnitude of fluorescence quenching attributable to pathways other than photochemistry (mainly thermal dissipation)
Gas exchange	
V_{cmax} (μmol m^{-2} s^{-1})	Maximum rate of carboxylation by ribulose-1,5-bisphosphate carboxylase/oxygenase
J_{max} (μmol m^{-2} s^{-1})	Light saturated maximum rate of electron transport
R_{day} (μmol m^{-2} s^{-1})	Release of CO_2 (in the light) by processes other than photorespiration

2. Materials and methods

2.1. Field site description

The Niwot Forest research area is located at the University of Colorado's Mountain Research Station (40° 01′ 58′ N, 105° 32′ 47′ W) approximately 6 km east of the Continental Divide at an elevation of 3000 m. The forest consists of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), and lodgepole pine (*Pinus contorta* Dougl. ex Loud.). Tree density is 16, 10 and 9 per 100 m^2 for spruce, fir and pine, respectively (Monson et al., 2002). Scaffolding towers facilitate access to the upper crown of the spruce trees studied in this paper.

2.2. Fluorescence

Fluorescence parameters were measured with a PAM-2100 (Heinz Walz GmbH, Effeltrich, Germany) portable chlorophyll fluorescence instrument. The instrument was fitted with the manufacturer's leaf-clip holder (2030-B). During daytime fluorescence measurements, the instrument was operated in saturation pulse mode. A brief (0.8 s) pulse of high intensity light (5000 μmol m^{-2} s^{-1} PAR, $\lambda < 710$ nm) was activated for determination of F_m' (maximum fluorescence yield of a light adapted sample). The saturation pulse closes all open photosystem II reaction centres thereby momentarily preventing fluorescence quenching attributable to photochemistry. Following the saturation pulse the sample is covered with a black cloth, to block ambient light, and exposed to a far red light (emission peak $\lambda =$ 735 nm). The far red light selectively excites photosystem I and enhances the reoxidation rate of photosystem II acceptors which facilitates accurate determination of minimal fluorescence yield of a light adapted sample (F_o'). F_o' was then determined as the lowest fluorescence yield resulting from exposure to the measuring light (0.1 μmol m^{-2} s^{-1} PAR, $\lambda = 650$ nm). Photosynthetic efficiency of a light adapted sample is then determined using the ratio of variable fluorescence (defined as F_m'–F_o', or F_v') to maximum fluorescence F_m'.

During predawn fluorescence measurements, the foliage was covered with the black cloth immediately after attaching the leaf clip and throughout measurement. Predawn determination of minimal fluorescence of a dark adapted sample (F_o) does not require the far red light and occurs before determination of maximal fluorescence of a dark adapted sample (F_m). Photosynthetic efficiency of a dark adapted sample is then determined using the ratio of variable fluorescence (defined as F_m–F_o, or F_v) to maximum fluorescence F_m. The literature contains numerous formulae that compare these light adapted (F_o', F_m') and dark adapted (F_o, F_m) values to provide information about thermal dissipation and photosynthetic efficiency (Maxwell and Johnson, 2000). In the present study, we chose three commonly used and well known formulas to describe thermal dissipation and photosynthetic efficiency (see Table 1).

2.3. Gas exchange

Gas exchange measurements were made using a portable photosynthesis system (CIRAS 2, PP Systems, Hitchin, UK) and conifer cuvette (PLC5). For A/C_i curves, the manufacturer's tungsten halogen light unit was fitted to the conifer cuvette and powered by a 30-amp battery. A photosynthetic photon flux density of 1250 μmol m^{-2} s^{-1} was sufficient to remove the light

limitation of CO_2 assimilation. Measurements were collected early in the day (0830–1130) to ensure that the prevailing high humidity kept stomata open (Parsons et al., 1997).

A/C_i curves were obtained as described in Parsons et al. (1997). Ambient CO_2 concentrations were first decreased, and then increased, so that stomatal closure at high CO_2 was minimized. Ten data points per response curve were obtained to ensure at least four points above, and also below, the inflection point. The following sequence of CO_2 levels produced a reasonable curve for determination of R_d, V_{cmax}, and J_{max} (342, 275, 150, 100, 550, 800, 1100, 1500 2000 ppm). The program Photosyn Assistant (version 1.1, Dundee Scientific, Scotland, UK) was used to estimate the parameters R_d, V_{cmax}, and J_{max} from A/C_i curves. The program utilizes the mechanistic model of photosynthesis proposed by Farquhar et al. (1980) and includes modifications by von Caemmerer and Farquhar, (1981); Sharkey, (1985); Harley and Sharkey, (1991); and Harley et al. (1992). In addition, the temperature dependence of Rubisco Kinetics parameters (K_c, K_o and τ) were modeled using the approach of Harley et al. (1992) and Wullschleger. (1993).

2.4. Foliar area determination

Projected needle area was determined photographically after harvesting foliage. Needles were spread out on a white board and digitally photographed alongside a reference object of known area. Using Image J particle analysis software from the National Institute of Health (Abramoff et al., 2004), images were analyzed for reference object and needle pixel area. Projected needle area (cm^2) was then determined from needle pixel area and the relationship between known area and pixel area of the reference object.

2.5. Percent N and N content

Needles were harvested from trees and dried to constant mass at 60°C in a convection oven. Dried samples were ground to a fine powder and analyzed for %N (mass basis) by a Carlo Erba CHN analyzer (Carlo Erba, Milan Italy). To determine N content of harvested shoots (N_a: g N m^{-2}) the %N of needles (divided by 100) was multiplied by shoot needle weight and that product was divided by shoot projected needle area.

2.6. Treatment solutions

At each tree, an N-branch received $NH_4^+NO_3^-$ (10 mg N l^{-1}) in an ion-matrix solution that was representative of mean common ion concentrations in site precipitation. A control branch (C) at each tree received only the ion-matrix solution (no N); a background branch (B) received no solutions other than natural precipitation. Three years of National Atmospheric Deposition Program (NADP) data were used to estimate the volume weighted mean concentrations of 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 S l^{-1}) in growing-season wet deposition. These common precipitation ions, at their respective volume weighted concentrations, were applied to C- and N-treated branches. C- and N-treated branches were sprayed until saturated (onset of dripping). Saturation resulted after spraying approximately 0.20–0.25 l of solution to each branch. Fourteen such sprays were administered between 20 June 2004 and 6 August 2004.

The concentrations of NH_4^+ and NO_3^- in N solutions were determined based on three years of wet deposition event sampling data (Tomaszewski et al., 2003). A pairwise comparison of concentration data from 43 wet deposition events across the 2000–2002 period indicated that the concentrations of NH_4^+ and NO_3^- were not statistically different (mean difference of 0.028 mg N l^{-1}; $p = 0.49$). The mean concentration of inorganic N in growing-season wet deposition at the Niwot Forest was about 1 mg inorganic N l^{-1}. An $NH_4^+NO_3^-$ treatment concentration of 10 mg N l^{-1} (5 mg N l^{-1} as NH_4^+, 5 mg N l^{-1} as NO_3^-) was chosen to ensure that a reasonable number of spray applications would enhance the growing-season wet N deposition to these branches by half or more. Measurements of branch area, the volume of water applied to each branch, and the concentration of N in applied solutions were used to estimate that wet N deposition to these branches was increased, on average for the three N-branches, by 1.4 kg N ha^{-1}. Wet N deposition was measured during the growing season of 2004 using the methods described in Tomaszewski et al., (2003). Growing-season wet N deposition totaled 2.4 kg N ha^{-1}. Thus, wet N deposition to N-treated branches was experimentally increased by ~60%.

2.7. Experimental design and data analysis

Three upper-crown branches from each of three spruce trees were selected to investigate the effects of canopy wet-deposited N on the photosynthetic response variables listed in Table 1. Branches and shoots were selected with similar orientations and light environments so that response variable differences among treatment levels (if any) would be the result of branch B, C and N treatments rather than branch light environment. Branches with an eastward orientation were chosen. New growth (<1 yr. old) and old growth (≥1 yr. old) shoots were selected from the terminal 15–20 cm of the branch tip so that shoots experienced similar within-crown shading. The similar crown position, branch orientation, and shoot location provided similar light environments.

The mean response variable value of shoots within a branch (three shoots per branch) represents the response variable value for that treatment level (B, C and N) within that tree for that sampling time. Each treatment level was represented within each of the three studied trees in an effort to block the effect that an individual tree might have on the response variables investigated. Data were analyzed by two-way ANOVA with trees 1, 2 and 3 constituting the first factor and branch treatment level (B, C

Table 2. Old growth response variable means for background (B), control (C) and nitrogen (N) treated spruce shoots

Response variable		Treatment mean			% Difference
		B	C	N	N – (BC)
F_v/F_m					
	Active treatment	0.752 a	0.750 a	0.772 a	2.8%
	Post treatment	0.740 a	0.734 a	0.762 a	3.4%
F_v'/F_m'					
	Active treatment	0.524 a	0.509 a	0.576 b	11.5%
	Post treatment	0.552 a	0.566 a	0.601 a	7.5%
NPQ					
	Active treatment	1.478 a	1.719 a	1.414 a	−11.5%
	Post treatment	1.143 a	0.859 a	1.064 a	6.3%
V_{cmax}	End of season	8.8 a	8.3 a	9.8 b	14.6%
J_{max}	End of season	41.0 a	42.3 a	46.3 a	11.2%
R_{day}	End of season	2.7 a	2.30 a	3.0 a	20.0%
N_a	End of season	2.24 a	2.47 a	2.54 a	7.9%

Common letters within a row indicate no significant difference between the treatments according to Tukey's Honestly Significant Difference (HSD) procedure. With this method, there is a 5% risk of calling one or more pairs significantly different when their actual difference equals zero. The units for nitrogen content (mass of nitrogen per unit projected needle area) were N_a = g N m^{-2}. All other units were the same as those presented in Table 1.

and N) constituting the second factor. The two-way ANOVA utilizes type III sums of squares, thereby assessing the contribution of each factor after having removed the influence of the other factor. When treatment level effects were significant ($p < 0.05$), multiple range comparisons between treatment levels were made using Tukey's Honestly Significant Difference Procedure. Two-way ANOVA and multiple range comparisons were performed using the program STATGRAPHICS Centurion.

The frequency of fluorescence measurements and N treatments throughout the growing season permitted the partitioning of fluorescence data into an active- and post-treatment period with distinct temporal/treatment characteristics. In the active-treatment period, which represents the period from 20 June 2004 to the 6 August 2004, C- and N branches were treated every 3–5 d. Fluorescence was frequently measured during this period resulting in 10 daytime fluorescence and 11 predawn fluorescence measurements for old growth. Previous to 30 July 2004, new growth foliage was too fragile to handle and as a result only three daytime and three predawn fluorescence measurements were taken. Non-photochemical quenching (NPQ) was determined for days when both predawn and daytime fluorescence were measured because the calculation of NPQ requires that both predawn F_m and daytime F_m' are available (Table 1). Three new growth and 11 old growth NPQ measurements were determined for the active-treatment period.

The post-treatment period began several weeks after the final N treatment (6 August 2004) and continued through to the final fluorescence measurement of 4 October 2004. During the post-treatment period, eight predawn and eight daytime old growth fluorescence measurements were taken as well as 10 predawn and 13 daytime measurements of new growth. While analysis of the post-treatment period tests for treatment level effects that carry beyond the period when branches were actively treated with N, analysis of the active-treatment period tests for treatment level effects that occur within days of treatment.

Although fluorescence data were obtained throughout the growing season, gas exchange analysis of shoots was performed once. Infrared gas analysis is dependent on accurate needle area measurements that require the destructive harvesting of shoots. To avoid the overharvesting of shoots within branches, gas exchange analysis was limited to end-of-season only. Thereafter, shoots were harvested for determination of projected needle area, dry weight, and %N.

3. Results and discussion

3.1. Old growth response to N treatment

N-treated shoots had greater mean values of F_v/F_m and F_v'/F_m' than B- and C-shoots with the percentage enhancement of N-treated shoots relative to the combined B- and C-shoot means being about 3 and 10% for F_v/F_m and F_v'/F_m', respectively (Table 2). The largest percent difference of 11–12% was for F_v'/F_m' during the active-treatment period and was significant at $p < 0.05$. Greater F_v'/F_m' often indicates less thermal dissipation of excess absorbed energy and coincides with lower values of

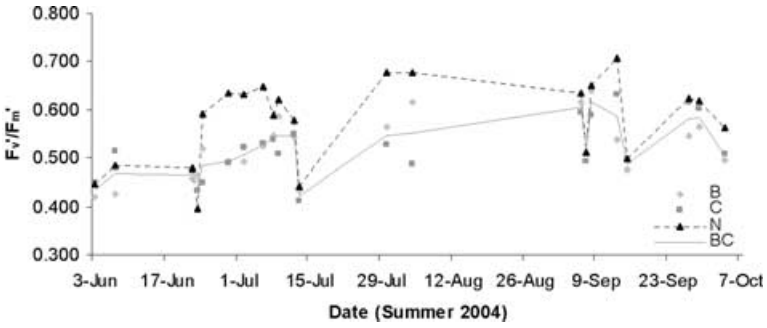

Fig. 1. Plot of mean B-, C-, and N-treated shoots' daily mean values of maximum observed photosynthetic efficiency (F_v'/F_m'). The grey line connects the mean value of the combined B- and C-treated shoots across dates. The black line connects the mean value of N-treated shoots across dates. Note the marked increase of the N-treated shoots F_v'/F_m' during the active-treatment period from 20 June 2004 to 6 August 2004.

NPQ. Significant treatment level differences were not observed for NPQ during the active- or post-treatment period, although the trend of 11–12% less NPQ for N-treated shoots during the active-treatment period was in agreement with the F_v'/F_m' result (Table 2).

The active treatment F_v'/F_m' result was the only significant treatment level difference evident in the active- and post-treatment fluorescence periods and suggests that the influence of N-treatment on photosynthetic efficiency was strongest during the 5-d period following N treatment. A study at the Niwot Forest (Sievering et al., 2007– part I) found that a portion of the variability in daytime net ecosystem CO_2 exchange (NEE), during the three-day period following precipitation, was positively correlated with canopy N uptake. The fact that N-treatment, in this study, exhibited the strongest influence on F_v'/F_m' during the 5-d period following treatment supports that the correlation between NEE and canopy N uptake is in fact due to N rather than a confounding variable such as water. In addition, this indicates that the greater photosynthetic efficiency of N-treated shoots may correspond to increased carbon assimilation rates a conclusion that is not supported by sole consideration of F_v'/F_m'. The time-series shown in Figure 1 demonstrates how mean F_v'/F_m' of N-treated shoots was enhanced during the active-treatment period. In addition, while not significant at $p < 0.05$, the second lowest p-value ($p = 0.08$) from the active- and post-treatment periods was for the greater F_v/F_m of N-treated shoots versus B- and C-shoots in the active-treatment period. These results support enhanced photosynthetic efficiencies of N-treated old growth as a result of a short-term response to N treatment.

Among the gas exchange response variables, V_{cmax} exhibited a significant treatment effect with N-treated shoots having 14–15% greater V_{cmax} relative to combined B- and C-shoot means (Table 2). Results for J_{max} and R_{day} were not significant, despite mean J_{max} and mean R_{day} being 11 and 20% greater, respectively, for N-treated old growth shoots (Table 2). The old growth results indicate that photosynthetic parameters were stimulated by increased canopy N uptake at this forest. Greater R_{day} is expected to occur concomitantly with greater N availability and photosynthesis since foliar maintenance activities including enzyme turnover and the regulation of ion gradients would be larger in more productive foliage (Lambers et al., 1983). The fact that N-treated shoots' mean J_{max} and mean R_{day} were not significantly greater than B- and C-shoots' mean J_{max} and mean R_{day} indicates J_{max} and R_{day} were highly variable across treatment levels and that the experimental design may lack sufficient power to detect treatment level differences in J_{max} and R_{day}. Future efforts investigating the influence of atmospheric N deposition on photosynthesis would benefit from an experimental design with more study branches.

The N content (g N m^{-2}) of N-treated shoots, although not significant, was 8% larger than B- and C-treated shoots' N content. Increased N-supply is known to enhance N content and V_{cmax}, especially for conifers that utilize Rubisco as an N-storage compound in addition to its catalytic functions (Warren et al., 2003). Thus, the enhanced V_{cmax} of N-treated shoots may result from the incorporation of applied N into Rubisco.

3.2. New growth trends

New growth response variables did not exhibit significant treatment level effects, although the new growth trends for F_v/F_m, F_v'/F_m', and V_{cmax} compare favorably with the old growth results (Table 3). N-treatment level mean comparisons with B- and C-treatment levels showed that F_v/F_m, F_v'/F_m', as well as V_{cmax} of N-treated new growth were larger by 0.5–1%, 2–4% and 8%, respectively (Table 3).

The lack of significant treatment level effects for new growth may indicate that their response variables were not affected by N-treatment. Alternatively, the new growth response to N treatment may be confounded by various unknown factors that add unexplained variability to the analysis. For instance, marked differences in the stage of development of new growth foliage across shoots may introduce variability that goes unexplained by tree and treatment level factors. New growth response variables exhibit greater variability than old growth response variables during both the active- and post-treatment periods.

During the active-treatment period the standard error (*SE*) of new growth treatment level means for F_v'/F_m' ($SE = 0.034$) was four times the corresponding old growth standard error. Although new growth shoots had fully expanded by the beginning of the post-treatment period, it may be that full expansion of the needles did not result in new growth shoots having variability comparable

Table 3. New growth response variable means for background (B), control (C), and nitrogen (N) treated spruce shoots

Response variable		Treatment level mean			% Difference
		B	C	N	N – (BC)
F_v/F_m					
	Active treatment	0.791 a	0.797 a	0.799 a	0.6%
	Post treatment	0.717 a	0.742 a	0.735 a	0.8%
F_v'/F_m''					
	Active treatment	0.448 a	0.499 a	0.485 a	2.4%
	Post treatment	0.447 a	0.570 a	0.529 a	4.0%
NPQ					
	Active treatment	1.042 a	0.798 a	1.028 a	11.7%
	Post treatment	1.633 a	0.916 a	1.246 a	−2.2%
V_{cmax}	End of season	11.0 a	11.4 a	12.1 a	8.0%
J_{max}	End of season	51.7 a	51.3 a	57.1 a	10.9%
R_{day}	End of season	5.5 a	4.2 a	6.3 a	29.9%
N_a	End of season	2.40 a	2.37 a	2.43 a	1.9%

Common letters within a row indicate no significant difference between the treatments according to Tukey's Honestly Significant Difference (HSD) procedure. With this method, there is a 5% risk of calling one or more pairs significantly different when their actual difference equals zero. The units for nitrogen content (mass of nitrogen per unit projected needle area) were N_a = g N m^{-2}. All other units were the same as those presented in Table 1.

to old growth variability. In the post-treatment period, the standard error of new growth F_v/F_m treatment level means (SE = 0.012) was similar to old growth treatment level means (SE= 0.013). In contrast, new growth F_v'/F_m' and V_{cmax} during the post-treatment period had roughly two and ten times as much variation as old growth. While new growth F_v/F_m, F_v'/F_m', and V_{cmax} trends were similar to old growth results, future efforts to discern treatment level effects will require larger sample sizes, or more control (covariates that reflect developmental stage), if potential new growth treatment level effects are to be statistically detected.

3.3. Summary of results and proposed mechanisms

The enhanced photosynthetic efficiencies of N-treated old growth shoots indicate that N decreased the relative magnitude of thermal dissipation and increased photosynthetic efficiency. During the active-treatment period, when N-treatment occurred every 5 or fewer days, F_v'/F_m' was 11–12% greater for N-treated shoots. During the post-treatment period neither F_v/F_m nor F_v'/F_m' was significantly greater for N-treated shoots. V_{cmax} of N-treated shoots was 14–15% greater than B- and C-treated shoots. The enhanced V_{cmax} of N-treated shoots indicates that N either increased the maximum rate of carboxylation by ribulose-1,5-bisphosphate carboxylase/oxygenase and/or prevented a decline that would have otherwise occurred. The following discussion presents two possible explanations regarding the observed influence of N treatment of spruce foliage.

3.4. Explanation 1: enhancement of the photosynthetic apparatus

N-treatment may increase photosynthetic efficiencies and the maximum rate of carboxylation by enhancing production of the N-requiring photosynthetic apparatus components. An enhanced photosynthetic apparatus allows for light at greater irradiances to be utilized so that it is not damaging (Ort, 2001). Chlorophyll fluorescence studies demonstrate that when light is excessive and N is available photosynthetic capacity increases (Verhoeven et al., 1997; Cheng, 2003). Thus, more absorbed light is used by photochemistry, which lowers the proportion of absorbed energy being thermally dissipated and lowers the likelihood of photodamage. Explanation one proposes that N-treatment enhanced the production of photosynthetic apparatus components which led to proportionally more light being utilized in electron transport (increased F_v'/F_m') and reduced the necessity for thermal dissipation in N-treated, old growth shoots. Greater daytime photosynthetic efficiencies, along with reduced activation of thermal dissipation, in N-treated old growth may translate into proportionally less dark-sustained activation of these enzymes. As a consequence, greater predawn F_v/F_m means were observed. Increased Rubisco concentration, due to enhancement of the photosynthetic apparatus, may also account for the greater V_{cmax} of N-treated shoots.

3.5. Explanation 2: assimilation of NH_4^+/NO_3^-

The F_v/F_m, F_v'/F_m', and V_{cmax} response to N treatment may, alternatively, be explained by the mechanism of foliar NH_4^+ assimilation/detoxification. NH_4^+ (taken up by shoots) is thought to enter the cytoplasm of mesophyll cells where it is either assimilated by glutamine synthetase or transferred into the chloroplast for assimilation by another isoform of glutamine synthetase (Rennenberg and Gessler, 1999). Carbon assimilation and ATP production are known to increase in response to leaf uptake of NH_4^+ (Krupa, 2003). Thus, NH_4^+ uptake may increase the fraction of absorbed light utilized by photochemistry. Such an increase could serve to decrease the fraction of absorbed light being thermally dissipated. Light was potentially less excessive for N-treated shoots because these shoots were experiencing more photosynthesis. Thus, more of the absorbed light would be utilized and not be damaging. This could lead to reduced activation of thermal dissipation and greater F_v/F_m as well as F_v'/F_m'.

Likewise the assimilation of NO_3^- could lead to similar results. The conversion of NO_3^- to NO_2^-, in either the apoplast or cytoplasm, is followed by NO_2^- transport to the chloroplast

for reduction to NH_4^+ by nitrite reductase. The NH_4^+ is then subject to assimilation by glutamine synthetase. Thus, NO_3^- assimilation in the chloroplast consumes electrons and may thereby prevent increased thermal dissipation.

The relatively frequent N treatments may have maintained a condition where shoots were more or less continually assimilating NH_4^+ and/or NO3$^-$. The greater V_{cmax} of N-treated old growth is not readily explained by inorganic N assimilation, since V_{cmax} was measured several weeks after the final N-treatment. However, excess absorbed light is known to lead to the formation of superoxide that is capable of degrading macromolecules such as Rubisco (Desimone et al., 1996). The reduction of NO_3^- and the increased photosynthesis stimulated by NH_4^+ assimilation are two processes that may have utilized excess electrons from electron transport and tempered superoxide formation. Rubisco in N-treated old growth may have been protected from photooxidative damage relative to B- and C-treated shoots, thus leading to a lasting enhancement of V_{cmax}.

Finally, the two explanations above are not mutually exclusive. For instance, greater V_{cmax} could result from an enhanced photosynthetic apparatus whereas enhanced F_v/F_m and F_v'/F_m' could result from response to inorganic N assimilation. The relatively rapid response of F_v'/F_m' to N-treatment suggests that explanation two may contribute more to the observed results since assimilation of N would occur before incorporation of N in to the photosynthetic apparatus. In any case, treatment of spruce foliage with N resulted in greater photosynthetic efficiencies and greater carboxylation rates.

4. Conclusions

The Introduction presented several mechanisms whereby N deposition at forest canopies could increase or decrease photosynthesis and photosynthetic parameters. Decreased photosynthesis in response to N-treatment was not supported by these data. The significant differences and non-significant trends supported enhanced photosynthesis for N-treated shoots. N-treatment reduced the proportion of light that was thermally dissipated and significantly enhanced daytime photosynthetic efficiencies for old growth spruce shoots during the active-treatment period. Additionally, old growth N-treated spruce shoots contained 8% more N (although $p > 0.05$) on an area basis and had 14–15% greater maximum rates of carboxylation than B- and C-treated shoots. Both the assimilation of inorganic N and the incorporation of applied N into the photosynthetic apparatus likely account for these results.

Mean branch J_{max} and R_{day} were highly variable indicating that the experimental design may lack sufficient power to detect treatment level differences in J_{max} and R_{day}. In addition, new growth parameters generally exhibited more variability among branches than old growth parameters. While new growth F_v/F_m, F_v'/F_m', and V_{cmax} trends were similar to old growth results (N-treated means > B- and C-treated means), future efforts to discern the influence of N-treatment on new growth parameters would benefit from an experimental design with more study branches and statistical power. This study represents one of only a few chlorophyll fluorescence field studies at conifer forests and demonstrates the usefulness of fluorometry for identifying the influence of environmental pollutants on foliar metabolism and photosynthesis.

5. Acknowledgments

Thanks go to Mike Kline, Rob Rouse, and Lisa Smith for their help in the field and to William Adams and Barbara Demmig-Adams for instruction on chlorophyll fluorescence techniques. We thank the University of Colorado's Mountain Research Station staff, especially Chris Seibold for assisting with the preparation of treatment solutions. We also thank Russ Monson for continued use of his canopy access towers. This research was supported by DOE's NIGEC program under Coop Agreement No. DE-FC03-90ER61010 and continues to be supported by the Long-Term Ecological Research Program of the National Science Foundation (NSF).

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