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# Relationships Between Needle Nitrogen Concentration and Photosynthetic Responses of Douglas-Fir Seedlings to Elevated CO2 and Temperature

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# Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated $CO_2$ and temperature

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## Summary

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• Here we examined correlations between needle nitrogen concentration ([N]) and photosynthetic responses of Douglas-fir (*Pseudotsuga menziesii*) seedlings to growth in elevated temperatures and atmospheric carbon dioxide concentrations ([$CO_2$]).
• Seedlings were grown in sunlit, climate-controlled chambers at ambient or ambient +3.5°C and ambient or ambient +180 µmol mol$^{-1}$ $CO_2$ in a full factorial design. Photosynthetic parameters and needle [N] were measured six times over a 21-month period.
• Needle [N] varied seasonally, and accounted for 30–50% of the variation in photosynthetic parameters. Across measurement periods, elevated temperature increased needle [N] by 26% and light-saturated net photosynthetic rates by 17%. Elevated [$CO_2$] decreased needle [N] by 12%, and reduced net photosynthetic rates measured at a common [$CO_2$], maximum carboxylation activity ($V_{c,max}$) and electron transport capacity ($J_{max}$), indicating photosynthetic acclimatization. Even so, elevated [$CO_2$] enhanced net photosynthesis, and this effect increased with needle [N].
• These results suggest that needle [N] may regulate photosynthetic responses of Douglas-fir to climate change. Further, needle [N] may be altered by climate change. However, effects of elevated [$CO_2$] on photosynthesis may be similar across growth temperatures.

**Key words:** acclimatization, carbon dioxide, climate change, nitrogen, photosynthesis, *Pseudotsuga menziesii* (Douglas-fir), seasonal variation, temperature.

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## Introduction

Increasing atmospheric carbon dioxide concentrations ([$CO_2$]) and increasing temperatures associated with climate change are predicted to have profound impacts on terrestrial ecosystems (Norby *et al.*, 1999; Ward & Strain, 1999). Considerable attention has been devoted to plant physiological and growth responses to elevated [$CO_2$]. By contrast, despite the 2–5°C increase in mean annual temperature predicted to accompany increasing atmospheric [$CO_2$] over the next century (IPCC, 2001), relatively few studies have examined the combined long-term effects of elevated [$CO_2$] and temperature. Further, although plant responses to elevated [$CO_2$] vary with the

availability of essential resources (Curtis & Wang, 1998; Saxe *et al.*, 1998; Poorter & Pérez-Soba, 2001), little is known about the roles of these factors in mediating the combined effects of elevated [$CO_2$] and temperature on plants. Clearly, predictions of plant responses to climate change would benefit from an understanding of the influences of essential resources on these responses.

One of the key resources likely to regulate plant responses to climate change is nitrogen (N). Nitrogen often is the primary factor limiting plant growth under current climate conditions (Bormann & Likens, 1967; Vitousek & Howarth, 1991). Plant responses to growth in elevated [$CO_2$] generally decline with decreasing nitrogen availability, as demonstrated

under conditions ranging from growth chambers to free-rooted trees (Stitt & Krapp, 1999; Harmens *et al.*, 2000; Oren *et al.*, 2001; Poorter & Pérez-Soba, 2001; but see Lloyd & Farquhar, 1996). Characteristic photosynthetic responses of nitrogen-limited plants to elevated [$CO_2$] include reduced photosynthetic capacity and reallocation of nitrogen from photosynthetic to nonphotosynthetic components (Bowes, 1991; Gunderson & Wullschleger, 1994; Sage, 1994; Drake *et al.*, 1997). The extent of these responses varies considerably, reflecting differences among species, the duration of exposure to elevated [$CO_2$] and other factors (Poorter & Pérez-Soba, 2001). For example, changes in leaf N concentration ([N]) associated with leaf development and senescence are a major factor regulating temporal variability in the magnitude of these responses (Wang *et al.*, 1995; Wullschleger *et al.*, 1997; Turnbull *et al.*, 1998; Jach & Ceulemans, 2000).

The effects of nitrogen availability on photosynthetic responses to climate change may be mediated by effects of climate change on soil nitrogen availability. For example, increasing growth temperatures have been shown to increase soil N mineralization rates, increasing soil N availability (van Cleve *et al.*, 1990; van Breemen *et al.*, 1998). Increased soil N availability often leads to increased leaf [N] because of increased N uptake by plants (Kellomäki & Wang, 1997; Hobbie *et al.*, 2001). Because of the central role N plays in photosynthesis, leaf [N] is generally correlated with net photosynthetic rates (Field & Mooney, 1986; Evans, 1989), suggesting that net photosynthetic rates may increase if elevated growth temperatures increase soil N mineralization rates (Norby *et al.*, 1999).

Douglas-fir (*Pseudotsuga menziesii*), a dominant tree species in the Pacific north-west (Franklin & Dyrness, 1988; Hermann & Lavender, 1990), often grows in low-N soils (Gessel *et al.*, 1973). Needle [N] is correlated with net photosynthetic rates of Douglas-fir (Bond *et al.*, 1999; Ripullone *et al.*, 2003), as well as maximum carboxylation rate of Rubisco ($V_{c,max}$) and electron transport capacity ($J_{max}$; Ripullone *et al.* 2003). Net photosynthetic rates in Douglas-fir seedlings are generally increased by growth in elevated [$CO_2$] (Hollinger, 1987; Lewis *et al.*, 2001) and by elevated mean annual temperatures (Lewis *et al.*, 2001). However, these responses show significant seasonal variation (Lewis *et al.*, 1999) that is not explained by seasonal changes in temperature (Lewis *et al.*, 2001) or stomatal conductance (Lewis *et al.*, 2002b). Thus, because N availability may influence photosynthetic responses to climate change, and regulates growth responses of Douglas-fir to climate change (Olszyk *et al.*, 2003), the primary objective of this study was to examine relationships between needle [N] and photosynthetic responses of Douglas-fir seedlings to elevated [$CO_2$] and mean annual temperature. In addition, because needle [N] may vary seasonally, a related objective was to determine whether seasonal variation in photosynthetic responses to climate change paralleled seasonal changes in needle [N]. To examine mechanisms through which needle [N] may influence photosynthetic responses to climate

change, net photosynthetic rate vs intercellular [$CO_2$] curves were measured to monitor changes in $V_{c,max}$ and $J_{max}$ over a 21-month period.

## Materials and Methods

### Growth conditions

Douglas-fir (*P. menziesii* (Mirb.) Franco) seed lots were collected at five low-elevation seed zones (< 500 m) in the Coast Range, Willamette Valley and the west slopes of the Cascade Mountains around Corvallis, OR, USA. Seedlings were grown for 1 yr in seed beds and 1 yr in nursery beds. In June 1993, 14 seedlings were transplanted as bare-root, 2-yr-old stock into each (1 × 2 m surface area) chamber at the US Environmental Protection Agency's ecological research division in Corvallis, OR, USA. Each chamber consisted of a sun-lit upper compartment (1.3–1.5 m high) where air temperature, [$CO_2$] and vapor pressure deficit were monitored and controlled, and a lower soil lysimeter (0.9 m deep) filled with a native coarse-textured sandy loam in which soil temperature and moisture were monitored (Tingey *et al.*, 1996). Soil moisture content was controlled to reflect seasonal changes in soil moisture typical for the wet winter and dry summer climate in the Pacific north-west (Griffiths & Caldwell, 1990; Griffiths *et al.*, 1991). Weekly water additions to the ambient [$CO_2$] and ambient temperature treatment were calculated based on this predicted pattern of soil moisture content. All treatments received the same weekly water additions. Seedlings were grown under ambient light, and without supplemental nutrients.

Ambient [$CO_2$] and air temperature were monitored at an adjacent meteorological station. The chambers were controlled to continuously track ambient [$CO_2$] or ambient +200 μmol mol$^{-1}$ $CO_2$ and ambient air temperature or ambient +4°C (Tingey *et al.*, 1996). Target dew point depression was based on ambient conditions and controlled to track equivalent vapor pressure deficits across treatments. Actual chamber conditions across the course of the experiment differed slightly from targets. The elevated [$CO_2$] treatment averaged 180 μmol mol$^{-1}$ $CO_2$ above ambient, with a 6% coefficient of variation for the differences in [$CO_2$] across all days (Olszyk *et al.*, 1998a). The elevated temperature treatment averaged 3.5°C above ambient, with a 12% coefficient of variation for the temperature differences across all days, and the vapor pressure deficit of chamber air in the elevated temperature treatment averaged 0.10 kPa above ambient (Olszyk *et al.*, 1998a).

The experimental design was a full factorial with three replicate chambers in each of the four treatment combinations: ambient [$CO_2$] and ambient temperature; ambient [$CO_2$] and elevated temperature; elevated [$CO_2$] and ambient temperature; and, elevated [$CO_2$] and elevated temperature. Treatments were applied 24 h per day beginning in August 1993 and continuing until the end of the study in July 1997. Mid-day (10:00–14:00 hours Pacific Standard Time) [$CO_2$] during the

1996 growing season typically ranged between 360 µmol mol$^{-1}$ and 400 µmol mol$^{-1}$ in the ambient [$CO_2$] treatment.

## Physiological measurements

Needle-level net photosynthetic rates were measured using an infrared gas analyser built into a leaf cuvette in an open-flow gas exchange system (LI-6400; Li-Cor, Lincoln, NE, USA). Measurements began in November 1995, 27 months after treatments were initiated. Additional measurements were made in February, March and October 1996, and March and July 1997. All measurements at a given measurement period were completed within a 7-d period. All measurements were made on intact fully expanded, unshaded needles from the most recent fully expanded cohort. In 1996, the mean date at which new needles reached full expansion was June 28 and did not significantly vary between treatments (Olszyk et al., 1998b). As a result, needles from the 1995 cohort were used for measurements made in November 1995 and February and March 1996, while the 1996 cohort was used for the remainder of the experiment.

Needles were arranged in the cuvette such that self-shading was minimized and all needles were parallel to the plane of the leaf chamber. Projected surface area of the measured needles was estimated using measurements of needle length and width. All measurements were made using ambient light. Photosynthetic photon flux densities (PPFD) at the upper needle surface generally ranged between 1200 and 2000 µmol m$^{-2}$ s$^{-1}$. No measurements were made at PPFD below 800 µmol m$^{-2}$ s$^{-1}$. A PPFD above 800 µmol m$^{-2}$ s$^{-1}$ has been shown to be saturating for photosynthesis in Douglas-fir (Bond et al., 1999; Lewis et al., 1999; Lewis et al., 2000).

The air-stream entering the cuvette was maintained at the desired [$CO_2$] using the LI-6400 computer-controlled $CO_2$ mixing system. Needle, cuvette and air temperatures were measured with thermocouples linked to the LI-6400 computer. Needle temperature was maintained at the target temperature using a computer-controlled Peltier module mounted on the cuvette. Needle and cuvette air temperatures generally were similar during measurements. The needle-to-air vapor pressure deficit in the cuvette was maintained at the target vapor pressure deficit by regulating the airflow rate, and by using desiccant to scrub the incoming air-stream as necessary. For a given measurement period, the target needle temperature and vapor pressure deficit for the ambient temperature treatment reflected average ambient conditions between 10:00 hours and 14:00 hours. Across $CO_2$ treatments, target needle temperatures for the elevated temperature treatment were 4.0°C higher than for the ambient treatment. The target vapor pressure deficit was the same for all treatments. The actual cuvette conditions deviated somewhat from actual chamber conditions because cuvette conditions were regulated to match the target chamber conditions, which themselves varied somewhat from actual chamber conditions (see above). Further,

although average cuvette measurement [$CO_2$] and needle temperatures matched target conditions, actual vapor pressure deficits were 0.3 kPa higher, on average, in the elevated temperature treatment than in the ambient temperature treatment. Although differences in vapor pressure deficit may influence photosynthesis through effects on stomatal conductance, across measurement periods neither stomatal conductance nor vapor pressure deficit significantly affected net photosynthetic rates in these seedlings (Lewis et al., 2002b).

Before each measurement, needles were equilibrated in the cuvette at saturating PPFD, the growth [$CO_2$], and the target temperature and vapor pressure deficit. Photosynthetic vs intercellular [$CO_2$] ($A-C_i$) curves were measured at all dates except the final harvest, when measurements were made at only the growth [$CO_2$]. At all dates, light-saturated net photosynthetic rates at the growth [$CO_2$] ($A_{growth}$) were determined at 350 or 550 µmol mol$^{-1}$ $CO_2$ for the ambient and elevated [$CO_2$] treatments, respectively. For $A-C_i$ curves, measurements were made at cuvette chamber [$CO_2$] of 80, 170, 260, 350, 450, 550, 700, 850, and 1000 µmol mol$^{-1}$. Data from $A-C_i$ curves were used to compare treatment effects on $A_{growth}$, the maximum carboxylation rate of Rubisco ($V_{c,max}$) and the capacity of electron transport mediated ribulose bisphosphate (RuBP) regeneration ($J_{max}$), as well as on light-saturated net photosynthetic rates at a common [$CO_2$] of 350 ($A_{350}$) or 550 µmol mol$^{-1}$ ($A_{550}$). Measurements were made once needles equilibrated at the target [$CO_2$]. Needles were considered equilibrated once the total coefficient of variation for gas exchange parameters was less than 1% for one minute. In general, the equilibration period lasted c. 5 min. For a given measurement day, measurements were initiated at c. 09:00 hours Pacific Standard Time, and typically were completed by 12:00 hours Pacific Standard Time.

A biochemical model of photosynthesis was used to calculate $V_{c,max}$ and $J_{max}$ from $A-C_i$ curves using (Farquhar et al., 1980; von Caemmerer & Farquhar, 1981):

$$A = \min\{A_c, A_q\} - R_d$$

($A$ is the net photosynthetic rate, $A_c$ and $A_q$ are the photosynthetic rates limited by Rubisco activity and by electron transport rate, respectively; min {} refers to the minimum of the two rates; $R_d$ is the daytime respiration rate resulting from processes other than photorespiration). The model was parameterized and run following Lewis et al. (1994) using the nonlinear regression model function in SYSTAT (SPSS, Evanston, IL, USA).

## Needle nitrogen concentration

The 1995 and 1996 needle cohorts were sampled for [N] analysis three times during the first year of growth of each cohort. All collections were made within 2 wk of corresponding gas exchange measurements. At all collections except

the final harvest (July 1997), 30–35 first-year needles were collected from around the crown of each tree in each chamber, with needles from all trees pooled to obtain one sample per chamber. At the final harvest, needles from the 1996 cohort were collected from 20 branches from around the crown of each of four trees per chamber, with all needles from a given chamber pooled to obtain one sample per chamber. After collection, needles were dried at 60°C and ground to pass through a 40-mesh screen. Samples were analysed for [N] using a Carlo Erba NC2500 elemental analyser (Finnigan MAT, Bremen, Germany). The internal standard was pine needles (NIST-SRM 1575; US National Institute of Standard and Technology, Standard Reference Materials Program). The coefficient of variation for quality control and duplicate samples for needle N analyses was less than 2%.

### Statistical analyses

Treatment effects on seasonal patterns in leaf biochemical and physiological properties were analysed using repeated measures analysis of variance with growth [$CO_2$] and temperature as the between-subjects factors and measurement period as the within-subjects factor. Regression analyses were conducted to examine relationships between needle [N] and photosynthetic properties within and between $CO_2$ and temperature treatments. Analyses were performed using the multivariate general linear model function (MGLH) in SYSTAT (SPSS). In general, needle gas exchange measurements were performed on one seedling in each chamber per measurement period. Individual branches were not repeatedly sampled over time, and across the study period measurements were made on several different seedlings from each chamber. Because the chamber was the experimental unit, measurements on multiple branches and seedlings from a chamber at a given measurement period were combined and the mean value used in the analyses. One chamber each in the ambient [$CO_2$], elevated temperature treatment and the elevated [$CO_2$], ambient temperature treatment were excluded from the analyses because of extensive insect damage to seedlings in these chambers.

## Results

Across the 21-month study period, growth in elevated temperature was associated with significant increases in needle [N] in first-year needles, compared with the ambient temperature treatment (Fig. 1a; $P = 0.021$). By contrast, growth in elevated [$CO_2$] was associated with significant reductions in needle [N] compared to the ambient [$CO_2$] treatment (Fig. 2a; $P = 0.025$). Across $CO_2$ and temperature treatments, needle [N] exhibited significant ($P = 0.035$) variation between measurement periods (Figs 1a and 2a). Needle [N] generally was highest in the winter and spring, and lowest during the summer.
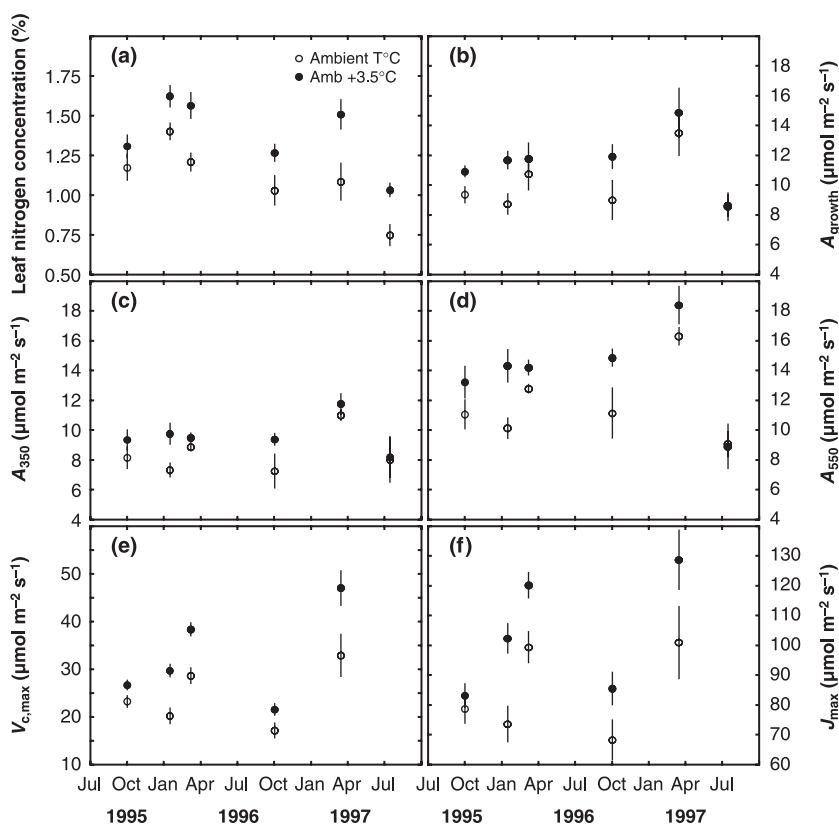


**Fig. 1** Effects of growth temperature on seasonal patterns of mean (± SE) needle nitrogen concentrations of Douglas-fir (*Pseudotsuga menziesii*) seedlings (a), light-saturated net photosynthetic rates at the growth [$CO_2$] ($A_{growth}$) (b), light-saturated net photosynthetic rates at 350 μmol mol$^{-1}$ $CO_2$ ($A_{350}$) (c), light-saturated net photosynthetic rates at 550 μmol mol$^{-1}$ $CO_2$ ($A_{550}$) (d), $V_{c,max}$ (e) and $J_{max}$ (f). Data are combined across $CO_2$ treatments. The ambient temperature treatment tracked the ambient air temperature at an adjacent meteorological station. Across the study period, actual air temperatures in the elevated temperature treatment were 3.5°C higher on average compared with the ambient temperature treatment. Measurements between November 1995 and July 1996 were made on the 1995 needle cohort, while the 1996 needle cohort was measured between August 1996 and July 1997. For all measurement periods, $n = 4$ for the ambient treatment (open circles), $n = 6$ for the ambient +3.5°C treatment (closed circles).

**Fig. 2** Effects of $CO_2$ supply on seasonal patterns of mean (± SE) needle nitrogen concentrations of Douglas-fir (*Pseudotsuga menziesii*) seedlings (a), light-saturated net photosynthetic rates at the growth [$CO_2$] ($A_{growth}$) (b), light-saturated net photosynthetic rates at 350 µmol mol$^{-1}$ $CO_2$ ($A_{350}$) (c), light-saturated net photosynthetic rates at 550 µmol mol$^{-1}$ $CO_2$ ($A_{550}$) (d), $V_{c,max}$ (e) and $J_{max}$ (f). Data are combined across temperature treatments. The ambient $CO_2$ treatment tracked ambient [$CO_2$] at an adjacent meteorological station; actual [$CO_2$] in the elevated $CO_2$ treatment was *c.* 180 µmol mol$^{-1}$ higher on average across the study period. Measurements between November 1995 and July 1996 were made on the 1995 needle cohort, while the 1996 needle cohort was measured between August 1996 and July 1997. Photosynthetic measurements in July 1997 were made at the growth [$CO_2$] only. For all measurement periods, $n$ = 5 for the ambient treatment (open squares) and for the ambient +180 µmol mol$^{-1}$ $CO_2$ treatment (closed squares).
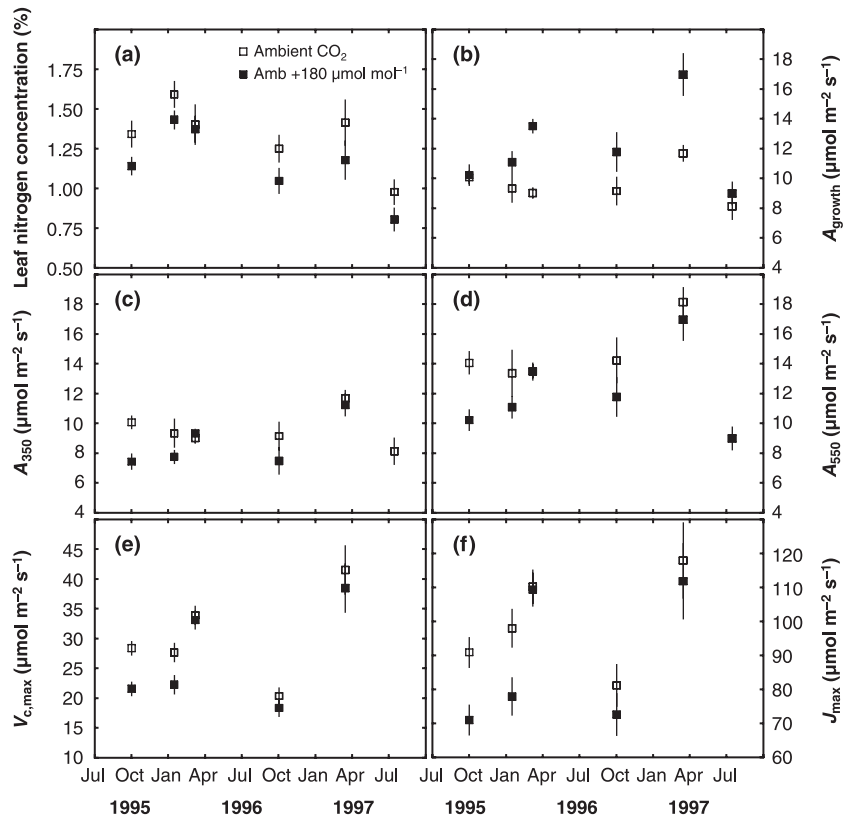
Elevated temperature (Fig. 1b–f) was associated with significant ($P$ = 0.089 in all cases) increases across the study period in net photosynthetic rates at the growth [$CO_2$] ($A_{growth}$), at 350 µmol mol$^{-1}$ $CO_2$ ($A_{350}$) and at 550 µmol mol$^{-1}$ $CO_2$ ($A_{550}$), maximum carboxylation activity of Rubisco ($V_{c,max}$) and electron transport-mediated RuBP regeneration capacity ($J_{max}$). By contrast, there were significant interactions ($P$ = 0.060 in all cases) between $CO_2$ treatment and measurement period (Fig. 2b–f) on $A_{growth}$, $A_{350}$, $A_{550}$, $V_{c,max}$, and $J_{max}$. Growth in elevated [$CO_2$] generally increased $A_{growth}$ but reduced $A_{350}$, $A_{550}$, $V_{c,max}$, and $J_{max}$. For all parameters, values generally peaked each spring in the elevated [$CO_2$] treatment but exhibited relatively less seasonal variation in the ambient [$CO_2$] treatment. As a result, the stimulatory effect of elevated [$CO_2$] on $A_{growth}$ generally increased from fall to spring, associated with lessening of the inhibitory effect of elevated [$CO_2$] on $A_{350}$, $A_{550}$, $V_{c,max}$ and $J_{max}$. There were no other significant interactions between $CO_2$ treatment, temperature treatment, and measurement period ($P$ = 0.298 in all cases).

A significantly greater increase was seen in $A_{growth}$ with increasing needle [N] in elevated compared with ambient [$CO_2$] (Fig. 3a; $P$ < 0.001). A comparison of the slopes indicates that a unit increase in needle [N] was associated with a *c.* 70% greater increase in $A_{growth}$ at elevated compared with ambient [$CO_2$]. It significantly increased with increasing needle [N] across temperature treatments ($P$ < 0.001); however,

the slope of the relationship between $A_{growth}$ and needle [N] did not significantly vary between temperature treatments (Fig. 3b; $P$ = 0.688). The relationships between needle [N] and $A_{350}$, $A_{550}$, $V_{c,max}$ or $J_{max}$ did not significantly vary between $CO_2$ or temperature treatments. Across $CO_2$ and temperature treatments, $A_{350}$, $A_{550}$, $V_{c,max}$, and $J_{max}$ significantly increased with increasing needle [N] concentration (Fig. 4a–d; $P$ < 0.001 in all cases). Adjusted $r^2$ values indicated that needle [N] accounted for 27.1% of the variation in $A350$, 31.8% of the variation in $A_{550}$, 37.1% of the variation in $V_{c,max}$, and 43.9% of the variation in $J_{max}$. Consequently, the observed differences in these parameters with temperature (Fig. 1) likely are the result of changes in leaf [N] rather than a direct effect of temperature treatment.

## Discussion

Needle [N] significantly varied between seasons, generally peaking in the winter and spring. Growth in elevated temperature increased needle [N] 26% and net photosynthetic rates 17%, on average, across the 21-month study period compared with the ambient temperature treatment. Increases in needle [N] were associated with a significant increase in the response of net photosynthetic rates to growth in elevated [$CO_2$]. Variation in needle [N] accounted for approximately 50% of the variation in net photosynthetic rates between $CO_2$
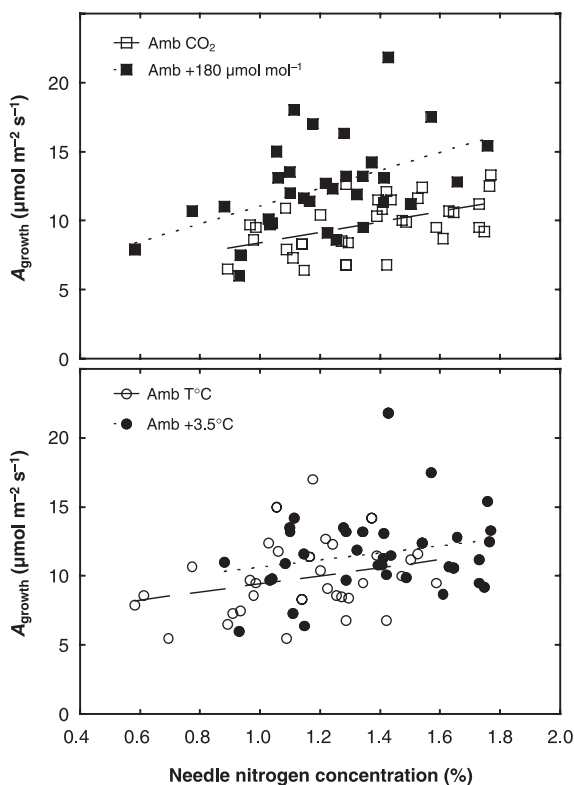
**Fig. 3** Effects of $CO_2$ supply (a) and temperature treatment (b) on the relationship between light-saturated net photosynthetic rate at the growth [$CO_2$] ($A_{growth}$) and needle [N] of Douglas-fir (*Pseudotsuga menziesii*) seedlings. There was a significant interaction between $CO_2$ treatment and needle [N] on $A_{growth}$ ($r^2 = 0.491$ for the combined model). Across temperature treatments, $A_{growth}$ was significantly correlated with needle [N] ($r^2 = 0.300$), and the slope of the relationship did not significantly vary between temperature treatments. The corresponding equations for the ambient and elevated [$CO_2$] treatments, respectively, are: $A_{growth} = 4.65 + 3.75 \times$ needle [N]; and, $A_{growth} = 4.59 + 6.46 \times$ needle [N]. For the ambient and elevated temperature treatments, the corresponding equations, respectively, are: $A_{growth} = 6.49 + 2.92 \times$ needle [N]; and, $A_{growth} = 8.08 + 2.29 \times$ needle [N]. (a) Open squares, ambient treatment; closed squares, ambient +180 µmol mol$^{-1}$ $CO_2$ treatment. (b) Open circles, ambient treatment; closed circles, ambient +3.5°C treatment.

treatments over the study period. Increases in the response of photosynthesis to elevated [$CO_2$] with increasing needle [N], coupled with increased needle [N] in the elevated temperature treatment, suggest that the effects of elevated [$CO_2$] on photosynthesis increased with increasing growth temperature, paralleling the effects of growth temperature on needle [N]. However, the interactive effects of elevated [$CO_2$] and temperature on photosynthesis were not statistically significant. Thus, effects of elevated [$CO_2$] on photosynthetic processes were generally similar across temperature treatments in this study.

Increasing mean growth temperature has been shown to increase the relative response of photosynthesis to elevated [$CO_2$] in other studies (Callaway *et al.*, 1994; Kellomäki &

Wang, 1996; Koike *et al.*, 1996; but see Teskey, 1997; Wayne *et al.*, 1998), as is predicted based on the relative effects of increasing temperature and [$CO_2$] on the carboxylation efficiency of Rubisco (Long, 1991). Elevated growth temperature has also been shown to enhance photosynthetic responses to elevated [$CO_2$] by increasing needle [N] (Kellomäki & Wang, 1997). While our results are partly consistent with these findings in that photosynthetic responses to elevated [$CO_2$] increased with increasing needle [N], the lack of significant interaction between elevated [$CO_2$] and temperature on photosynthesis indicates that other factors must also regulate plant responses to climate change. Thus, in addition to the potential effects of elevated [$CO_2$] and temperature on the carboxylation efficiency of Rubisco, the effects of climate change on carbon uptake are likely to reflect complex interactions with other factors, such as needle [N], that influence photosynthesis and that may be altered by elevated [$CO_2$] or temperature.

The relationship between changes in needle [N] and photosynthetic responses to elevated [$CO_2$] also resulted in substantial seasonality in the response of $A_{growth}$ to elevated [$CO_2$]. Peaks in needle [N] in the winter and spring were associated with relatively large responses of $A_{growth}$ to elevated [$CO_2$], while comparatively low needle [N] in the summer was associated with a relatively small response (Fig. 2a,b). The observed relationship between seasonal changes in leaf [N] and the response of net photosynthetic rates to elevated [$CO_2$] is consistent with other studies on trees, and indicate that temporal changes in leaf [N] are a key factor regulating plant responses to climate change (Curtis & Teeri, 1992; Jach & Ceulemans, 2000).

The comparatively small response of $A_{growth}$ to elevated [$CO_2$] during the summer suggests that the stimulatory effect of elevated [$CO_2$] on annual carbon uptake by Douglas-fir seedlings was relatively low during the summer. A limitation to these results is that only first-year needles were examined. Needle [N] of first-year needles has been shown to rise during the winter and decline during the summer, as occurred in this study, owing to seasonal patterns of allocation and remobilization (Fife & Nambiar, 1984; Helmisaari, 1992; Millard & Proe, 1992). By contrast, older cohorts show little N accumulation in the winter, and thus may be expected to show less of a wintertime enhancement of photosynthetic responses to elevated [$CO_2$]. Further, older needle cohorts in Douglas-fir have lower needle [N] than younger cohorts (Hobbie *et al.*, 2001). These constraints on the effect of elevated [$CO_2$] on annual carbon uptake may partly account for the lack of significant growth responses of these seedlings to elevated [$CO_2$] (Olszyk *et al.*, 1998a; Olszyk *et al.*, 2003).

Increases in the response of $A_{growth}$ to elevated [$CO_2$] with increasing needle [N] were primarily due to a shift in electron transport capacity rather than Rubisco activity, as indicated by greater changes in $J_{max}$ and $A_{550}$ with increasing needle [N] compared with the changes in $V_{c,max}$ and $A_{350}$. For example,

**Fig. 4** The relationships between needle [N] concentration and light-saturated net photosynthetic rates of Douglas-fir (*Pseudotsuga menziesii*) seedlings at 350 $\mu$mol mol$^{-1}$ $CO_2$ ($A_{350}$) (a), light-saturated net photosynthetic rates at 550 $\mu$mol mol$^{-1}$ $CO_2$ ($A_{550}$) (b), $V_{c,max}$ (c), and $J_{max}$ (d) across $CO_2$ and temperature treatments. (a) $A_{350}$ = 3.50 + 4.41 × needle [N], $r^2$ = 0.271. (b) $A_{550}$ = 4.17 + 7.07 × needle [N], $r^2$ = 0.318. (c) $V_{c,max}$ = −6.45 + 25.78 × needle [N], $r^2$ = 0.371. (d) $J_{max}$ = −1.28 + 70.02 × needle [N], $r^2$ = 0.439.
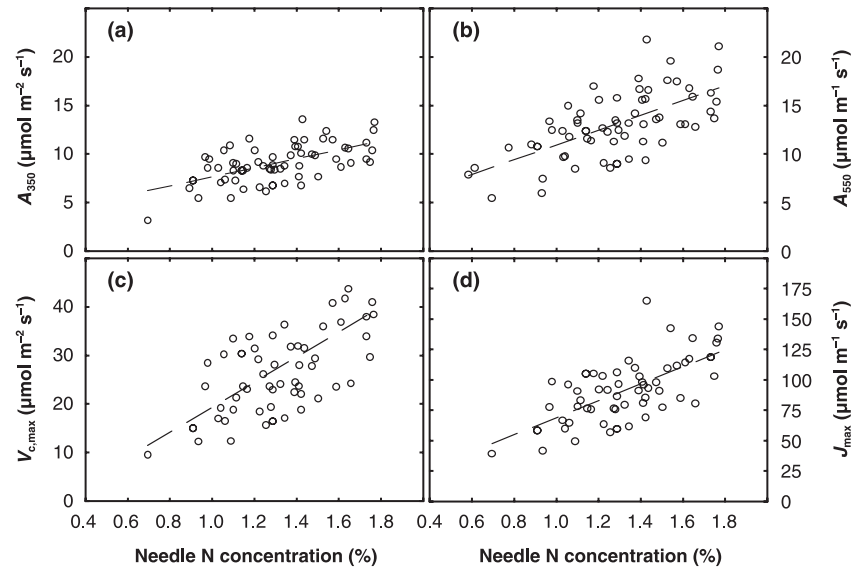
relative to $A_{350}$, $A_{550}$ increased 60% more rapidly with increasing needle [N] (Fig. 4). Increasing [$CO_2$] is predicted to shift control of photosynthesis from Rubisco activity towards electron-transport-mediated RuBP regeneration by increasing substrate availability for carboxylation and reducing the relative amount, and energetic cost, of photorespiration (Jordan & Ogren, 1984; Sharkey, 1988; Woodrow & Berry, 1988; Hikosaka & Hirose, 1998). Indeed, the stimulatory effect of elevated [$CO_2$] on Rubisco carboxylation is large enough that growth in elevated [$CO_2$] may induce reductions in photosynthetic capacity, often referred to as photosynthetic acclimatization, while still enhancing net photosynthetic rates (Sage, 1994; Woodrow, 1994; Medlyn, 1996; Tissue *et al.*, 1999).

Although growth in elevated [$CO_2$] increased net photosynthetic rates 12% on average across the study, there was clear evidence of photosynthetic acclimatization in the elevated [$CO_2$] treatment. Net photosynthetic rates measured at a common [$CO_2$], as well as $V_{c,max}$ and $J_{max}$, were reduced approximately 10–15%, on average, in the elevated [$CO_2$] treatment (Fig. 1). Similar reductions were observed in chlorophyll concentrations in the seedlings grown in elevated [$CO_2$] compared with the seedlings in the ambient [$CO_2$] treatment (Ormrod *et al.*, 1999). Studies on a range of other tree species have also observed a reduction in photosynthetic capacity despite a stimulation of net photosynthetic rates by growth in elevated [$CO_2$] (Norby *et al.*, 1999). Thus, for Douglas-fir and at least some other tree species, long-term photosynthetic responses to elevated [$CO_2$] may be substantially smaller than predicted from short-term studies.

Photosynthetic acclimatization was observed even though the seedlings in this study were grown in a large soil volume, and the fine root distribution pattern in the chambers was similar to that observed in same-age Douglas-fir seedling grown in similar soils in the field (M. G. Johnson, pers. comm.). Photosynthetic acclimatization to elevated [$CO_2$] has frequently been

observed in laboratory studies (Gunderson & Wullschleger, 1994; Curtis & Wang, 1998), and limited rooting volume has been identified as a key factor leading to acclimatization through effects on carbohydrate source–sink balance (Arp, 1991; Stitt, 1991; Thomas & Strain, 1991). However, acclimatization to elevated [$CO_2$] has also been demonstrated in several field studies (Lewis *et al.*, 1996; Rey & Jarvis, 1998; Tissue *et al.*, 1999; Griffin *et al.*, 2000; Bernacchi *et al.*, 2003), indicating that this phenomenon is not simply an artifact of limited rooting volume. Rather, acclimatization may reflect a variety of factors that influence carbohydrate source–sink balance, including low growth rates (Poorter, 1998) and ontogeny (Coleman *et al.*, 1994; Lewis *et al.*, 2002a). Other factors that have been shown to influence acclimation include nutrient allocation (McConnaughay *et al.*, 1993) and changes in leaf morphology (Luo *et al.*, 1994).

In the present study, changes in N allocation may have been a key factor driving photosynthetic acclimatization to elevated [$CO_2$]. The 10–15% reduction in photosynthetic capacity in elevated [$CO_2$] across the study period was paralleled by a 12% reduction, on average, in needle [N]. Reductions in leaf [N] are commonly seen during long-term exposure to elevated [$CO_2$] (Curtis & Wang, 1998), and generally reflect reallocation of N from photosynthetic machinery to other parts of the plant (Tissue *et al.*, 1993; Sage, 1994; Griffin & Seemann, 1996; Wolfe *et al.*, 1998; Tingey *et al.*, 2003) or a dilution effect due to starch and sugar accumulation in leaves (Field *et al.*, 1992). Reductions in needle [N] were associated with reallocation of N to nonphotosynthetic tissue in these seedlings (Hobbie *et al.*, 2001; Tingey *et al.*, 2003). Further, although needle carbon concentrations did not significantly vary with $CO_2$ treatment, needle carbohydrate concentrations were significantly greater in the elevated $CO_2$ treatment (Tingey *et al.*, 2003). Increased needle carbohydrate concentrations without associated increases in needle carbon concentrations

suggest that elevated $[CO_2]$ did not affect needle [N] through a dilution effect but rather through reallocation in response to sink limitation associated with the lack of growth response to elevated $[CO_2]$.

By contrast to the reduction in photosynthetic capacity in the elevated $[CO_2]$ treatment, growth in elevated temperature was associated with increased photosynthetic capacity, in conjunction with increased needle [N] (Fig. 1). Growth in elevated temperature also significantly increased chlorophyll concentrations (Ormrod *et al.*, 1999), and significantly reduced needle carbohydrate concentrations of these seedlings, although needle carbon concentrations did not significantly vary between temperature treatments (Tingey *et al.*, 2003). Increased needle [N] with increased growth temperature may result from increased soil nitrogen availability due to increased soil nitrogen mineralization rates (Rygiewicz *et al.*, 2000; Hobbie *et al.*, 2001), as has been observed in other studies (van Cleve *et al.*, 1990; van Breemen *et al.*, 1998). However, increased N uptake was not observed in this study (Tingey *et al.*, 2003). Thus, these results suggest that, as with elevated $[CO_2]$, changes in N allocation rather than dilution effects on needle [N] or changes in nitrogen assimilation were a primary driver of photosynthetic responses to elevated temperature.

In summary, photosynthetic responses of Douglas-fir seedlings to growth in ambient +180 μmol mol$^{-1}$ $CO_2$ showed similar trends across growth temperatures. In addition, these results suggest that needle [N] may regulate photosynthetic responses of Douglas-fir seedlings to climate change. Seasonal increases in needle [N] were associated with a significant increase in photosynthetic responses to growth in elevated $[CO_2]$. Further, changes in needle [N] accounted for nearly 50% of the variation between $[CO_2]$ treatments in net photosynthetic rates over the 21-month study period. Needle [N] also accounted for *c.* 30% of the variation in $A_{350}$ and $A_{550}$, and for *c.* 40% of the variation in $V_{c,max}$ and $J_{max}$. The link between seasonal patterns in needle [N] and the response of $A_{growth}$ to elevated $[CO_2]$ suggest that the effect of elevated $[CO_2]$ on annual carbon uptake by Douglas-fir seedlings was lowest during the summer. Seasonality in photosynthetic responses of Douglas-fir to elevated $[CO_2]$ may also be influenced by seasonal patterns in leaf temperature (Lewis *et al.*, 2001) and ontogeny (Lewis *et al.*, 1999). These constraints may partly account for the lack of significant growth responses of these seedlings to elevated $[CO_2]$ (Olszyk *et al.*, 1998a; Olszyk *et al.*, 2003). Thus, the effects of elevated $[CO_2]$ on carbon uptake in Douglas-fir seedlings are likely to reflect complex interactions with other factors, such as needle [N], that may be altered by elevated $[CO_2]$ or temperature.

## Acknowledgements

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