Photosynthesis–nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus × euroamericana* in a mini-stand experiment

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Summary   We compared photosynthesis–nitrogen relationships of one broad-leaved (poplar; *Populus × euroamericana* (Dole) Guinier) and one conifer (Douglas-fir; *Pseudotsuga menziesii* (Mirb.) Franco) species. Plants were grown in large pots to allow free root development and were kept well watered. We determined effects of low, intermediate and high nitrogen supply rates on area-based leaf nitrogen (N_a) and chlorophyll concentrations, leaf mass per area (LMA), light-saturated photosynthesis (A_{max}), maximum carboxylation (V_{cmax}) and electron transport rate (J_{max}), photosynthetic nitrogen-use efficiency (PNUE), and proportions of leaf N in active Rubisco (P_R), bioenergetic pools (P_B) and the light-harvesting complex (P_{LH}). Nitrogen supply significantly affected leaf N_a. Leaf mass per area did not differ between species and was unaffected by the N treatments. In both species, there was a positive correlation between leaf N_a and chlorophyll concentration, and between leaf N_a and the photosynthetic parameters A_{max}, J_{max} and V_{cmax}. At comparable leaf N_a, however, poplar showed twofold higher PNUE and a threefold steeper slope of the A_{max}–nitrogen relationship than Douglas-fir. Leaf N_a was negatively correlated with PNUE in Douglas-fir but not in poplar. Leaf N_a was also negatively correlated with P_R, P_B and P_{LH} in Douglas-fir, whereas in poplar, a negative correlation was found only for P_{LH}. Parameter P_R was significantly higher in poplar than in Douglas-fir. The ratio of CO_2 concentration in the intercellular space to that in ambient air was higher in poplar than in Douglas-fir. Overall, our data suggest that differences in the photosynthesis–nitrogen relationship and PNUE between Douglas-fir and poplar primarily reflect a different investment of N to active Rubisco, and possibly a different constraint to CO_2 diffusion.

Keywords: conifer, hardwoods, leaf mass per area, light-saturated photosynthesis, nitrogen partitioning, photosynthetic nitrogen-use efficiency.

Introduction

The relationship between light-saturated photosynthesis (A_{max}) and leaf nitrogen concentration on an area basis (N_a) has been widely assessed (Field and Mooney 1986, Evans 1989, Reich et al. 1994) and included in physiologically based ecosystem models (Aber et al. 1995). However, neither the ratio of A_{max} to leaf N_a (photosynthetic nitrogen-use efficiency, PNUE) nor the slope of the A_{max}–nitrogen relationship is stable across tree species. Higher PNUE and a steeper slope of the A_{max}–nitrogen relationship are generally observed in deciduous tree species, which preferentially grow on nutrient-rich soils, than in conifer species, which are often restricted to nutrient-poor sites (Field and Mooney 1986, Reich et al. 1994, 1995). It has been suggested that high investment of nitrogen in the photosynthetic apparatus has positive returns only if abundant site resources allow plants to maximize assimilation and growth (Chapin et al. 1990, Reich et al. 1992).

Accounting properly for interspecific differences in the photosynthesis–nitrogen relationship may be helpful for predicting the response of forest vegetation to nitrogen deposition and climate change (Nadelhoffer et al. 1999, Jarvis and Linder 2000, Oren et al. 2001), and for the functional interpretation of canopy chemistry assessed by hyperspectral remote sensing (Martin and Aber 1997). Inter-specific differences in the photosynthesis–nitrogen relationship have been attributed to a variety of factors including: (1) leaf structure (Poorter and Evans 1998, Reich et al. 1998); (2) allocation of nitrogen to components of photosynthetic machinery (Evans 1989, Niinemets and Tenhunen 1997); and (3) limitations to CO_2 diffusion (Field and Mooney 1986, Lloyd et al. 1992, Hikosaka et al. 1998). Leaf structure—usually expressed by leaf mass per area (LMA)—which affects processes like light absorption (Terashima and Hikosaka 1995), nitrogen allocation (Field and Mooney 1986, Evans 1989) and internal CO_2 diffusion (Lloyd et al. 1992), has often accounted well for differences in

photosynthesis–nitrogen relationships. For instance, species with relatively high LMA have lower PNUE and smaller changes in A_{max} per unit of leaf nitrogen than species with lower LMA (Reich and Walters 1994, Reich et al. 1995, Poorter and Evans 1998). However, little information is available on processes such as allocation of nitrogen to components of the photosynthetic apparatus (Hikosaka and Terashima 1995, Niinemets and Tenhunen 1997, Poorter and Evans 1998) and CO_2 diffusion to carboxylation sites (Hikosaka et al. 1998). Furthermore, it is not known whether such processes can explain interspecific differences in the photosynthesis–nitrogen relationship.

We studied a broad-leaved (*Populus × euroamericana* (Dole) Guinier) species and a conifer (*Pseudotsuga menziesii* (Mirb.) Franco) species that had similar LMA values in a preliminary trial (F. Ripullone, unpublished data). Plants differing in leaf nitrogen concentration were obtained by using different nitrogen supply rates and were used to test the following hypotheses: (1) poplar has a higher PNUE and a steeper A_{max}–nitrogen relationship than Douglas-fir; and (2) intra-leaf nitrogen allocation plays an important role in determining interspecific differences. To mimic more natural conditions, the plants were grown in large pots to allow free root development and the formation of mini-stands.

Materials and methods

Plant material and experimental treatments

The experiment was carried out in the experimental greenhouse and nursery of the University of Basilicata, Potenza, Italy. In March 2000, 2-year-old Douglas-fir seedlings were selected for dimensional uniformity (height = 41.7 ± 0.51 cm, mean ± standard error, $n = 20$) and planted in nine large cylindrical pots (diameter = 130 cm, height = 70 cm) according to a square spacing design (28 × 28 cm, 16 seedlings per pot). Pots were located in a greenhouse, where day/night temperatures and relative humidity were maintained at 24/19 °C and 60%, respectively, throughout the experiment. In June 2000, stem cuttings of poplar (cv I-214) were selected for uniformity (height = 20.4 ± 0.46 cm, mean ± standard error, $n = 30$) and planted in nine large, square parallelepiped pots (side length = 220 cm, height = 70 cm) according to a square spacing design (58 × 58 cm, 16 plants per pot). Pots were located in the nursery, below a transparent plastic roof that prevented soil recharge by natural rainfall. Day/night temperature and relative humidity were in the range 20–30/15–18 °C and 40–60%, respectively, over the duration of the experiment. All pots were filled with sieved (2 mm) siliceous sand, and a 5-cm thick layer of perlite was placed on top of the sand to minimize evaporation. Clear days prevailed during the experiment, with a photosynthetic photon flux density (PPFD) of about 900 µmol m^{-2} s^{-1} at the leaf level.

Three pots were assigned randomly to each of three fertilization treatments: low (LN), intermediate (MN) and high (HN) nitrogen supply. Nitrogen was supplied as ammonium nitrate (NH_4NO_3) in liquid solution (pH adjusted to 5.5). Each week throughout the growing season, Douglas-fir plants were supplied with 0.005 g (LN), 0.02 g (MN) or 0.04 g (HN) of nitrogen, whereas poplar plants were supplied with 0.3 g (LN), 0.9 g (MN) or 1.9 g (HN) of nitrogen. Calculation of total amounts of nitrogen to be supplied in the different treatments was based on initial tissue nitrogen concentration and expected biomass increase. Macroelements in the solution were adjusted with respect to the amount of nitrogen as suggested by Ingestad (1979) for a range of tree species. Plants were kept well watered throughout the experiment.

Photosynthesis

Net photosynthesis (A) was measured with a portable infrared gas analyzer (CIRAS 1, PP Systems, Hitchin, U.K.) at different concentrations of ambient CO_2 (c_a), to explore the dependence of A on intercellular CO_2 concentration (c_i). Measurements were made on 26 Douglas-fir plants (nine plants from the LN and HN treatments, eight plants from the MN treatment) and 14 poplar plants (six and eight plants from the LN and HN treatments, respectively) in early October 2000. Only plants growing in the central portion of each pot were sampled, and measurements were made on one fully illuminated shoot or leaf per plant, selected from the upper crown portion. Gas exchange of Douglas-fir shoots was measured with a conifer type chamber (PLC-conifer, PP Systems). A broadleaf type chamber (PLC-broad, PP Systems) that enclosed 3.24 cm^2 of leaf surface was used for poplar leaves. Chambers were illuminated by two 50-W halogen lamps (OSRAM 41870 WFL), supplying a PPFD of 1600 µmol m^{-2} s^{-1} at the shoot or leaf level. For Douglas-fir, lamps were positioned to minimize within-shoot shading. Starting at a carbon dioxide concentration ($[CO_2]$) of 370 µmol mol^{-1}, chamber $[CO_2]$ was lowered to 20 µmol mol^{-1} in 3–4 steps and then increased to 1600–2000 µmol mol^{-1} in three steps. Data were recorded after steady-state conditions had been attained for at least 5 min at each $[CO_2]$. Temperature inside the cuvette was maintained at 26 ± 2 °C for Douglas-fir and 24 ± 2 °C for poplar.

Leaf mass, nitrogen and chlorophyll

Douglas-fir needle area (projected) was determined with an LI-3000 area meter (Li-Cor, Lincoln, NE). Measurements were made on two subsamples of at least 30 needles detached from shoots immediately after completion of the photosynthesis measurements. Needles were then dried at 70 °C for 48 h and weighed to the nearest 1 mg, to compute leaf mass per area (LMA; g cm^{-2}). For poplar, LMA was computed from dry mass and projected area of four fully illuminated leaves sampled in the upper part of the crown of each plant.

Dried needles and leaves were finely ground and analyzed for total nitrogen according to the Kjeldahl method. Chlorophylls were extracted and analyzed according to Moran (1982): for Douglas-fir we used another subsample of 30 needles, and for poplar we used two 5 cm^2 leaf disks punched from the leaves measured for photosynthesis. Needles and leaf disks were frozen in liquid nitrogen and stored at –40 °C until analysis.

Photosynthetic parameters and nitrogen partitioning

Net photosynthesis (A) was expressed on a projected area basis. The equations proposed by Farquhar et al. (1980) were fitted on A/c_i curves by nonlinear least square regression procedures for estimating in vivo maximum carboxylation (V_{cmax}) and electron transport rate (J_{max}), on the lower ($c_i < 250$ μmol mol^{-1}) and upper ($c_i \geq 500$ μmol mol^{-1}) parts of the A/c_i curve, respectively. Light-saturated photosynthesis at c_i ~370 μmol mol^{-1} was considered to be the maximum assimilation rate (A_{max}). Estimates of J_{max} and V_{cmax} were based on the kinetic constants of von Caemmerer et al. (1994), and both parameters were referenced to 25 °C with temperature-dependence equations (Walcroft et al. 1997).

Photosynthetic nitrogen-use efficiency (PNUE) was calculated as the ratio between A_{max} and area-based leaf nitrogen concentration (N_a). Estimates of nitrogen partitioning among photosynthetic components, i.e., active Rubisco (P_R), bioenergetic pools (P_B) and the light-harvesting complex (P_{LH}), were estimated from values of V_{cmax}, J_{max} and chlorophyll concentration, according to the equations and procedures reported by Niinemets and Tenhunen (1997).

Statistical analysis

Effects of nitrogen treatments and species were assessed by factorial analysis of variance (ANOVA) and means were compared by the Student-Newman-Keuls test. Slopes of regression equations were compared according to Gomez and Gomez (1984); in cases where slopes were not significantly different, we also looked at intercept differences. All analyses were carried out with SPSS statistical software (Version 6.1.3 for Windows).

Results

In both species, nitrogen supply significantly affected leaf N_a (Table 1), although differences between plants in the MN and HN treatments were not significant. Leaf N_a differed between species, with poplar having higher values than Douglas-fir ($P < 0.001$). Similar results were also obtained when leaf nitrogen concentration was expressed on a mass basis (data not shown). In contrast, leaf mass per unit area (LMA) did not differ between species and was unaffected by the treatments (Table 1) or leaf N_a (Figure 1). In both species, a positive correlation was found between leaf chlorophyll concentration and leaf N_a (Figure 2), and the slopes of the linear regressions were similar for the two species.

Figure 3 shows typical A/c_i curves for Douglas-fir and poplar plants in the HN and LN treatments. Photosynthetic parameters derived from the A/c_i curves were analyzed in relation to leaf N_a. In both species, significant positive correlations were observed between leaf N_a and the photosynthetic parameters A_{max}, J_{max} and V_{cmax} (Figure 4). Over the range of leaf N_a common to both species (2.0 to 2.7 g m^{-2}), poplar showed higher A_{max} and V_{cmax} than Douglas-fir ($P < 0.001$), whereas no significant difference between species was observed for J_{max}. Slopes
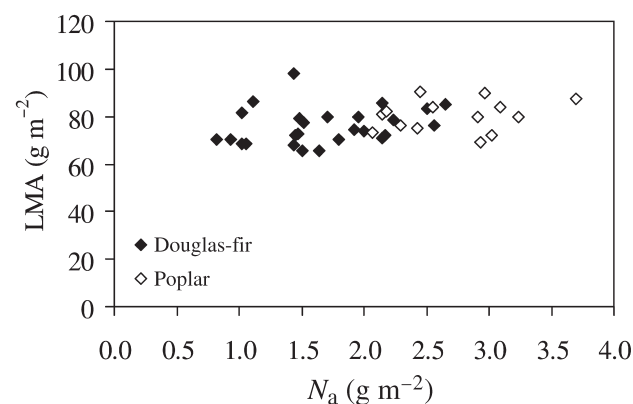

Figure 1. Relationships between leaf mass per area (LMA) and area-based leaf nitrogen concentration (N_a) in Douglas-fir and poplar.
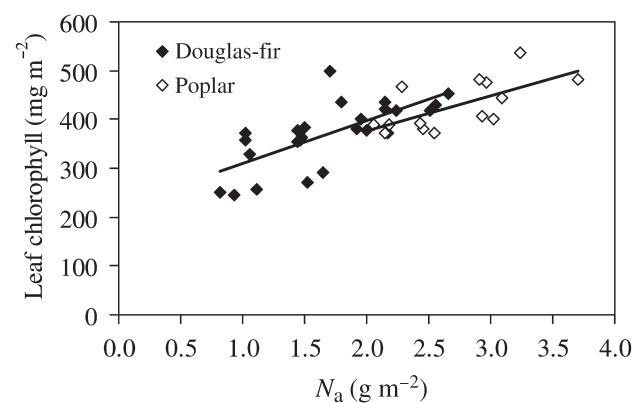

Figure 2. Relationships between area-based leaf chlorophyll and leaf nitrogen (N_a) concentrations in Douglas-fir and poplar. The regression relationships are: Douglas-fir, Chl = 87.31x + 222.7 ($r^2 = 0.49$, $P < 0.001$); and poplar, Chl = 71.95x + 232.6 ($r^2 = 0.45$, $P < 0.05$). Neither the slopes nor the intercepts differed significantly between species ($P < 0.05$).

Table 1. Effects of nitrogen treatments on area-based leaf nitrogen concentration (N_a) and leaf mass per area (LMA) in Douglas-fir and poplar at the time of gas exchange measurements. Abbreviations: LN = low nitrogen supply; MN = intermediate nitrogen supply; and HN = high nitrogen supply. Mean values not sharing common letters are significantly different according to the Student-Newmann-Keuls test ($P < 0.05$).

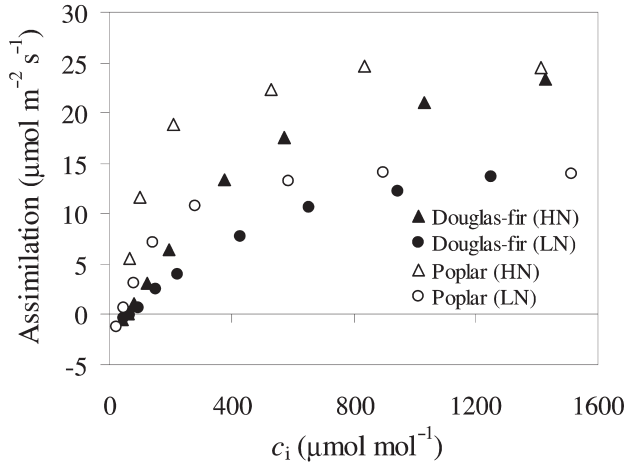| Species and treatment	Leaf N_a (g m^{-2})	LMA (g m^{-2})
Douglas-fir		
LN	1.11 b	78 a
MN	1.80 a	74 a
HN	2.10 a	76 a
Poplar		
LN	2.26 b	80 a
MN	2.80 a	77 a
HN	3.06 a	82 a

Figure 3. Typical A/c_i curves for Douglas-fir and poplar plants in the high-nitrogen (HN) and low-nitrogen (LN) treatments.

of A_{max}–N_a and V_{cmax}–N_a relationships were significantly higher in poplar than in Douglas-fir. The photosynthetic parameters A_{max}, J_{max} and V_{cmax} were not significantly correlated with LMA in either species.

Strong correlations were found between J_{max} and V_{cmax} in both species, but the relationships differed between species, with Douglas-fir showing a significantly steeper regression line than poplar (Figure 5). The ratio between J_{max} and V_{cmax} was significantly higher in Douglas-fir than in poplar (2.7 ± 0.2 versus 1.5 ± 0.1; see also Figure 3) and it was unaffected by leaf N_a in either species.

Photosynthetic nitrogen-use efficiency (PNUE) was negatively correlated with leaf N_a in Douglas-fir but not in poplar (Figure 6). Over the range of leaf N_a common to both species, PNUE was significantly higher in poplar than in Douglas-fir ($P < 0.001$).

Estimated proportions of leaf nitrogen in light-harvesting (P_{LH}), bioenergetic pools (P_B) and active Rubisco (P_R) were negatively correlated with leaf N_a in Douglas-fir (Figure 7), whereas in poplar a negative correlation was found only for P_{LH}. Over the range of leaf N_a common to both species, P_{LH} and P_B did not differ between species, whereas P_R was significantly higher in poplar than in Douglas-fir ($P < 0.001$).

The ratio of $[CO_2]$ in the intercellular space (c_i) to that in ambient air (c_a) decreased slightly with leaf N_a in both Douglas-fir and poplar (Figure 8). Although the c_i/c_a ratio was not significantly different between species over the range of leaf N_a common to both species, there was a significant difference between the regression intercepts ($P < 0.01$).

Discussion

Strong correlations between photosynthesis and area-based leaf N concentration (N_a) were observed in both Douglas-fir and poplar. At comparable leaf N_a, however, poplar showed twofold higher PNUE and threefold steeper slope of the A_{max}–nitrogen relationship than Douglas-fir. We discuss factors
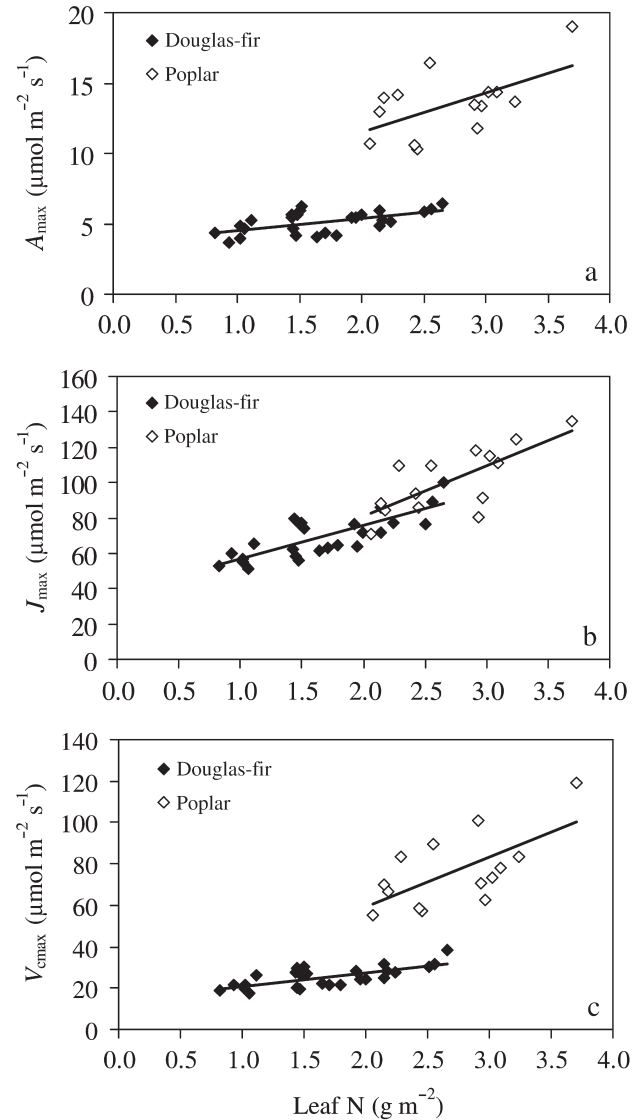

Figure 4. Relationships between (a) maximum assimilation rate (A_{max}), (b) maximum electron transport rate (J_{max}), and (c) maximum carboxylation rate of Rubisco (V_{cmax}) and leaf nitrogen concentration (N) in Douglas-fir and poplar. The regression relationships are: Douglas-fir, $A_{max} = 0.844^a x + 3.70$ ($r^2 = 0.35$, $P < 0.01$), $J_{max} = 18.88^a x + 37.93^a$ ($r^2 = 0.63$, $P < 0.001$), $V_{cmax} = 6.771^a x + 13.93$ ($r^2 = 0.50$, $P < 0.001$); and poplar, $A_{max} = 2.792^b x + 5.956$ ($r^2 = 0.33$, $P < 0.05$), $J_{max} = 28.59^a x + 23.79^a$ ($r^2 = 0.55$, $P < 0.01$), $V_{cmax} = 24.2^b x + 10.77$ ($r^2 = 0.42$, $P < 0.01$). Different letters in the equations indicate that slopes or intercepts are significantly different between species at $P < 0.05$.

(LMA, nitrogen allocation, CO_2 diffusion to carboxylation sites) that could account for these differences.

In our experiment, LMA did not differ between species. Furthermore, LMA did not change with leaf N_a, which contrasts with the negative correlations between LMA and leaf N_a reported by others (Dijkstra 1990, Cornelissen et al. 1997, Grassi et al. 2002). Mechanisms underlying the scaling of LMA with leaf N_a may change with species or experimental conditions (Reich and Walters 1994, Garnier et al. 1997,
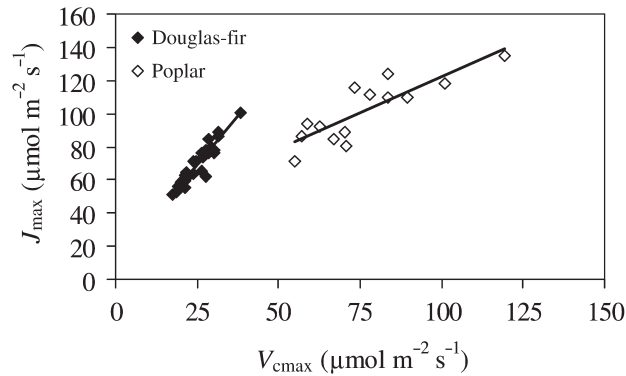
Figure 5. Relationships between maximum carboxylation rate of Rubisco (V_{cmax}) and maximum electron transport rate (J_{max}) for Douglas-fir and poplar. The regression relationships are: Douglas-fir, $J_{max} = 2.34^a x + 10.32$ ($r^2 = 0.89$, $P < 0.001$); and poplar, $J_{max} = 0.879^b x + 34.17$ ($r^2 = 0.72$, $P < 0.001$). Different letters in the equations indicate that slopes are significantly different between species at $P < 0.05$.
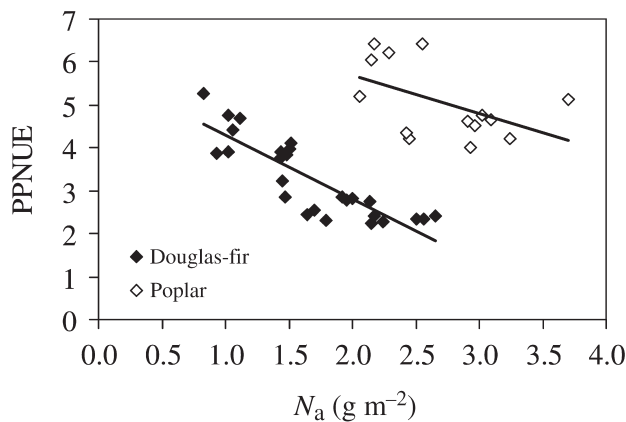
Figure 6. Relationships between potential photosynthetic nitrogen-use efficiency (PPNUE) and area-based leaf nitrogen concentration (N_a) in Douglas-fir and poplar. The regression relationships are: Douglas-fir, PPNUE $= -1.50x + 5.80$ ($r^2 = 0.73$, $P < 0.001$); and poplar, PPNUE $= -0.903x + 7.508$ ($r^2 = 0.24$, $P > 0.05$).

Figure 7. (a) Percent of leaf nitrogen (N) in light-harvesting (P_{LH}), (b) percent of leaf N in bioenergetic pools (P_B) and (c) percent of leaf N in Rubisco (P_R) versus leaf nitrogen concentration (N) in Douglas-fir and poplar. The regression relationships are: Douglas-fir, $P_{LH} = -4.725^a x + 20.56^a$ ($r^2 = 0.65$, $P < 0.01$), $P_B = -0.972^a x + 5.646$ ($r^2 = 0.64$, $P < 0.01$), $P_R = -3.725^a x + 20.72^a$ ($r^2 = 0.62$, $P < 0.01$); and poplar, $P_{LH} = -1.922^a x + 13.64^a$ ($r^2 = 0.47$, $P < 0.05$), $P_B = -0.224^b x + 3.75$ ($r^2 = 0.11$, $P > 0.05$), $P_R = -1.587^a x + 26.42^a$ ($r^2 = 0.04$, $P > 0.05$). Different letters in the equations indicate that slopes or intercepts are significantly different between species at $P < 0.05$.

Niinemets 1999) because nitrogen may affect LMA components, i.e., leaf thickness and density, and interactive effects may arise. For instance, high nitrogen supply could enhance cell enlargement and thus favor the production of thicker leaves; however, low nitrogen supply often results in higher LMA because of starch accumulation (Thompson et al. 1988).

Invariance of LMA with species and leaf N_a has interesting implications. First, it suggests that LMA cannot explain the observed differences in the photosynthesis–nitrogen relationship between our study species, as suggested by Reich et al. (1995) when interpreting differences between hardwoods and conifers, with conifers usually having higher LMA than hardwoods. Second, it allows analysis of photosynthesis–nitrogen relationships without the confounding effect of LMA as a covariate (Niinemets et al. 1998, Sims et al. 1998, Le Roux et
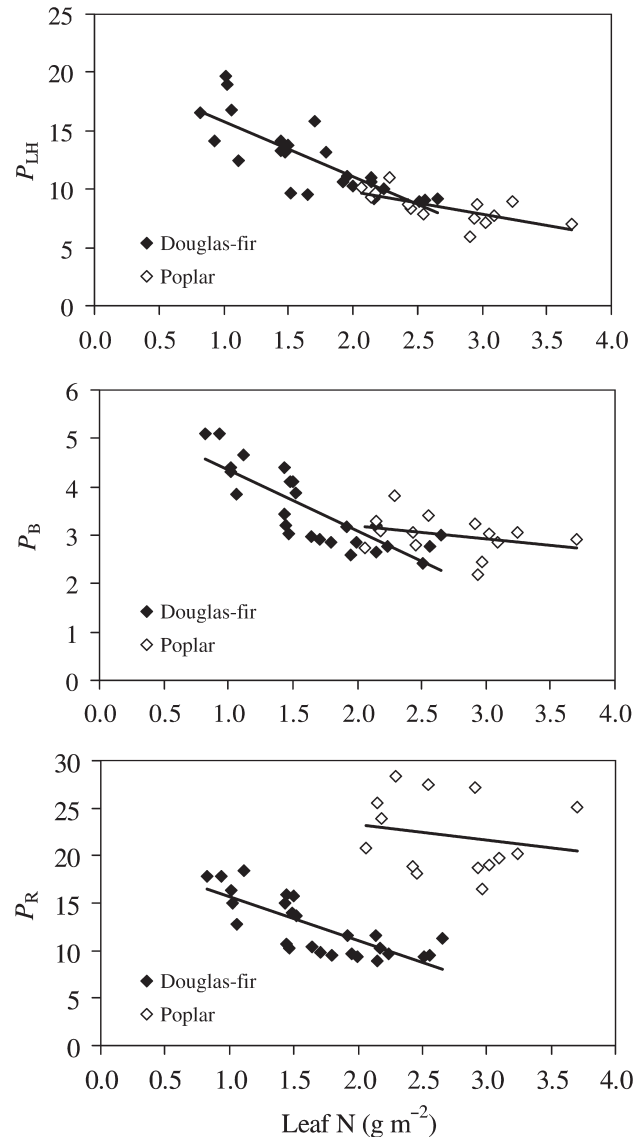
al. 1999, Grassi and Bagnaresi 2001) and independently of the units used (i.e., area-based or mass-based photosynthesis–nitrogen relationship) (Reich and Walters 1994). However, because we did not separate the LMA components, i.e., leaf thickness and density, we cannot exclude the possibility that there were differences in these two components.

Intra-leaf nitrogen allocation differed markedly between species, indicating that it may be a factor underlying species differences in the photosynthesis–nitrogen relationship and
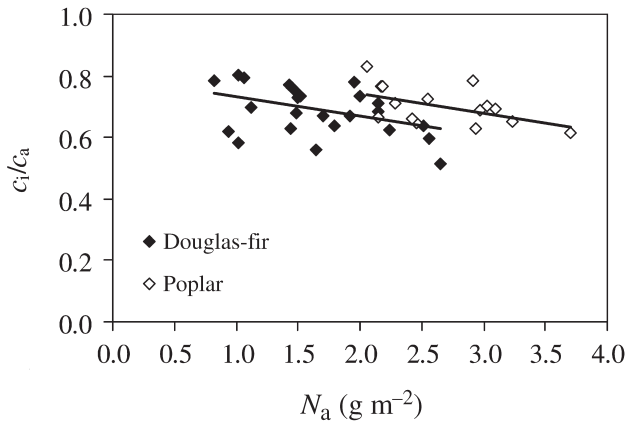
Figure 8. Relationships between the c_i/c_a ratio and area-based leaf nitrogen concentration (N_a) in Douglas-fir and poplar. The regression relationships are: Douglas-fir, $c_i/c_a = -0.063^a x + 0.793^b$ ($r^2 = 0.17$, $P < 0.05$); and poplar, $c_i/c_a = -0.066^a x + 0.876^a$ ($r^2 = 0.25$, $P > 0.05$). Different letters in the equations indicate that slopes or intercepts are significantly different between species at $P < 0.05$.

PNUE. At comparable leaf N_a, we estimated that poplar invested 50% more nitrogen in photosynthetic machinery than Douglas-fir. In particular, the proportion of nitrogen allocated to active Rubisco (estimated as P_R) was notably higher in poplar than in Douglas-fir. Overall, allocation patterns in our study species were similar to those reported for conifers (Brown et al. 1996, Turnbull et al. 1998) and hardwoods (Evans 1989, Niinemets and Tenhunen 1997). It has frequently been suggested that the lower PNUE observed in conifers compared with hardwoods may reflect inherently low investments of nitrogen in the photosynthetic apparatus (Wullschleger 1993, Reich et al. 1995, Brown et al. 1996), possibly as a consequence of long leaf life span and the associated nitrogen investment in compounds required for longevity and defense (Field and Mooney 1986, Aerts 1990, Hikosaka et al. 1998).

Leaf N_a may trigger nitrogen allocation among photosynthetic components to optimize resource partitioning (Hikosaka and Terashima 1995). It has been reported that, with increasing nitrogen availability, allocation of nitrogen to photosynthetic apparatus increases in herbaceous species (Evans 1989, Makino et al. 1992, 1994) but not in trees (Bauer et al. 2001). The lower P_R values found in Douglas-fir than in poplar with increasing leaf N_a suggest that an increasing proportion of Rubisco is inactive and possibly serves as a storage protein under conditions when nitrogen availability exceeds growth requirements of Douglas-fir (Millard 1988, Chapin et al. 1990, Stitt and Shulze 1994). Overall, our data confirm that, compared with broad-leaved species, conifers are unable to efficiently exploit large amounts of available nitrogen for growth.

Limitations to CO_2 diffusion represent another possible determinant of species differences in photosynthesis–nitrogen relationships and PNUE. Based on gas exchange measurements, the difference in c_i/c_a between Douglas-fir and poplar was small. However, based on Farquhar's model (Farquhar et

al. 1980) and the relationships between c_i/c_a and leaf N_a (Figure 8), we calculated that if Douglas-fir had the same c_i/c_a ratio as poplar, its photosynthetic rate would have been, on average, 18% higher than observed. Furthermore, working on the same material, F. Ripullone et al. (unpublished observations) found a significant difference in the stable isotope composition (Δ) of leaves of the two species ($\Delta = 19.0 \pm 0.8$ for Douglas-fir and 22.3 ± 0.5 for poplar) and estimated a larger difference between species in the long-term $\delta^{13}C$ values of c_i/c_a (0.646‰ for Douglas-fir and 0.792‰ for poplar, $P < 0.01$) compared with values obtained from gas exchange measurements. These results suggest additional interspecific differences in internal conductance. Because J_{max} is almost insensitive to changes in intercellular resistance, species differences in intercellular transfer resistance could partly explain the finding that the J_{max}–N_a relationship was similar for Douglas-fir and poplar, whereas the V_{cmax}–N_a relationship differed between species (Figure 4). In accordance with the finding of Hikosaka et al. (1998), the c_i/c_a ratio decreased with leaf N_a in both species.

In conclusion, our data confirm previous findings of lower A_{max}–PNUE and less steeply sloping A_{max}–nitrogen relationships in coniferous species compared with deciduous species (Reich et al. 1994, 1995). Previous studies have concluded that differences between functional groups are modulated primarily by differences in LMA (Field and Mooney 1986, Poorter and Evans 1998, Reich et al. 1998). In contrast, we found that differences between Douglas-fir and poplar primarily reflect differences in nitrogen investment in Rubisco, and possibly differences in the constraint to CO_2 diffusion.

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