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ORIGINAL PAPER

Influence of ammonium and nitrate supply on growth, dry matter partitioning, N uptake and photosynthetic capacity of *Pinus radiata* seedlings

Horacio E. Bown · Michael S. Watt ·
Peter W. Clinton · Euan G. Mason

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Abstract Growth and physiological responses of *Pinus radiata* D. Don seedlings to a combination of N supply regimes (low N $= 1.78$ mol m^{-3}, high N $= 7.14$ mol m^{-3}) and ammonium:nitrate ratios (80:20, 50:50 and 20:80; molar basis) were assessed in a hydroponic experiment run over the course of 105 days. Highly significant ($P < 0.001$) increases in seedling diameter, height, leaf area and dry mass occurred at lower ammonium:nitrate ratios and were two to fourfold greater than the non-significant (for diameter) to marginally significant ($P < 0.05$ for other dimensions) increases in these dimensions that occurred with greater N supply. Increases in N supply resulted in a highly significant ($P < 0.001$) reduction in biomass partitioning to roots and highly significant ($P < 0.001$) increases in allocation to foliage. The ammonium:nitrate ratio was not found to significantly change biomass partitioning to either foliage, stems or roots. Ammonium and nitrate uptake was significantly influenced by N supply and N form and conformed to ammonium and nitrate concentrations in nutrient solution. Uptake rates of ammonium were twice those of nitrate at comparable concentrations suggesting that *P. radiata* is in the lower end of the ratio of uptake of ammonium to nitrate reported for conifers (range from 2 to 20 mol mol^{-1}). Despite this, plants growing in high ammonium:nitrate ratios were smaller, exhibited luxurious N consumption and lower N use efficiency. Differences in productivity among treatments were partially explained by greater rates of light-saturated photosynthesis associated with nitrate nutrition.

Keywords Ammonium · Growth · Isotopes · Nitrate · Photosynthesis · Radiata pine

Introduction

Nitrogen (N) availability is the primary factor limiting productivity in most natural and managed ecosystems (Berendse and Aerts 1987; Aerts and Chapin 2000). Although some plants are reliant on organic forms of N (Ohlund and Nasholm 2004) most N is supplied to plants through ammonification and nitrification (Haynes and Goh 1978; Bloom 1985; Chapin et al. 1987; Marschner 1995). Nitrification plays a minor role in climax communities whereas in most disturbed and cultivated soils, where early successional species dominate, it may assume a major role (Haynes and Goh 1978). Consequently, plants exhibit great differences in their ability to take up and use ammonium and nitrate as sources of N (Haynes and Goh 1978), which reflects the environment to which the species are adapted (Kronzucker et al. 1997, 2003; Min et al. 1999).

Conifers are usually reported to grow faster under ammonium than nitrate (McFee and Stone 1968; van Den Driessche 1971; Kronzucker et al. 1997). However, this generalisation could be biased as most research has been undertaken in temperate and boreal ecosystems in the

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H. E. Bown
Facultad de Ciencias Forestales, Universidad de Chile, Casilla, 9206 Santiago, Chile

M. S. Watt (✉) · P. W. Clinton
ScionResearch, PO Box 29237, Christchurch, New Zealand
e-mail: michael.watt@scionresearch.com

E. G. Mason
School of Forestry, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

Northern Hemisphere (e.g. Lavoie et al. 1992; Downs et al. 1993) or mature plantation forests in the Southern Hemisphere where nitrification is minimal (e.g. Adams and Attiwill 1982a, b). Exceptions to this generalisation have been noted. For instance *Pinus radiata* D. Don, the most widely planted conifer species in the Southern Hemisphere (Lewis and Ferguson 1993), shows enhanced growth on disturbed sites, such as old-fields and pastures (Skinner and Attiwill 1981) that are high in nitrate (Haynes and Goh 1978; Vitousek et al. 1989; Parfitt et al. 2003). Despite this, previous work with *P. radiata* has shown poor growth for this species when nitrate is the sole N source (i.e., McFee and Stone 1968). This apparent paradox may be due to the often noted increase in plant growth performance under a mixture of ammonium and nitrate rather than either source alone (Haynes and Goh 1978; Chapin et al. 1987; Marschner 1995; Warren and Adams 2002a; Rothstein and Cregg 2005). Further research is required to clarify how varying ratios of ammonium:nitrate influence growth of *P. radiata*.

The influence of N supply and N form on biomass partitioning is also of considerable interest and controversy. Afforestation with fast growing species such as *P. radiata* represents an effective means of offsetting carbon dioxide emissions to meet national commitments under the Kyoto protocol (Dixon et al. 1994). Despite the importance of root biomass as a contributing component to the carbon budget, below ground biomass remains one of the most poorly quantified components in terrestrial ecosystems (Clark et al. 2001; Gower et al. 2001; Litton et al. 2007). As this component accounts for a significant proportion of the total plantation biomass it is imperative that our understanding of how soil and environmental factors influence this component is improved (Cairns et al. 1997; Kurz et al. 1996; Peichl and Arain 2006). A large body of research has investigated the effects of soil N availability on allocation to roots, and this research generally shows a reduction in biomass allocation to roots as N supply increases (Beets and Whitehead 1996; Santantonio and Santantonio 1987), although exceptions have been noted (King et al. 1999; Nadelhoffer et al. 1985). In contrast, little research has investigated how N form affects allocation to roots and the few studies that have been undertaken are contradictory suggesting that N form may (Kruse et al. 2003, 2010) or may not (Zerihun et al. 1998) have an effect on C allocation to roots.

The aim of the study was to examine the influence of a factorial combination of N form and N supply on growth, nitrogen uptake, nitrogen use efficiency (NUE) and photosynthetic capacity of *P. radiata* seedlings. We also assessed whether N-level and N-form treatments brought about changes in C partitioning between shoots and roots.

Materials and methods

Plant material

Pinus radiata D. Don seedlings were hydroponically grown under a factorial combination of N supply regimes (low $N = 1.78\ \mathrm{mol\ m^{-3}}$ and high $N = 7.14\ \mathrm{mol\ m^{-3}}$) and ammonium to nitrate ratios (80:20, 50:50 and 20:80). The high N regime was defined as 100 ppm (7.14 mM) as this concentration has been shown to provide optimal growth in *Pinus pinaster* Ait (Ingestad 1979). The low-N regime was chosen as one-fourth of the high-N concentration. The ammonium to nitrate ratios are expressed on a molar basis, e.g. a ratio 80:20 contains 4 mol of NH_4^+ for each mol of NO_3^- in nutrient solution. Following Ingestad (1979), nutrients other than N were provided at 0.420 mol m^{-3} P, 0.512 mol m^{-3} K, 0.250 mol m^{-3} Ca, 0.411 mol m^{-3} Mg, 0.281 mol m^{-3} S, 12.535 mmol m^{-3} Fe, 0.459 mmol m^{-3} Zn, 0.472 mmol m^{-3} Cu, 7.281 mmol m^{-3} Mn, 0.072 mmol m^{-3} Mo, 18.501 mmol m^{-3} B, 0.846 mmol m^{-3} Cl and 0.130 mmol m^{-3} Na (Table 1).

Seeds were germinated in free-draining containers with vermiculite, watered daily with $CaSO_4$ at 10 mol m^{-3}, and then cultured hydroponically for 2 weeks at ¼ and ½ strength Ingestad (1971, 1979) complete nutrient solution before starting the treatments. A total of 192 similar-size seedlings were randomly transplanted, in groups of four, within forty-eight 4.25 dm^3 light-tight root boxes. Lids, which were approximately 20 × 20 cm, were perforated in four corners with 4-cm diameter holes, at a square spacing 13 × 13 cm, for the trees to grow through. Foam-plugs were inserted for each plant at the root–shoot junction, and placed hanging from the lids of the root boxes to support the seedlings. Roots were immersed in 4-L treatment solutions that were changed weekly during the experiment. Root boxes were continuously aerated by immersing flexible tubing into treatment solutions through the centre of the lid which was connected to an air-pump (Aqua One, Model SR, Southampton, UK). Boxes were assigned randomly to nutrient treatments so that treatments were replicated eight times (8 boxes × 2 N supply regimes × 3 ammonium to nitrate ratios).

Plants were equally divided, with respect to treatment, into two controlled growth cabinets (Contherm Phytotron Climate Simulator, Petone, New Zealand). The environment was set to a 22/18°C day/night temperature, 16/8 h day/night regime, with irradiance of 333 ± 49 μmol $m^{-2}\ s^{-1}$ photosynthetically active radiation and a constant relative humidity of 75%.

The experiment was run from December 19, 2004 to April 2, 2005. Plants were destructively harvested at four dates at 32, 57, 77 and 105 days after seedling establishment in the root boxes. At each date one plant per root box

Table 1 Chemical composition of treatment solutions in which *Pinus radiata* seedlings were immersed during 105 days

Compound	Common solution (mg l^{-1})	Treatments (mg l^{-1})					
		Low N (1.78 mM)			High N (7.14 mM)		
		80/20	50/50	20/80	80/20	50/50	20/80
NH_4NO_3	–	0.00	17.59	0.00	74.98	136.08	108.56
$(NH_4)_2SO_4$	–	36.06	25.38	22.10	36.06	30.35	0.00
$(NH_4)_2HPO_4$	–	30.66	17.86	0.00	55.43	55.43	0.00
NH_4Cl	–	21.37	0.00	0.00	177.42	34.31	0.00
KNO_3	–	32.75	22.95	9.53	51.72	51.72	9.53
$Ca(NO_3)_2{\cdot}4H_2O$	–	0.00	26.36	63.61	0.00	58.92	253.99
$Mg(NO_3)_2{\cdot}6H_2O$	–	0.00	24.28	97.65	0.00	94.39	272.99
KH_2PO_4	–	25.52	38.72	57.12	0.00	0.00	57.12
$CaCO_3$	–	24.97	13.80	0.00	24.97	0.00	0.00
MgO	–	17.05	9.78	0.00	17.05	0.00	0.00
$MgSO_4{\cdot}7H_2O$	–	0.00	19.91	26.04	0.00	10.66	67.26
HNO_3	3.200	–	–	–	–	–	–
FeEDTA	4.600	–	–	–	–	–	–
$MnSO_4{\cdot}4H_2O$	1.624	–	–	–	–	–	–
H_3BO_3	1.140	–	–	–	–	–	–
$CuCl_2{\cdot}2H_2O$	0.082	–	–	–	–	–	–
$ZnSO_4{\cdot}7H_2O$	0.128	–	–	–	–	–	–
$Na_2MoO_4{\cdot}2H_2O$	0.016	–	–	–	–	–	–
$NiSO_4{\cdot}6H_2O$	0.026	–	–	–	–	–	–

Solutions were permanently aerated and changed weekly. Treatments were made up as a combination of two levels of nitrogen (LN = 1.78 mM, HN = 7.14 mM) and three ratios of NH_4^+ to NO_3^- (80:20, 50:50 and 20:80)

was randomly selected and harvested without replacement. A total of 48 plants, comprising 8 plants from each treatment combination, were harvested during each of these times. Dry mass of roots, stems and foliage were determined after oven-drying at 70°C to constant mass. Tissue nitrogen concentration at day 105 was determined based on Kjeldahl digestion and colorimetric methods using a Segmented Flow Analyser (SKALAR Analytical BV, Breda, The Netherlands) at Veritec Laboratories, Rotorua. Plant net N uptake was calculated as the difference between tissue N content at day 105 and day 0.

Ammonium and nitrate uptake and water use measurements

Nutrient solution was sampled (50 cm^3) at the beginning and at the end of each of 7 weeks ($n = 168$ observations) from day 54 to the end of the experiment on day 105, on 24 root boxes (4 boxes per nutrient treatment per week). Uptake rates were determined over a 7-day period from ammonium and nitrate depletion in nutrient solution. Uptake rates were expressed in micromoles of N per gram of dry root tissue to account for differences in root mass between root boxes. Ammonium and nitrate concentration in sample solutions were determined by steam-distillation methods (Bremner 1965). Values of pH were determined in all sample solutions with a pH and conductivity meter. By comparison of initial and final hydroponic solution in three root boxes without plants over a week, ammonium volatilisation was found to be negligible. Water depletion by evapo-transpiration was measured weekly in all root boxes using a graduated cylinder and used to determine long-term water use efficiency (WUE) as plant dry matter/water consumption.

Stable isotope analysis

Oven-dried samples from harvest at day 105 were ball-milled, and the carbon and nitrogen composition determined using a mass spectrometer at the Stable Isotope Laboratory at the University of Waikato, New Zealand. Isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) was calculated according to the following expression,

$$\delta = [(R/R_{\mathrm{std}}) - 1] \times 1{,}000 \qquad (1)$$

where R is the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample and R_{std} is the isotope ratio of the reference material (PeeDee belemnite, PDB, 11237.2×10^{-6} and air, respectively). $\delta^{15}N$ for treatment solutions were calculated based on $\delta^{15}N$ of salts and their proportions in each treatment solution. For plant tissue, we present N isotope composition as $\Delta\delta^{15}N$ relative to the source treatment solution (i.e., $\Delta\delta^{15}N = \delta^{15}N_{plant} - \delta^{15}N_{source}$). Carbon and nitrogen isotope discrimination was determined following the convention of Farquhar and Richards (1984), as,

$$\Delta = (\delta_{air} - \delta_{plant})/(1 + \delta_{plant}). \quad (2)$$

Gas exchange measurements and calculations

The rate of light-saturated photosynthesis at ambient CO_2 concentration (A_{sat}) was measured in six to eight plants per treatment, near the experiment end, from March 21 to March 29, 2005 using a portable photosynthesis system (Model 6400, Li-Cor, Lincoln, NE, USA). For each plant, three fascicles were placed inside a 6 cm^2 cuvette avoiding shading between needles. Temperature in the growth chamber and the cuvette was maintained at 20°C while leaf-to-air vapour pressure deficit (D) ranged from 1 to 1.5 kPa. Foliage samples were left to equilibrate for 10 min at ambient external CO_2 concentration, C_a (360 μmol mol^{-1}), with saturating irradiance, Q (400–700 nm), maintained at 2,000 μmol m^{-2} s^{-1}. Once values of photosynthesis (A), intercellular CO_2 concentration (C_i) and stomatal conductance (g_s) became stable (coefficient of variation $\leq$2%) values of A_{sat} were recorded. Following the measurement of A_{sat}, foliage samples were carefully removed from the cuvette and cut to match the leaf area exposed to gas exchange. Total surface area of needles was determined based on water volume displacement as described by Johnson (1984).

Nitrogen foliage concentrations were expressed on an leaf area basis (N_a) while photosynthetic nitrogen use efficiencies (E_N) were defined as A_{sat}/N_a. Water use efficiency (E_W) was determined as the ratio of A_{sat} to transpiration (E), while long-term NUE was determined as the ratio of total plant dry mass and N content at the experiment end (day 105).

Data analysis

All analyses were undertaken at the plant level using SAS (SAS Institute Inc., Cary, NC, USA). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. The main and interactive effects of N supply and N form on growth and physiological responses at day 105 were examined by analysis of variance and covariance. Adjusted means were presented in the results when analysis showed the covariate initial plant mass (for the variable plant growth) and N_m (for the variable $\Delta^{15}N$) to be significant. Tukey's least significant difference test was used to distinguish among individual means where applicable at a significance level of $P \leq 0.05$.

Plant size (mass) may confound the effect of treatments on dry matter partitioning among roots, stems and foliage, and therefore allometric analysis was carried out. Allometric analysis was used to remove the influence of growth on allometry, so that the direct influence of N supply and N form on allocation could be determined independent of plant size. Using data from all harvests at days 32, 57, 77 and 105 (192 plants), the relationship between a particular component, y (root, stem and foliage dry mass), and total plant mass, x, was modelled using the following log linear model; $\ln y = b_0 + b_1 \ln x$. Analysis of covariance was used to determine if slopes or intercepts of fitted equations significantly differed between treatment. Using these models predictions of allocation to foliage, stems and roots were made as a function of plant biomass and N supply. Analysis of covariance was also used to determine how treatment influenced fractional root, stem and foliage masses, using total plant mass as the covariate, at days 32, 57, 77 and 105 (192 plants). These analyses also investigated the effect of treatment on root:shoot ratio, defined as the ratio of below ground biomass and above-ground biomass.

The main and interactive effects of N supply and N form on weekly measurements of ammonium and nitrate uptake and pH over 7 weeks were modelled using a mixed effects model that accounted for the repeated measurements made within the experiment. Using averages determined over the 7-week period this data were also analysed using analysis of variance. As both analyses resulted in equivalent results findings from only the simpler analysis of variance are presented.

Results

Treatment influences on growth

There were highly significant ($F_{2,41} > 9.51$, $P < 0.001$) increases in seedling diameter, height, leaf area and dry mass as the ammonium:nitrate ratios decreased from 80:20 to 20:80 (Table 2). These increases were two to fourfold greater than the non-significant (for diameter) to marginally significant ($F_{1,41} > 4.57$, $P < 0.05$ for other dimensions) increases in these metrics that occurred with greater N supply (Table 2). None of these plant characteristics were significantly influenced by the interaction of N form and N supply ($F_{2,41} > 1.95$, $P > 0.16$).

Stem, foliage and root N concentrations were significantly higher in plants grown in ammonium than nitrate-dominated solutions. However, plant N uptake was greater in nitrate-dominated solutions as the plants were larger (Table 2). The leaf area to mass ratio (M) was not significantly influenced by the main or interactive effects of N

Table 2 Plant, foliage and photosynthetic characteristics of *Pinus radiata* seedlings growing in a factorial combination of N supply regimes (LN = 1.78 mol m^{-3}, HN = 7.14 mol m^{-3}) and NH_4^+:NO_3^- ratios (80:20, 50:50 and 20:80)

Variable	N-form ammonium:nitrate ratio (*F*)			N supply (*S*)		ANOVA statistics		
	80:20	50:50	20:80	LN	HN	*S*	*F*	$S \times F$
Diameter growth (mm)	9.4 ± 0.3 b	10.0 ± 0.3 b	11.6 ± 0.3 a	10.2 ± 0.2 a	10.6 ± 0.4 a	NS	***	NS
Height growth (mm)	269 ± 11 b	303 ± 13 b	346 ± 13 a	291 ± 10 b	323 ± 13 a	*	***	NS
Plant mass growth (g)	22.8 ± 1.4 c	27.3 ± 1.8 b	36.2 ± 2.0 a	26.8 ± 1.4 b	31.1 ± 2.1 a	*	***	NS
Root mass fraction	0.276 ± 0.007 a	0.287 ± 0,006 a	27.8 ± 0.6 a	0.294 ± 0.005 b	0.266 ± 0.005 a	***	NS	NS
Stem mass fraction	0.136 ± 0.004 a	0.134 ± 0.004 a	0.145 ± 0.004 a	0.138 ± 0.003 a	0.139 ± 0.003 a	NS	NS	NS
Foliage mass fraction	0.588 ± 0.006 a	0.579 ± 0.006 a	0.577 ± 0.006 a	0.567 ± 0.005 b	0.595 ± 0.004 a	***	NS	NS
Root:shoot ratio	0.387 ± 0.013 a	0.409 ± 0.012 a	0.391 ± 0.012 a	0.425 ± 0.009 b	0.367 ± 0.010 a	***	NS	NS
Leaf area growth (m^2)	0.26 ± 0.02 b	0.27 ± 0.02 a	0.37 ± 0.03 a	0.27 ± 0.02 b	0.33 ± 0.02 a	*	***	NS
Plant net N uptake (mmol)	38.6 ± 3.0 b	42.7 ± 3.3 ab	49.5 ± 3.7 a	35.3 ± 1.4 b	52.5 ± 2.9 a	***	*	NS
M (m^2 kg^{-1})	18.9 ± 0.4 a	18.2 ± 0.7 a	18.5 ± 0.9 a	18.4 ± 0.5 a	18.6 ± 0.6 a	NS	NS	NS
N_m (mmol g^{-1})	1.78 ± 0.10 a	1.67 ± 0.07 a	1.35 ± 0.06 b	1.40 ± 0.06 b	1.80 ± 0.06 a	***	***	NS
N_a (mmol m^{-2})	94.3 ± 4.8 a	93.1 ± 4.9 a	74.8 ± 4.7 b	76.3 ± 3.2 b	98.7 ± 4.9 a	***	***	NS
g_s (mmol m^{-2} s^{-1})	50.0 ± 8.2 a	65.6 ± 7.9 a	78.0 ± 9.2 a	60.9 ± 6.9 a	67.2 ± 7.3 a	NS	NS	NS
A_{sat} (μmol m^{-2} s^{-1})	6.2 ± 0.5 b	8.0 ± 0.5 a	9.5 ± 0.7 a	7.3 ± 0.5 a	8.3 ± 0.6 a	NS	**	NS
E_N (μmol mol^{-1} s^{-1})	63.7 ± 4.7 c	89.7 ± 8.0 b	120.2 ± 7.0 a	94.6 ± 7.8 a	86.7 ± 7.0 a	NS	***	NS
NUE (g g^{-1})	43.7 ± 2.1 b	46.8 ± 2.0 b	54.0 ± 2.1 a	54.2 ± 1.6 a	42.0 ± 1.2 b	***	***	NS
$\Delta^{15}N$ (‰)	0.62 ± 0.28 a	−0.09 ± 0.25 a	−0.75 ± 0.29 b	−1.22 ± 0.23 b	1.07 ± 0.25 a	***	*	NS
E_W (mmol mol^{-1})	5.9 ± 0.5 a	6.3 ± 0.4 a	5.3 ± 0.4 a	5.8 ± 0.4 a	5.9 ± 0.3 a	NS	NS	NS
WUE (mg g^{-1})	2.86 ± 0.10 a	2.78 ± 0.12 a	2.87 ± 0.10 a	2.87 ± 0.09 a	2.80 ± 0.08 a	NS	NS	NS
$\Delta^{13}C$ (‰)	15.8 ± 0.4 a	16.4 ± 0.2 a	16.6 ± 0.3 a	16.3 ± 0.3 a	16.2 ± 0.2 a	NS	NS	NS
Long-term C_i/C_a (%)	50.6 ± 1.6 a	53.1 ± 0.8 a	54.0 ± 1.3 a	52.8 ± 1.1 a	52.4 ± 1.0 a	NS	NS	NS

Values are presented as means (±1 SE) of seedling growth in basal diameter, height, mass and leaf area, seedling net N uptake, leaf area to mass ratio (M), foliage N concentration on a mass (N_m) and area (N_a) basis, stomatal conductance to CO_2 diffusion (g_s), light-saturated rate of photosynthesis at ambient CO_2 (A_{sat}), instantaneous photosynthetic nitrogen use efficiency (E_N), long-term nitrogen use efficiency (NUE), ^{15}N foliage isotope discrimination ($\Delta^{15}N$), instantaneous photosynthetic water use efficiency (E_W), long-term water use efficiency (WUE), ^{13}C foliage isotope discrimination ($\Delta^{13}C$) and long-term intercellular to ambient CO_2 concentration (C_i/C_a); $n = 7$–8 for all plant and foliage variables and $n = 6$–8 for all photosynthetic variables. All variables shown are reported at the end of the experiment at day 105, apart from component biomasses and root:shoot ratio in which data were taken from destructive harvests at four dates at 32, 57, 77 and 105 days after seedling establishment. Significance of main effects of N supply (*S*) and N form (*F*) or the interaction between N supply and N form ($S \times F$) are shown as: *NS* non-significant; * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$. Separation of means was determined by a Tukey test where applicable. Different letters within N supply regimes or N forms indicate that means were significantly different at $P < 0.05$. Covariates (given in brackets) were significant for plant mass growth (initial plant mass), component biomasses (total biomass) and $\Delta^{15}N$ (N_m) and therefore adjusted means are presented

supply and N form and was on average (± SE) 18.5 ± 0.4 m^2 kg^{-1}.

Treatment influences on photosynthetic characteristics

The rate of photosynthesis at saturating irradiance and ambient CO_2 (A_{sat}) was significantly influenced by N form ($F_{2,36} = 7.64$, $P = 0.002$) but not by N supply ($F_{1,36} = 0.96$, $P = 0.33$) or their interaction ($F_{2,36} = 0.43$, $P = 0.65$). Values of A_{sat} were significantly greater by 53% at 20:80 (9.5 ± 0.7 μmol m^{-2} s^{-1}) than at 80:20 (6.2 ± 0.5 μmol m^{-2} s^{-1}). Values of A_{sat} did exhibit an insignificant increase with nutrient supply from 7.3 ± 0.5 μmol m^{-2} s^{-1} at low N to 8.3 ± 0.6 μmol m^{-2} s^{-1} at high-N supply regimes (Table 2). Stomatal conductance to CO_2 diffusion (g_s) was not significantly influenced by the main or interactive effects of N supply and N form ($F_{5,36} = 1.17$, $P = 0.34$). However, despite this insignificance g_s did increase with N supply (from 61 mmol m^{-2} s^{-1} at low N to 67 mmol m^{-2} s^{-1} at high N) and as the ammonium:nitrate ratio decreased (from 50 mmol m^{-2} s^{-1} at 80:20 to 78 mmol m^{-2} s^{-1} at 20:80) (Table 2).

Values of E_N, NUE, and leaf ^{15}N discrimination ($\Delta^{15}N$) were strongly influenced by N form ($F_{2,36–41} > 4.7$, $P < 0.02$), while all these characteristics apart from E_N ($F_{1,36} = 2.41$, $P = 0.13$), were significantly influenced by N supply ($F_{1,39–41} > 34$, $P < 0.001$). The interaction between N form and N supply on all these characteristics was not significant (Table 2). Values of E_N and NUE significantly increased from high N to low N and as the proportion of ammonium in nutrient solution decreased (or nitrate increased) (Table 2). Values of Δ^{15}N followed the opposite pattern to E_N and NUE significantly increasing from low N to high N and as the proportion of ammonium in nutrient solution increased (or nitrate decreased) (Table 2). The effect of N supply was almost twice than that of N form on Δ^{15}N values (Table 2).

Values of photosynthetic water use efficiency (E_W), long-term WUE, foliage C isotope discrimination (Δ^{13}C) and long-term C_i/C_a values were not significantly influenced by main or interactive effects of N supply and N form ($F_{5,35–41} = 0.18–0.84$, $P > 0.52$) (Table 2).

Treatment influences on dry matter partitioning

After correcting for the effects of plant biomass, allocation to roots was significantly higher, while allocation to foliage was significantly lower in the low N compared to the high-N supply regime. The N form had no significant influence on allocation (Fig. 1). Slopes ($F_{1,184} > 4.3$, $P < 0.04$), but not intercepts ($F_{1,184} < 0.85$, $P > 0.35$), of the linear relationships between log-transformed foliage and root mass to log-transformed total plant mass were significantly different between N supply regimes (Fig. 1a, e). Stem mass fraction was not significantly influenced by N supply (Fig. 1c). Using

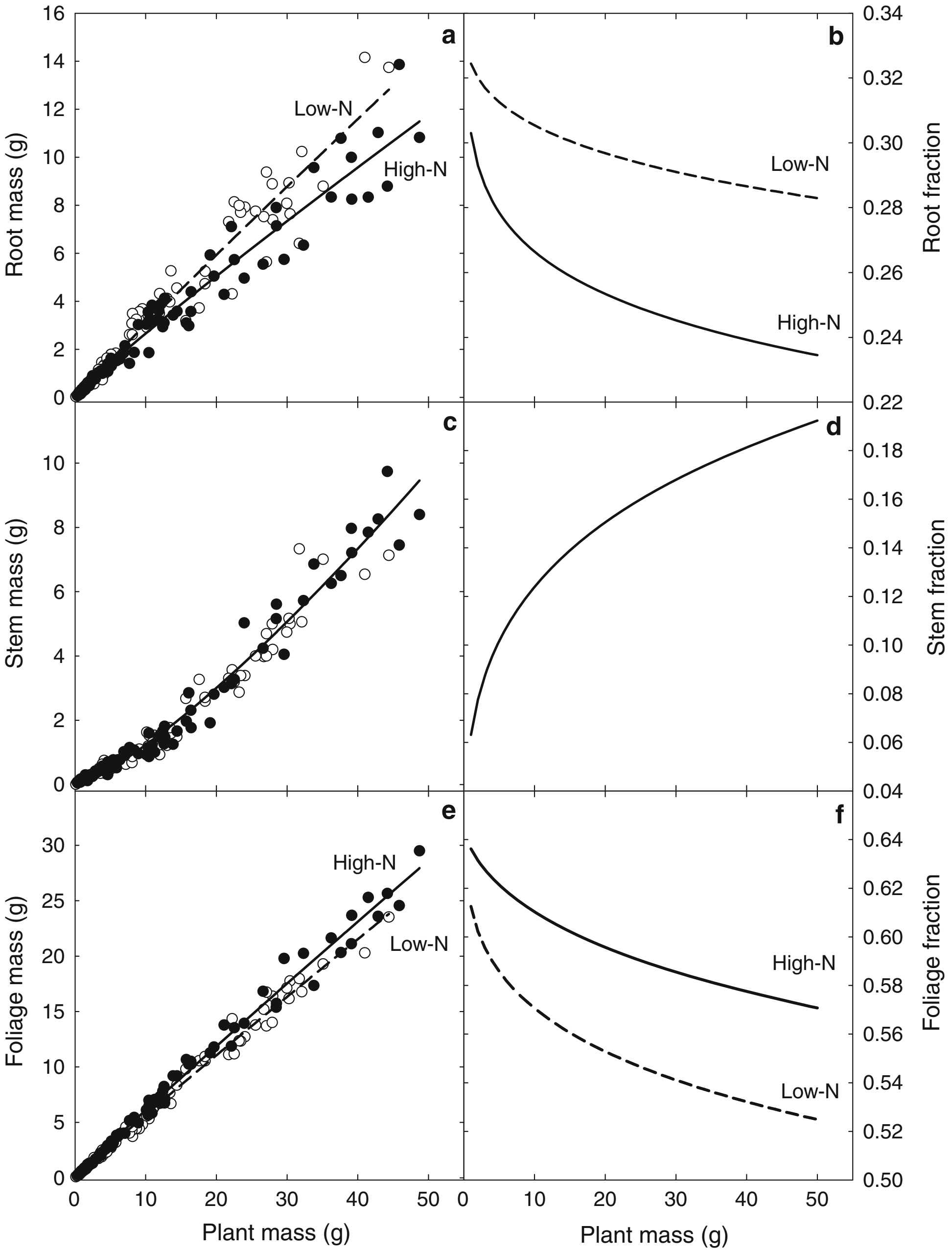

Fig. 1 On the *left side*, relationships between plant mass (W_T) and **a** root (W_R), **c** stem (W_S) and **e** foliage (W_F) mass as influenced by N supply regimes ($n = 182$). *Open symbols* and *dashed lines* represent low N (1.78 mol m^{-3}) and closed symbols and solid lines high N (7.14 mol m^{-3}), that differ between N supply for **a** and **e**, but not **c**. **a** $W_R = 0.3277\ W_T^{0.9667}$, $r^2 = 0.97$, $P < 0.001$ (LN); $W_R = 0.3176\ W_T^{0.9234}$, $r^2 = 0.98$, $P < 0.001$ (HN). **c** $W_S = 0.0637\ W_T^{1.2866}$, $r^2 = 0.98$, $P < 0.001$. **e** $W_F = 0.6187\ W_T^{0.9622}$, $r^2 = 0.99$, $P < 0.001$ (LN); $W_F = 0.6668\ W_T^{0.9612}$, $r^2 = 0.99$, $P < 0.001$ (HN). All these relationships were not influenced by N form ($P > 0.05$) and additionally, the relationship between W_S and W_T was not influenced by N supply regime and therefore a single equation was fitted. On the *right side*, relationships between plant mass and **b** root fraction, **d** stem fraction and **f** foliage fraction as influenced by N supply regimes. These values were calculated based on equations fitted in **a**, **c** and **e**

these models predicted variation in fractional masses of roots, stems and foliage as a function of seedling size (mass), and N supply, are shown in Fig. 1b, d, f, respectively. These figures show divergence in treatment allocation to roots and foliage with increasing seedling biomass.

Analyses undertaken using allometric analysis agree with results from the analysis of covariance undertaken on fractional biomasses. Least square means from these models show a significant decrease in root mass fraction with N supply that was on average (±1 SE) 0.294 ± 0.005 at LN and 0.266 ± 0.005 at HN supply (Table 2). In contrast, foliage mass fraction significantly increased with N supply, and was 0.567 ± 0.005 at LN and 0.595 ± 0.004 at HN supply (Table 2). These changes were reflected in the highly significant variation in the root:shoot ratio, that decreased from 0.425 ± 0.011 at LN supply to 0.367 ± 0.009 at HN supply ($F_{1,181} = 15.07$, $P < 0.001$). Stem mass fraction was not significantly influenced by main or interactive effects of N supply and N form ($F_{6,181} = 1.63$, $P = 0.14$) being on average 0.138 ± 0.002 (Table 2).

Treatment influences on ammonium and nitrate uptake

Ammonium, nitrate and ammonium plus nitrate uptake (over 24 h) were significantly influenced by N supply ($F_{1,18} > 11$, $P < 0.004$) and N form ($F_{2,18} > 11$, $P < 0.001$), but not their interaction ($F_{2,18} < 2.4$, $P > 0.12$) with the exception of nitrate uptake ($F_{2,18} = 7.8$, $P = 0.004$). The relative rates of ammonium and nitrate uptake conformed to ammonium and nitrate concentrations in nutrient solutions (Fig. 2a, b). However, the rate of ammonium uptake was on average 2.0 ± 0.2 times greater than that of nitrate at comparable concentrations, and the difference tended to increase with N supply from 1.65 ± 0.15 at LN (50:50) to 2.31 ± 0.30 at HN (50:50). This preferential uptake of ammonium was also reflected in the ratio of ammonium to nitrate uptake across the range in N form solutions, from 0.74 ± 0.18 at a ammonium:nitrate ratio of 20:80 to 6.04 ± 0.82 at a ammonium:nitrate ratio of 80:20. Analysis of covariance showed that nitrate uptake did not influence ammonium uptake and vice versa ($F_{1,17} = 2.11$, $P = 0.16$).

Ammonium plus nitrate uptake (24 h) significantly increased by 72% with N supply from 187 ± 12 µmol g^{-1} at LN to about 322 ± 19 µmol g^{-1} at HN. Seedlings grown at a greater proportion of ammonium exhibited luxurious consumption of nitrogen (Fig. 2c), that translated into greater tissue N concentration (Table 1). Total N uptake increased by 56% from 202 ± 18 µmol g^{-1} for plants grown at 20:80 to 315 ± 41 µmol g $^{-1}$ at 80:20.

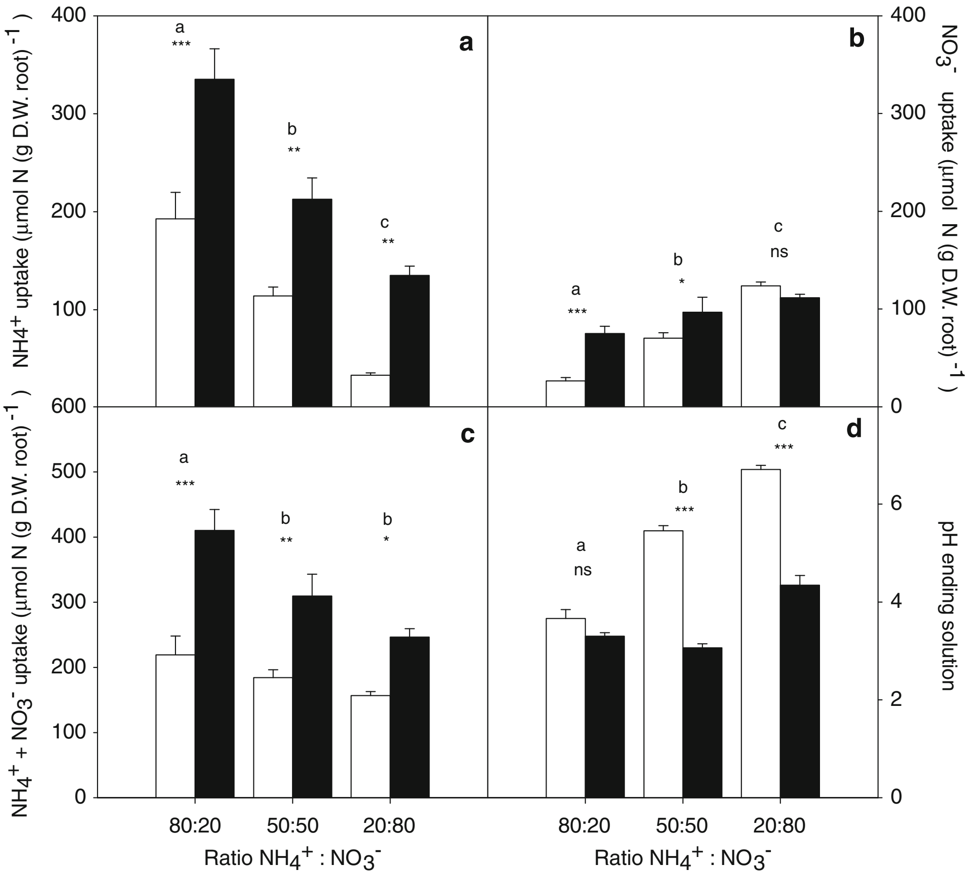

Fig. 2 Influence of NH_4^+:NO_3^- ratio under low (*open bars*) and high (*filled bars*) N supply on **a** ammonium, **b** nitrate, **c** ammonium plus nitrate uptake (24 h) and **d** pH of nutrient solution (at the end of a week). Values are presented as means (±1 SE; $n = 4$) for each treatment. *Different letters* indicate significant differences between NH_4^+:NO_3^- ratios at $P < 0.05$. Differences between N supply regimes within the same NH_4^+:NO_3^- ratio are shown as: ns, non-significant; * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$. Interactive effects between N supply regime and NH_4^+:NO_3^- ratio were not significant ($P > 0.05$) for ammonium and ammonium plus nitrate uptake, but were significant ($P < 0.05$) for nitrate uptake and pH

The pH of nutrient solution measured at the end of each week was significantly influenced by N supply ($F_{1,18} = 257$, $P < 0.001$), N form ($F_{2,18} = 126$, $P < 0.001$) and their interaction ($F_{2,18} = 40$, $P < 0.001$) (Fig. 2d). Values of pH were adjusted to lie between 5.0 and 5.5 at the beginning of each week. We observed a decrease in ending pH with N supply being on average 5.3 ± 0.4 at LN compared to 3.5 ± 0.1 at HN. Ending pH also increased with [NO_3^-] and decreased with [NH_4^+] in nutrient treatments being on average 3.5 ± 0.1 at 80:20 compared to 5.5 ± 0.5 at 20:80, respectively.

Discussion

This study suggests that *P. radiata* seedlings are well adapted to use both NH_4^+ and NO_3^- as sources of N, which may partly explain the success of this species over a wide range of ecological conditions. This is consistent with a large body of evidence that shows most species grow better with a mixture of ammonium and nitrate rather than either source alone (e.g. van Den Driessche 1971; Cox and Reisenauer 1973; Bigg and Daniel 1978; Haynes and Goh 1978; Chapin et al. 1987; Marschner 1995; Warren and Adams 2002a; Rothstein and Cregg 2005). In a previous study, McFee and Stone (1968) showed that *P. radiata* performed poorly when supplied with nitrate as the sole source of N. Although we did not include a nitrate-only control, previous research on other species suggests that sole use of nitrate results in lower productivity. When nitrate is supplied as the sole N source for *P. pinaster* growth is reduced to a greater extent than that explained by slower uptake or lower photosynthetic rates (Warren and Adams 2002a). Among the likely reasons for this are nutrient imbalances (Haynes and Goh 1978; Marschner 1995), greater synthesis of organic acids to restore imbalance of hydroxyl ions (Raven and Smith 1976; Warren and Adams 2002a) and excessive carbon loss to the growing media (Vuorinen et al. 1995).

In this study, seedlings of *P. radiata* developed more rapidly under nitrate- compared to ammonium-dominated N supply. Differences in productivity were at least partially explained by greater photosynthetic rates in plants grown in nitrate-dominated solutions. Similarly, Rothstein and Cregg (2005) found that photosynthesis rates of *Abies fraseri* declined markedly under ammonium compared to nitrate-dominated N supply. Bloom et al. (1989) suggested that nitrate assimilation was not competitive with carbon fixation in barley plants grown at high irradiance suggesting that chloroplast electron transport has a capacity beyond that immediately required for carbon fixation. Similarly, Zerihun et al. (1998) in a study of energy costs of N form acquisition showed that nitrate assimilation in well-illuminated leaves might not be much more expensive than ammonium acquisition. Thus, additional energetic costs associated with nitrate nutrition might be offset by excess reductants supplied from a surplus in electron transport (Bloom et al. 1989) or by up-regulation of photosynthesis as suggested by Rothstein and Cregg (2005) and also by the results of this study.

Few studies have characterised the influence of N form on biomass partitioning in forest species (e.g. Heiskanen 2005; Bauer and Berntson 2001), and we are unaware of any investigation considering N form after accounting for the effect of plant size. Using allometric analysis we found that partitioning to root and stems, and root:shoot ratio was influenced by N supply regime but not by N form. This result is relevant as there is an on-going debate as whether N form may (e.g. Kruse et al. 2003, 2010) or may not (e.g. Zerihun et al. 1998) influence shoot–root biomass partitioning.

Seedlings growing under ammonium-dominated solutions were smaller, exhibited luxurious consumption of N and lower N use efficiency. Several authors have reported luxurious consumption of N associated with ammonium nutrition (McFee and Stone 1968; van Den Driessche 1971; van Den Driessche and Dangerfield 1975; Haynes and Goh 1978; Flaig and Mohr 1991; Lavoie et al. 1992; Malhi et al. 1988; Warren and Adams 2002a). Independent of N form, photosynthesis rates are known to be closely related to foliar nitrogen (Field and Mooney 1986; Walcroft et al. 1997; Grassi et al. 2002; Ripullone et al. 2003), which is explained by the high proportion of total nitrogen partitioned to the carboxylating enzyme Rubisco (Sage and Pearcy 1987; Evans 1989; Warren and Adams 2002b; Takashima et al. 2004). Considering N form, the results of the study show that photosynthetic rates were lower and foliar nitrogen greater in plants growing under ammonium rather than nitrate-dominated solutions, suggesting that N partitioning to active Rubisco decreased while N storage increased with ammonium nutrition. Warren and Adams (2002a) showed that Rubisco concentration remained constant in seedlings of *P. pinaster* supplied with ammonium, nitrate or a mixture, while foliage N concentration increased with ammonium nutrition, providing further evidence of N storage associated with ammonium nutrition in conifers (e.g. Flaig and Mohr 1991; Lavoie et al. 1992; Kronzucker et al. 1997). Although we found that ammonium-dominated plants showed enhanced N uptake, and nitrogen seems to accumulate in leaves without a concomitant effect on photosynthesis and growth, it is possible that some other factor became growth-limiting. The element that is most likely to become limiting is phosphorus for which uptake is strongly pH-dependent. However, this seems unlikely as plant level foliage phosphorus concentrations at the end of the experiment ranged from 0.255 and

0.429%, which markedly exceed the value of 0.12% thought to be optimal for *P. radiata* (Turner and Lambert 1986).

There is an on-going discussion whether foliage sucrose, foliage protein concentration or plant/shoot N controls the biomass partitioned between roots and shoots (Andrews et al. 2007; Hermans et al. 2007). Hermans et al. (2007) argues that plant root:shoot increases with N deficiency as shoot N concentration decreases, and also noted that nitrate content in leaves is directly related to N supply and negatively correlated to the carbon partitioned to the root. Hermans et al. (2007) propose that an increase in shoot sucrose leads to an increase in plant root:shoot when sucrose can be translocated to the root. Andrews et al. (2007), on the other hand, proposes that leaf soluble protein concentration drives the root:shoot ratio regardless of N form (NO_3^-, NH_4^+, glutamine, among others). Hermans et al. (2007) and Andrews et al. (2007) positions are reconciled by recognising that carbon partitioning between shoots and roots are co-limited by the availability of C and N substrates, and that the shoot C:N ratio, determines the relative partitioning of C for shoot metabolism or phloem export. Plants growing under ammonium-dominated solutions in our study were smaller, exhibited luxurious consumption of N, had a greater N concentration and did not exhibit changes in C partitioning to roots or shoots compared to other mixtures of ammonium and nitrate. Only the N supply regime changed the proportion of carbon partitioned to roots. It seems likely that not all C assimilated through photosynthesis and not all N content at the leaf is involved as a signal for carbon allocation. Plants growing in the ammonium-dominated solutions exhibited high foliage N concentration and they did not exhibit smaller root:shoot ratios, possibly as leaf soluble proteins were uniform across N-form treatments (Andrews's hypothesis). On the other hand, plants growing in nitrate-dominated solutions exhibited greater photosynthesis rates compared to other mixtures of ammonium and nitrate and did not exhibit differences in C partitioned to roots, indicating that probably not all C assimilated, maybe shoot sucrose only, is involved in signalling root:shoot partitioning (Herman's hypothesis). The carbon fraction partitioned to roots in this study was independent of N form suggesting that unravelling drivers behind carbon allocation require further experiments involving shoot and root C and N fractionation of plants growing under different N sources.

Ammonium uptake was about twofold greater than that of nitrate (on a molar basis) at comparable concentrations in nutrient solutions. Kronzucker et al. (1997) proposed that the ability to use ammonium and nitrate depends on the species successional stage, with early successional species growing better on nitrate (more disturbed soils) and late successional species growing better on ammonium (less disturbed soils). The empirical evidence strongly supports this theory (e.g. Krajina et al. 1973; Hangs et al. 2003; Chen et al. 2005). Thus, the ratio of ammonium to nitrate uptake on a molar basis might be used as an index of this successional ability, with values close to 1 mol mol^{-1} (equal uptake of ammonium and nitrate) occurring in agricultural species and values in the range between 2.9 and 20 mol mol^{-1} for conifers (Kronzucker et al. 1997, 2003). This would suggest that *P. radiata* occurs at the lower end of the range given for conifers from Kronzucker's work, partly explaining its enhanced early growth on disturbed sites such as old-fields and pastures (Skinner and Attiwill 1981) where light is not usually as limiting as it is in mature undisturbed forests. Therefore, conifer species could be ordered using the ammonium:nitrate uptake ratio (Φ, mol mol^{-1}) as a surrogate for their capacity to use nitrate, e.g. *P. radiata* ($\Phi = 2$, this study) < *Pseudotsuga menziesii* ($\Phi = 3$, Kronzucker et al. 2003) = *Pinus sylvestris* ($\Phi = 3$, Flaig and Mohr 1992) < *Picea abies* ($\Phi = 4$, Buchman et al. 1995) < *Pinus contorta* ($\Phi = 6$, Min et al. 2000) < *Pinus strobus* ($\Phi = 12$, Bauer and Berntson 2001) < *Picea glauca* ($\Phi = 20$, Kronzucker et al. 1997), among others.

Nitrate and ammonium uptake were independent of each other within the range of concentrations used in this study. Some studies suggest that high ammonium concentration will reduce nitrate reductase activity in shoots and roots and therefore inhibit nitrate uptake (Haynes and Goh 1978; Downs et al. 1993; Sagi and Lips 1998). Others, like Flaig and Mohr (1992), found that ammonium and nitrate were taken up at the same rate in seedlings of *P. sylvestris* as if they were applied separately suggesting that ammonium does not inhibit nitrate uptake (the same as this study) at least in longer-term studies than those involved in uptake kinetics.

Although pH was set to a value of between 5.0 and 5.5 on a weekly basis by changing the solutions, the pH did systematically vary in different treatments over the course of the week. We observed a decrease in ending pH with increasing N supply. In response to N form there was increase in pH with increasing [NO_3^-] and a decrease with increasing [NH_4^+]. Previous research has shown the pH of the nutrient solution changes the rate of plant uptake of ammonium versus nitrate (van Den Driessche 1978). However, results presented here clearly show that the relative rates of ammonium and nitrate uptake largely conformed to the ammonium and nitrate concentrations in nutrient solution. It is therefore likely that treatment variation in results was attributable to variation in N form and supply and not confounded by treatment variation in pH.

In conclusion, we examined the effects of N supply and N form on growth, uptake and photosynthetic characteristics of *P. radiata* seedlings grown at high irradiance. Plants grown in ammonium-dominated solutions were smaller, had lower photosynthetic rates and exhibited luxurious consumption of N. Uptake rates of ammonium were about twofold greater on a molar basis than those of nitrate at comparable concentrations, suggesting that *P. radiata* occurs at the lower end of the ammonium:nitrate uptake ratio expected for conifers (2.9–20 mol mol^{-1}). This may help to explain success of this species in fertile disturbed sites such as ex-pastures where nitrate may represent a high proportion of available N.

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