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Light regulation of root and leaf NO_3^- uptake and reduction in the floating macrophyte *Lemna minor*

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Summary

• The regulation of NO_3^- uptake kinetics and reduction in relation to long- and short-term changes in irradiance was explored in roots and photosynthetic tissues of *Lemna minor*.

• The NO_3^- uptake kinetics, nitrate reductase activity, plant morphology, chlorophyll and tissue NO_3^-, organic-N, starch and sugars were measured on roots and fronds of *L. minor* grown at four combinations of irradiance- and NO_3^- availability.

• Long-term acclimatizations paralleled those of terrestrial plants. Short-term changes in irradiance, however, changed frond NO_3^- uptake proportionally with frond chlorophyll and N content, indicating a relationship between responsiveness and the metabolic potential of the plants. Root uptake changed to balance the change in frond uptake keeping whole plant uptake varying by < 40%. Nitrate reductase activity was primarily located in the roots and was correlated with frond uptake, indicating a transport of NO_3^- from shoot to root before reduction.

• This study shows that irradiance can affect the contribution of root and leaf uptake by aquatic plants and that roots play a major role in NO_3^- reduction despite large foliar uptake.

Key words: acclimatization, allocation, floating macrophyte, irradiance, *Lemna minor*, nitrate reductase activity, nitrate uptake, root vs shoot uptake.

Introduction

The understanding of nitrate uptake and assimilation in plants and its regulation has advanced considerably during the past decade (Forde & Clarkson, 1999; Kaiser & Huber, 2001; Forde, 2002; Stitt *et al.*, 2002; Foyer *et al.*, 2003). Two of the main environmental factors of importance for the regulation of nitrate uptake and assimilation are nitrate and light (Forde & Clarkson, 1999). Nitrate induces both the transcription of nitrate transporters and of nitrate reductase (NR), the primary nitrate reducing enzyme (Forde & Clarkson, 1999; Forde, 2000). When nitrate is available in excess to plant demand, the transporters and nitrate reductase are feedback regulated by products of nitrate assimilation and the transporters possibly also by nitrate itself (Sivasankar & Oaks, 1996; Gojon *et al.*, 1998; Forde, 2000; Orsel *et al.*, 2002). Light affects nitrate uptake both as a signal activating transporters and nitrate reductase and as a resource affecting plant photosynthesis and growth (Cheng *et al.*, 1992; Lillo *et al.*, 1998; Forde, 2000; Klein *et al.*, 2000; Kaiser & Huber, 2001).

The effect of short-term changes in irradiance on nitrate uptake has been most intensely studied in micro-algae, where NO_3^- uptake as a function of irradiance has been shown to follow a saturation curve similar to the light–photosynthesis curve (Priscu *et al.*, 1991; Muggli & Smith, 1993; Kristiansen *et al.*, 1998). The increased NO_3^- uptake at increased irradiance is believed to be related to the increased photosynthetic rates that enhance the plants demand for nitrogen and at the same time provide the energy and carbon skeletons needed (Priscu *et al.*, 1991). Long-term effects of irradiance on nitrate uptake appear to be mediated through the effect of irradiance on plant growth rates and plant demand for nitrogen (Imsande & Touraine, 1994). Thus, plants grown at high irradiance have higher growth rates and higher nitrate uptake rates and reduction capacity, and vice versa.

In terrestrial plants, nitrate is taken up by the root and can be assimilated by both root and shoot (Gojon *et al.*, 1994). Thus, it has been suggested that the effect of photon flux density on root NO_3^- uptake is mediated through phloem-transported compounds, of which sugars are considered the most important in stimulating nitrate uptake, whereas amino acids such as glutamine and asparagine are considered important factors for downregulating uptake (Delhon *et al.*, 1996; Sivasankar & Oaks, 1996; Gojon *et al.*, 1998; Forde, 2000, 2002). In aquatic macrophytes both roots and shoots possess the ability to take up nutrients, including nitrate, although the relative importance of the two uptake routes differs with growth conditions and nutrient ion species (Barko & Smart, 1981; Thursby & Harlin, 1984; Cedergreen, 2002). The mechanisms regulating the balance between root and shoot uptake is not known in any details, although it appears that the relative availability of nutrients to root and shoot is of importance. However, effects of other environmental factors have only attracted little, if any, attention (Barko & Smart, 1981; Thursby & Harlin, 1984; Cedergreen, 2002; Cedergreen, 2003).

If aquatic macrophytes respond to increased irradiance in the same way as terrestrial plants do, it is to be expected that both short- and long-term changes in irradiance and an associated change in nitrogen demand by the plants, will be reflected in adjustments in shoot nitrate uptake and assimilation rates that are proportional to the change in photosynthesis. For root nitrate uptake two opposite-directed scenarios can be suggested. Uptake and assimilation may either increase in response to increased irradiance, as a consequence of increased flux of photosynthates from shoot to root, or decrease, as a consequence of increased flux of reduced N-compounds following an increased NO_3^- uptake and assimilation by the shoot. Nitrate reduction can potentially take place in both roots and shoots of aquatic macrophytes (Cedergreen, 2003). Considering the dependence of nitrate reduction on energy and carbon skeletons, however, shoot reduction is expected to be predominant, at least in situations of high shoot uptake.

It was the aim of this study to investigate the effect of irradiance on the division of nitrate uptake and reduction between root and shoot of an aquatic macrophyte. We wished to assess both short-term responses and the long-term acclimatizations to changes in irradiance. As a model organism we used *Lemna minor*; a small floating macrophyte with a simple morphological organization consisting of one leaf-like frond and one unbranched root. *Lemna minor* is a cosmopolitan inhabitant of stagnant, nutrient-rich waters (Hillman, 1961). The floating nature of the plants allows easy manipulation of nitrate availability to root and frond while still keeping the plants under near-natural growth conditions. The long-term acclimatization of nitrate uptake and assimilation was assessed by growing the plants at combinations of high and low irradiance and two nitrate availabilities.

Materials and Methods

Plant culture

Lemna minor L. was collected from a small eutrophic pond in, Resenborg plantage, West Jutland, Denmark. Epiphytes were removed from the plants by washing with a 0.5% hypochlorite solution (Landolt & Kandeler, 1987), and a culture of the epiphyte-free plants was thereafter kept in artificial growth media in autoclaved Erlenmeyer flasks sealed with cotton wool. Once a week, a few fronds were transferred using a sterilized needle to new flasks with fresh autoclaved medium. The epiphyte-free cultures were kept in a growth cabinet at a temperature cycle of 20°C light/15°C dark, and a photon flux density of 400 μmol m^{-2} s^{-1} (PAR) provided by metal halide bulbs (Osram 250 W) in a 16 h light/8 h dark cycle. The growth medium was adapted from media described by Landolt and Kandeler (1987) and contained: 1.65 mol m^{-3} $MgSO_4$, 1.00 mol m^{-3} $CaCl_2$, 0.65 mol m^{-3} NaH_2PO_4, 0.50 mol m^{-3} K_2SO_4, 0.50 mol m^{-3} KNO_3, 0.16 mol m^{-3} K_2CO_3 and 27 mmol m^{-3} Fe-ethylenediaminetetraacetic acid (EDTA), 5.77 mmol m^{-3} H_3BO_3, 1.13 mmol m^{-3} $MnCl_2$, 0.19 mmol m^{-3} $ZnSO_4$, 0.08 mmol m^{-3} $CuSO_4$ and 0.05 mmol m^{-3} Na_2MoO_4.

For experiments, plants from the epiphyte-free culture were placed in 10 l growth medium in containers (70 × 30 × 10 cm, length × width × height) that were placed in a growth cabinet under the following irradiance and NO_3^- regimes: (1) 300 μmol m^{-2} s^{-1} (PAR) and 500 mmol m^{-3} NO_3^- (HIHN); (2) 300 μmol m^{-2} s^{-1} (PAR) and 10 mmol m^{-3} NO_3^- (HILN); (3) 30 μmol m^{-2} s^{-1} (PAR) and 500 mmol m^{-3} NO_3^- (LIHN); and (4) 30 μmol m^{-2} s^{-1} (PAR) and 10 mmol m^{-3} NO_3^- (LILN). The NO_3^- concentrations were measured and adjusted daily and the growth medium changed twice a week. Plant density was kept at *c.* 100 g fresh wt m^{-2} by frequent harvesting and the plants were allowed to grow under treatment conditions for 3 wk before determination of morphology, growth rate, NO_3^- uptake kinetics, nitrate reductase activity (NRA), C : N ratio and tissue content of nitrate, starch, sugars and chlorophyll.

Morphology and growth rate

Plant fresh weight and dry weight, frond surface area and weight, and root length and weight were determined on 10 samples from each treatment at the end of a 2-wk growth period following the 3-wk preincubation period. Each sample included *c.* 100 *L. minor* plants. Fresh weight was determined after gently blotting the plants with tissue paper. Frond surface area (one sided) was determined as projected area. Root length was measured on plants floating on the water surface of a narrow transparent Perspex container (1.5 cm wide). Root diameter was measured under a microscope (×100) on 20 roots from each treatment. Root surface area

was calculated from mean root length and diameter assuming cylindrical roots. Specific root area (SRA) and specific leaf area (SLA, one sided) were calculated from root and frond surface area divided by dry weight, using one-sided leaf area. Root : frond dry weight ratio was determined by separating roots and fronds before drying at 85°C for 24 h.

The relative growth rate of the *L. minor* populations were measured as net dry weight increment of five samples from each treatment and calculated as

$$(\log_e W_2 - \log_e W_1)t^{-1}$$

(W_1 and W_2 are initial and final population dry weight and t is incubation time in days). The initial dry weight was calculated from measured fresh weight and the dry weight : fresh weight ratio determined at the start of the experiment for plants comparable to the experimental material.

Nitrate uptake kinetics

Nitrate uptake kinetics was determined for roots and entire plants at high (300 μmol m^{-2} s^{-1}) and low irradiance (30 μmol m^{-2} s^{-1}). Plants were exposed to the irradiance used during uptake experiments 3 h before the onset of the dark period preceding the day where the kinetic measurements were made. On that day the measurements were initiated 5 h into the light cycle. This procedure allowed us to ignore a possible light-independent diurnal cycle in NO_3^- uptake (Peuke & Jeschke, 1998). Root uptake was determined by placing the plants in a net fixed to a circular (0.5 cm high, 5 cm diameter) Perspex frame floating on polystyrene foam, keeping the lower surface of the frond just above the water surface, leaving only the roots in the medium. Uptake by entire plants was measured on free-floating plants. Frond uptake rate was calculated as the difference in uptake rate of roots and entire plants.

Measurements were initiated by incubating 0.5–1.5 g f. wt plant material in 60 ml fresh growth medium containing 20 mmol NO_3^- m^{-3}. A magnetic follower ensured mixing of the solution. After 15 min, the NO_3^- concentration was determined spectrophotometrically at 202 nm by cycling part of the media through a flow-through cuvette (UV-1201 spectrophotometer; Shimadzu Corporation, Nagoya, Japan) (Oscarson *et al.*, 1988). The NO_3^- concentration was measured every 10 min until no further change in concentration was observed. The NO_3^- concentration of the incubation medium was then increased by adding aliquots of NO_3^- stock solutions (added as KNO_3). The nitrate concentration was increased in steps of *c.* 50 mmol m^{-3} to a final concentration of 200 mmol m^{-3}. At each concentration, NO_3^- depletion was followed and uptake rates were estimated from changes in NO_3^- concentration with time.

After uptake measurements the plants were freeze-dried for 24 h and weighed. The uptake capacity (V_{max}) was determined as the mean uptake rate at 200 mmol m^{-3} and the minimum concentration of uptake (C_{min}) as the concentration where net uptake was zero. The half saturation constant ($K_{1/2}$) was determined by subtracting C_{min} from the NO_3^- concentration (S) and fitting the data to the Michaelis-Menten equation:

$$V = V_{max}(S - C_{min})/(K_{1/2} + (S - C_{min}))$$

by nonlinear regression.

Tissue parameters: NO_3^- content, organic nitrogen and carbon content, NRA, starch, sugars and chlorophyll

For measurements of tissue nitrate reductase activity and content of nitrate, starch, sucrose and reducing sugars, plants were harvested and stored in liquid nitrogen or freeze-dried before analysis. Root and frond tissue nitrate content was determined on extracts of 10–20 mg freeze-dried plant material extracted for 30 min in 10 ml distilled water at 80°C, after which the samples were filtered and nitrate was measured using a Lachat flow injection analyser (QuikChem Method 10-107-04-1-C; QuikChem, Milwaukee, WI, USA). Tissue organic nitrogen and carbon content were determined on homogenized, freeze-dried material using a Fison NA 1500 CHN analyser (Fison, Rodano, Italy).

The nitrate reductase activity (NRA) was determined as the actual nitrate reductase activity (NR_{act}), analysed in the presence of Mg^{2+}, which is believed to prevent the dephosphorylation of inactivated nitrate reductase, and as maximal nitrate reductase activity (NR_{max}), analysed with EDTA as a substitute for Mg (MacKintosh *et al.*, 1995). The analysis was performed according to Scheible *et al.* (1997a), using an extraction buffer with 50 mol m^{-3} *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.5), 5 mol m^{-3} magnesium acetate, 1 mol m^{-3} EDTA, 2.5 mol m^{-3} dithiothreitol (DTT), 0.5% (w : v) bovine serum albumin (BSA) and 1% (w : v) polyvinylpolypyrrolidone (PVPP)-40 and an assay-mix consisting of 30 mol m^{-3} phosphate buffer (pH 7.5), 10 mol m^{-3} KNO_3, 0.30 mol m^{-3} NADH and 10 mol m^{-3} MgAc for NR_{act} or 5 mol m^{-3} EDTA for NR_{max}. Incubation time was 20 min The activation state of nitrate reductase was calculated as (NR_{act}/NR_{max}) × 100%. Because nitrate reductase activity can vary with buffer pH, effects of pH on NR_{max} was tested for plants grown at high irradiance and nitrate availability at six phosphate buffered assay-mix pH-levels ranging from 5.5 to 8.5. In addition NRA activity using NADPH as a reductant was tested according to Cathala *et al.* (1992). The results showed that an assay pH of 7.5 was optimal and that the activity of NADPH-reduced NRA was very low (max. 6%) and could be ignored.

Starch content was determined as follows: 2 ml cold 80% ethanol was added to *c.* 15 mg freeze-dried plant material and allowed to extract for 15 min at 80°C. The samples were centrifuged at 1000 ***g*** for 3 min and the supernatant removed and kept for analysis of sugars. The ethanol extraction procedure

was repeated until no chlorophyll could be detected in the supernatant (two or three times). Subsequently, the pellet was dried at 80°C, 2 ml distilled water added and the samples autoclaved 20 min at 120°C. After cooling, 0.5 ml HEPES (pH 6.9) with 100 EU α-amylase was added, the samples shaken lightly and incubated for 2 h at 37°C. Another 0.5 ml α-amylase solution was then added and the samples incubated for 2 h at 37°C before centrifugation at 1000 ***g*** for 3 min The maltose in the supernatant was converted to glucose by amyloglycosidase and the final glucose concentration was then measured by the hexokinase method (Carroll *et al.*, 1970): 10 µl amyloglycosidase, prepared by dialysing 1.4 ***g*** amyloglycosidase in 100 ml distilled water against a 35 mM phosphate buffer (pH 7.5) containing 0.1 mM DTT for 2 × 10 h at 4°C, was mixed with 20 µl of the supernatant and incubated for 15 min at 37°C. Iodonitrotetrazolium violet, 100 µl, was added and after 15 min at 37°C, OD at 492 nm was measured using an Automated Microplate Reader EL_x800 (Bio-Tek Instruments, Winooski, VT, USA). Starch standards were added before autoclaving and maltose and glucose standards added before mixing with amyloglycosidase.

Sucrose, glucose and fructose were measured on the ethanol extracts according to Walch-liu *et al.* (2000). Chlorophyll was removed from the extracts by adding about 10 mg ml^{-1} of activated charcoal, swirling and centrifuging at 18 000 ***g*** (centrifuge 5417C; Eppendorf, Hamburg, Germany). For sucrose analysis, 0.1 ml of the supernatant was mixed with 0.1 ml invertase solution (50 units ml^{-1}) and 0.8 ml 100 mol m^{-3} sodium acetate. The mixture was incubated for 2 h in a 30°C water bath to convert sucrose to glucose and fructose. For determination of glucose and fructose 0.2 ml supernatant was mixed with 0.8 ml 100 mol m^{-3} sodium acetate (pH 4.8).

Sucrose and the reducing sugar concentrations were subsequently determined by adding 5 ml colour solution (30 mol m^{-3} hydroxybenzoic acid hydrazide, 50 mol m^{-3} trisodium citrate, 10 mol m^{-3} $CaCl_2$, and 500 mol m^{-3} NaOH), boiling for 10 min in a water bath and measured spectrophotometrically at 415 nm (UV-160A UV-visible spectrophotometer; Shimadzu) on the cooled samples.

Chlorophyll *a* and *b* were measured spectrophotometrically on ethanol extracts of freeze-dried plant material at 665 nm and 649 nm and calculated according to $((Abs_{665\ or\ 649} - Abs_{750})E)/AW$, where E is the extraction volume in ml, A the absorption coefficient of chlorophyll in ethanol (83.4 l g^{-1} cm^{-1} for chlorophyll *a* and 53.8 l g^{-1} cm^{-1} for chlorophyll *b*) (Wintermans & De Mots, 1965) and W is the plant dry weight.

Statistics

The kinetic and tissue parameters were analysed by a three-way analysis of variance (ANOVA) where plant part (root, frond/root and entire plant), growth conditions (HIHN-, HILN-, LIHN- and LILN-grown plants) and measuring irradiance (HI, LI) constituted the factors. If interactions between factors were significant ($P < 0.05$), data were split into two-way and thereafter one-way ANOVA. Treatment differences for parameters tested by one-way ANOVA were tested by Fisher's LSD test ($P < 0.05$). Homogeneity of variance was tested by Cochran's test.

Results

Effects of growth conditions on growth and morphology

Lemna minor grown at different combinations of irradiance and NO_3^- availability displayed distinct differences in population growth rate, morphology and physiology (Table 1). Net population growth rate was 0.32 ± 0.02 d^{-1} for plants grown at high irradiance and high NO_3^- availability, decreased to 0.21 ± 0.04 d^{-1} for NO_3^- sufficient plants at low irradiance, and further to 0.13 ± 0.01 d^{-1} and 0.12 ± 0.01 d^{-1} for plants grown at low NO_3^- availability and in high and low irradiance, respectively. Morphologically, the high-N plants grown at low irradiance had large, thin and dark green fronds and short, thin roots, resulting in a large specific leaf and root area, and a small root dry weight proportion (Table 1). Physiologically plants grown at high NO_3^- availability and low irradiance had the highest total organic N and tissue NO_3^- concentrations, and the highest chlorophyll content. Starch reserves, however, were by far the lowest, as was the C : N ratio (Tables 1 and 2). By contrast, plants grown at low NO_3^-

Table 1 Phenotypic characteristics of *Lemna minor* grown for 3 wk in combinations of high and low irradiance and N availability

Plant growth conditions	Dry weight : fresh weight ratio	Root percentage of entire plant	SLA (cm^2 g^{-1} frond d. wt)	SRA (cm^2 g^{-1} root d. wt)	Chlorophyll *a* + *b* (mg g^{-1} plant d. wt)	Root N (% of root d. wt)	Frond N (% of frond d. wt)	C : N ratio
HIHN	0.07 ± 0.00^b	13 ± 2^b	322 ± 24^b	3140 ± 393^b	7.3 ± 0.1^c	4.4 ± 0.0^d	3.2 ± 0.1^c	12
HILN	0.12 ± 0.00^d	27 ± 1^d	245 ± 13^a	2624 ± 152^a	0.6 ± 0.1^a	0.9 ± 0.0^a	0.7 ± 0.0^a	50
LIHN	0.05 ± 0.01^a	6 ± 1^a	758 ± 26^c	5342 ± 801^c	17.3 ± 0.1^d	3.9 ± 0.1^c	3.9 ± 0.1^d	10
LILN	0.08 ± 0.00^c	20 ± 2^c	334 ± 18^b	3012 ± 259^b	4.8 ± 0.3^b	1.3 ± 0.0^b	1.4 ± 0.0^b	27

HIHN, High irradiance, high N; HILN, high irradiance, low N; LIHN, low irradiance, high N; LILN, low irradiance, low N; SLA, specific leaf area; SRA, specific root area. Significant differences between phenotypes were tested by Fisher's LSD test ($P < 0.05$, $n = 3$–10).

Table 2 Tissue parameters of roots and fronds of four *Lemna minor* phenotypes grown for 3 wk in combinations of high and low irradiance and N availability

Plant part	Plant growth conditions	Uptake conditions	Tissue NO_3^- (μmol g^{-1} d. wt)	NR_{act} (μmol NO_2^- g^{-1} d. wt h^{-1})	NR_{max} (μmol NO_2^- g^{-1} d. wt h^{-1})	Starch (mg g^{-1} d. wt)	Sucrose (mg g^{-1} d. wt)	Reducing sugars (mg g^{-1} d. wt)
Root	HIHN	HI	432	36.0 ± 0.4^b	38.8 ± 0.3^b	54	12	12
		LI	528 ± 3	26.6 ± 3.0^a	31.3 ± 0.9^a	8 ± 1	13 ± 1	11 ± 2
	HILN	HI	8 ± 0^a	1.9 ± 0.1^b	0.0 ± 0.0^a	35 ± 0^a	22 ± 2^a	24 ± 2^a
		LI	13 ± 0^b	1.3 ± 0.1^a	2.6 ± 3.7^a	39 ± 1^b	22 ± 1^a	25 ± 1^a
	LIHN	HI	505	15.0 ± 0.4^b	15.1 ± 0.7^a	32	13 ± 0	12 ± 0
		LI	1153	12.5 ± 1.5^b	4.4 ± 6.3^a	3	17	14
	LILN	HI	16 ± 0^a	2.8 ± 0.5^a	0.8 ± 0.8^a	30 ± 2^b	15 ± 2^a	16 ± 2^a
		LI	50^b	5.5 ± 0.1^b	6.0 ± 5.3^a	22 ± 0^a	13 ± 2^a	12 ± 2^a
Frond	HIHN	HI	42 ± 1^a	2.3 ± 0.2^b	2.7 ± 0.0^b	219 ± 1^b	49 ± 2^b	54 ± 2^b
		LI	70 ± 3^b	0.6 ± 0.1^a	0.3 ± 0.3^a	72 ± 9^a	36 ± 2^a	42 ± 3^a
	HILN	HI	4 ± 3^a	0.5 ± 0.1^a	0.0 ± 0.0^a	392 ± 7^a	46 ± 2^b	44 ± 1^b
		LI	2 ± 0^a	0.1 ± 0.1^b	0.5 ± 0.9^a	390 ± 8^a	26 ± 2^a	27 ± 4^a
	LIHN	HI	64 ± 3^a	1.2 ± 0.1^b	1.6 ± 0.4^a	134 ± 5^b	38 ± 1^b	45 ± 1^b
		LI	88 ± 5^b	0.0 ± 0.1^a	1.2 ± 1.2^a	29 ± 5^a	36 ± 3^b	41 ± 4^b
	LILN	HI	4 ± 1^a	0.0 ± 0.0^a	0.0 ± 0.0^a	197 ± 14^a	26 ± 1^a	29 ± 1^b
		LI	7 ± 1^b	0.4^a	0.8 ± 0.9^a	258 ± 24^b	25 ± 1^a	31 ± 4^b

The parameters were measured after 6–10 h of either high irradiance (HI) or low irradiance (LI). Conditions: HIHN, high irradiance, high N; HILN, high irradiance, low N; LIHN, low irradiance, high N; LILN, low irradiance, low N. Nr_{act}, actual nitrate reductase activity; Nr_{max}, maximal nitrate reductase activity. Letters indicate significant differences between irradiance treatments within each phenotype for root and frond, respectively (Fisher's LSD test, $P < 0.05$, $n = 1$–3).

availability and high irradiance were small, thick and pale with long thick roots, resulting in a low specific root- and leaf area and a high root dry weight proportion. These plants had low N (< 1.4%) and chlorophyll content and a very low tissue NO_3^- concentration, but had a high starch content and, as a result, a high C : N ratio. The plants grown under the two regimes not mentioned above, high irradiance and high NO_3^- availability and low irradiance and low-N, showed traits that were somewhere in between the two populations described (Tables 1 and 2). For plants from all four treatments, the nitrate reductase activity varied from near zero to about 40 μmol NO_2^- g^{-1} d. wt h^{-1} and followed the population growth rates, being highest for plants grown at high irradiance and high-N and lowest in the two low-N plant populations (Table 2).

NO_3^- uptake kinetics

The NO_3^- uptake rate of entire *L. minor* plants from all treatments followed Michaelis–Menten kinetics, as did the NO_3^- uptake by roots of plants grown at high irradiance ($r^2 = 0.48$–0.99, $n = 6$–19 per curve). The NO_3^- uptake kinetics by roots of low irradiance plants, however, followed Michaelis–Menten kinetics only when measured at low irradiance ($r^2 = 0.78$–0.98, $n = 9$–14 per curve). When measured at high irradiance, uptake rates were initially high but then decreased as uptake measurements proceeded, resulting in low uptake rates at high substrate concentrations, which were the final concentrations measured.

For entire plants the $K_{1/2}$ for NO_3^- uptake were in average 4.0 ± 2.6 mmol NO_3^- m^{-3} and ranged from 2.1 ± 0.5 mmol NO_3^- m^{-3} for HIHN-grown plants measured at low irradiance to 7.2 ± 2.3 mmol NO_3^- m^{-3} for LIHN-grown plants measured at high irradiance. The minimum concentrations of uptake (C_{min}) were, on average, 4.55 ± 2.72 mmol m^{-3}, ranging from 0.97 ± 0.66 mmol m^{-3} in LILN-grown plants measured at low irradiance to 8.45 ± 1.69 mmol m^{-3} in HILN-grown plants measured at high irradiance. Hence for all plants at both high and low irradiance and at growth N-concentrations, NO_3^- uptake rates were 70–100% of V_{max}. The only exception was HILN-grown plants where the uptake rate measured at high irradiance was only 26% of V_{max} at 10 mmol NO_3^- m^{-3} as a result of the large C_{min}. Thus, because at growth NO_3^- concentrations uptake rates are close to V_{max}, only uptake capacity will be used when comparing uptake kinetics of different tissues under different irradiance regimes, bearing in mind the exception of the HILN plants.

A close coupling between the relative growth rate of the plants and the NO_3^- uptake capacity was observed (Fig. 1). The short-term effects of irradiance on the NO_3^- uptake capacity by entire plants differed between treatments. While measuring irradiance had no effect on the NO_3^- uptake capacity of plants grown at high irradiance, uptake capacity of plants grown at low irradiance increased by 24% for LIHN-grown plants in response to increased measuring irradiance, while it decreased by 37% for LILN-grown plants (Fig. 1 and Table 3). Root uptake capacity, however, varied considerably

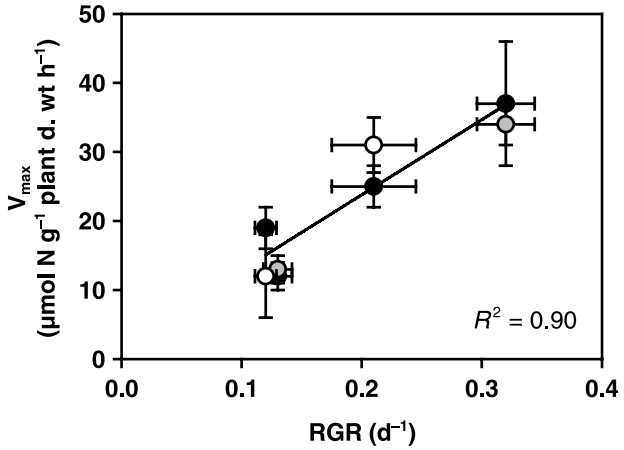

Fig. 1 Nitrate uptake capacity (V_{max}) of *Lemna minor* plants as a function of relative growth rates for plants grown at different irradiance and nitrate availability. Filled circles, uptake rates measured at growth irradiance; open circles, uptake measured at higher than ambient irradiance; tinted circles, uptake measured at lower than ambient irradiance. Data are mean ± SD; n = 5.

Table 3 Nitrate uptake capacity of entire plants and roots of *Lemna minor* grown for 3 wk in combinations of high and low irradiance and N availability

Uptake conditions		Uptake capacity of plants acclimatized to the following growth conditions (μmol NO_3^- g^{-1} whole-plant d. wt h^{-1})			
Plant part	Irradiance	HIHN	HILN	LIHN	LILN
Entire plants	HI	37 ± 9^{c}	12 ± 2^{b}	31 ± 4^{d}	12 ± 6^{b}
	LI	34 ± 3^{c}	13 ± 2^{b}	25 ± 3^{c}	19 ± 3^{c}
Roots	HI	2 ± 4^{a}	13 ± 3^{b}	1 ± 2^{a}	3 ± 3^{a}
	LI	13 ± 5^{b}	5 ± 1^{a}	15 ± 4^{b}	8 ± 3^{b}

The parameters were measured after 6–10 h of either high irradiance (HI) or low irradiance (LI). Conditions: HIHN, high irradiance, high N; HILN, high irradiance, low N; LIHN, low irradiance, high N; LILN, low irradiance, low N.

Letters indicate significant differences between entire plant and root NO_3^- uptake in high- and low irradiance for each phenotype (Fisher's LSD test, $P < 0.05$, n = 5–6).

in response to measuring irradiance. Plants grown at high irradiance increased root V_{max} by a factor 6 or decreased it with 50% when measured at low irradiance, for the high-N and low-N plants, respectively, while the plants grown at low irradiance decreased root uptake capacity when measured under high irradiance with app. 93% and 65% in the high- and low-N plants (Table 3). Because the uptake capacity of entire plants did not change by more than 40% in response to changed irradiance (Table 3), it was mainly the allocation of uptake between root and frond that changed. Hence, when expressed in absolute terms (μmol N g^{-1}plant d. wt h^{-1}), a negative, linear coupling (r^2 = 0.92) between the change in frond uptake rate and the change in root uptake rate was found (Fig. 2a). Focusing on fronds only, the change in NO_3^- uptake capacity of fronds when measured at high compared with low irradiance was proportional to both chlorophyll- and N-content of the frond (Fig. 2b,c).

Nitrate reductase activity and sugars

The activation state of nitrate reductase was, on average, 65 ± 34% and showed no consistent pattern in relation to growth treatments (data not shown). In the following NR_{act} will be used for nitrate reductase activity (NRA). Analysing the correlation between NRA and nitrate uptake capacity of roots and fronds separately revealed a significant correlation between frond uptake capacity and root and frond NRA (Fig. 3a,c), while there was no correlation between root uptake capacity and NRA (Fig. 3b,d). The nitrate reductase activity of the roots was in all treatments more than five times higher than the frond tissue activity, and the nitrate concentration was two to 13 times higher (Table 2). Because root biomass was low relative to frond biomass, the contribution of root NRA to total plant NRA was lower than indicated above, but was still, in average, about four times higher than frond NRA.

The sucrose and starch content was about 10 times higher in frond tissue than in root tissue and for both compartments the content tended to be lower at low treatment irradiance (Table 2). Also, in response to short-term changes in irradiance, an inverse relation between starch content and irradiance was observed, except for the LILN-grown plants where the starch content declined by about 25% when the plants were switched from low to high irradiance (Table 2).

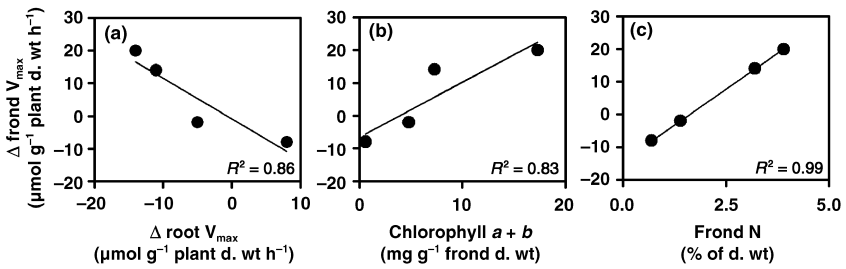

Fig. 2 The difference between frond uptake capacity (V_{max}) of *Lemna minor* measured in high (300 μmol m^{-2} s^{-1}) and low irradiance (30 μmol m^{-2} s^{-1}) expressed on a whole-plant dry weight basis as a function of (a) the difference in root V_{max} measured under high and low irradiance, (b) the chlorophyll $a + b$ content of the fronds and (c) the N content of the fronds.

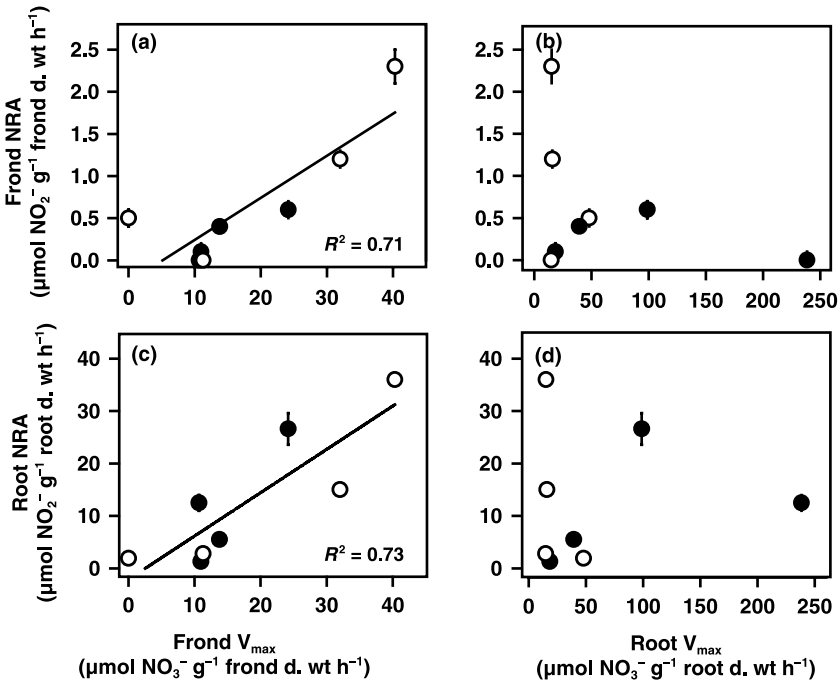

Fig. 3 The correlations between root and frond nitrate reductase activity (NRA) and root and frond uptake capacity (V_{max}) measured in high (open circles) and low (closed circles) irradiance. There was a significant correlation between frond V_{max} and both root and frond NRA ($P < 0.01$). NRA is mean ± SD; $n = 3$.

Discussion

A significant result of this study is the observation that changes in light regime alone can affect the balance between root and frond NO_3^- uptake of *L. minor*, even though the external NO_3^- concentration is the same for the two tissues. These results show that the balance between root and frond (or leaf) uptake by aquatic macrophytes might be regulated in a complex way and not only by the relative nutrient availability to roots and leaves, although the latter is of significance (Carignan, 1982). It is not clear from our study why *L. minor* responds by changing the relative uptake of nitrate through root and frond while maintaining total uptake, but the data suggest that the observed changes are coupled to photosynthesis. For fronds, the change in uptake capacity in response to short-term irradiance increase was closely coupled to frond chlorophyll and N content (Fig. 2b,c) and thereby, presumably, to the ability of the plant to absorb light energy and use it in carbon assimilation (Behaeghe & Impens, 1995). The regulation of root uptake is probably mediated by reduced N-compounds as root uptake decreased with increasing frond uptake, and vice versa (Fig. 2a), as is commonly observed for the regulation of root uptake in terrestrial plants (Cooper & Clarkson, 1989; Sivasankar & Oaks, 1996; Gojon *et al.*, 1998; Forde, 2002). The fact that the whole plant uptake was relatively constant under both irradiance regimes (Fig. 1, Table 3), indicates that whole plant demand for N did not change significantly within the timeframe of the experiment.

The changes in nitrate reductase activity in roots and fronds in response to changed irradiance did not reflect the changes in uptake of the two tissues, although NRA have been shown to be regulated by the same compounds as the high-affinity nitrate uptake system (Sivasankar & Oaks, 1996; Gojon *et al.*, 1998; Forde, 2000; Kaiser & Huber, 2001; Orsel *et al.*, 2002). On a whole-plant basis, nitrate reductase activity did increase with whole-plant uptake, indicating that the enzyme activity balanced plant uptake (or vice versa). Looking at root and frond NRA separately, however, revealed, on average, 16 times higher root NRA compared with frond NRA on a dry weight basis, comprising about 78% of total plant NRA, which contrasts with the 52% average contribution of roots to whole-plant uptake. This, combined with the correlation between root NRA and frond uptake (Fig. 3c), suggests that NO_3^- reduction in *L. minor* mainly takes place in the root. This is also supported by the close coupling between NRA and internal NO_3^- concentration, which corresponds well with the various findings of NO_3^- induction of nitrate reductase activity (Li & Oaks, 1993, 1995; Samuelson *et al.*, 1995). The lack of correlation between NRA and sugar content, which was approximately constant compared with the variations in NRA (Table 2), does not eliminate the possibility that sugars played a role in regulating NRA, as it is the flux, rather than the content, which is of importance (Delhon *et al.*, 1996; Kaiser & Huber, 2001). It must also be kept in mind that the NRA measured *in vitro* indicates the potential for nitrate reduction, but that nitrate reduction *in situ* can be substrate limited and is therefore not necessarily reflected in the *in vitro* measurements (Kaiser *et al.*, 2000). Studies of both *in vitro* and *in situ* nitrate reduction in *Lemna gibba* have, however, shown *in vitro* measurements to reflect *in situ* measurements relatively well (Ingemarsson, 1987).

The finding of considerably higher NRA in roots compared with fronds and the indications that NO_3^- taken up by fronds is at least partly reduced in roots are interesting, as the proximity of photosynthetically derived energy and carbon skeletons in fronds was expected to favour frond NO_3^- reduction over root reduction (Raven, 1985), at least as long as NO_3^- was taken up by fronds. This pattern was found for lettuce, where application of N to leaves was shown to increase leaf NRA while decreasing root NRA, even when grown at ample root NO_3^- supply (Hufton *et al.*, 1996). There are, to our knowledge, no studies documenting a transport of NO_3^- from shoots to roots, but reversed xylem flow is known from both roots and fruits exhibiting low transpiration rates (Lang & Thorpe, 1989; Sakuratani *et al.*, 1999). Reversed xylem flow could take place in *L. minor*, where leaf transpiration potentially can be covered by leaf water uptake. In submerged aquatic plants, where the transpiration is absent, acropetal water transport does take place and is closely coupled to light (Pedersen & Sand-Jensen, 1993). Further knowledge of the water movement in floating macrophytes is, however, needed to confirm the existence of reversed xylem flow. High root NRA was also observed in another *Lemna* species, *L. gibba*, under both natural conditions and when grown with 500 µmol NO_3^- m^{-3} (Cedergreen, 2003). The ecological significance of reducing NO_3^- in roots as opposed to shoots is unclear, but it seems, judging from the NRA distribution, to be a common trait among several aquatic species capable of taking up NO_3^- by shoots, and might be a plesiomorphic character of the terrestrial ancestors (Cedergreen, 2003).

The long-term acclimatizations of *L. minor* to the growth conditions showed that nitrate uptake was rate-saturated at the growth conditions being closely coupled to population growth rate. This corresponds well to previous studies showing a balanced response by the plants to ensure optimal use of the resources available (Cooper & Clarkson, 1989; Imsande & Touraine, 1994; Bazzaz, 1997). In addition to acclimatization of the nitrate uptake apparatus, *L. minor* acclimatized to the light and nitrate regimes through various morphological and physiological changes. These acclimatizations also followed the patterns known from terrestrial plants, where growth under low irradiance generally results in larger specific leaf area and less biomass allocation to roots, increased chlorophyll content on a dry weight basis and a smaller accumulation of starch than growth under high irradiance (McDonald *et al.*, 1986; Minotta & Pinzauti, 1996; Reich *et al.*, 1998), whereas acclimatizations to low nitrogen availability generally result in lower specific leaf area and more biomass allocated to roots, lower N, NO_3^- and chlorophyll content and higher starch reserves (Tables 1 and 2) (McDonald *et al.*, 1986; Rufty *et al.*, 1988; Skillman & Osmond, 1998; Peterson *et al.*, 1999). We consider the functional significance of these acclimatizations to be analogous to those of terrestrial plants, although the functional significance of an increased allocation of biomass to roots in response to either low N availability or high irradiance might seem less obvious for a plant such as *L. minor* that can take up nutrients through both root and frond. However, considering the investment needed to obtain a particular nutrient-assimilating surface by *L. minor*, this would be lower when biomass and energy are invested in root tissue rather than frond tissue (Cedergreen, 2002). Thus, in a situation of increased demand for assimilating surfaces for nutrient uptake, investment in root tissue would be more beneficial than investment in frond tissue.

In conclusion, the present study showed that short-term changes in irradiance changed the balance between root and frond NO_3^- uptake of *Lemna minor*, but that whole plant uptake remained approximately balanced to population growth rates. The high nitrate reductase activities of roots compared with fronds, suggests, together with the measured uptake rates by roots and fronds, that NO_3^- is transported from fronds to roots for reduction. This contrasts what is observed for terrestrial plants (Gojon *et al.*, 1994) and the division of NO_3^- uptake and reduction between roots and fronds of *L. minor* might represent an uptake and assimilation pattern unique to floating aquatic plants.

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References

Barko JW, Smart RM. 1981. Sediment-based nutrition of submersed macrophytes. *Aquatic Botany* **10**: 339–352.

Bazzaz FA. 1997. Allocation of resources in plants: State of the science and critical questions. In: Bazzaz FA, Grace JB, eds. *Plant resource allocation.* London, UK: Academic Press, 1–37.

Behaeghe NT, Impens I. 1995. Leaf nitrogen content as a predictor of photosynthetic capacity in ambient and global change conditions. *Journal of Biogeography* **22**: 177–183.

Carignan R. 1982. An empirical model to estimate the relative importance of roots in phosphorus uptake by aquatic macrophytes. *Canadian Journal of Fisheries and Aquatic Science* **39**: 243–247.

Carroll JJ, Smith N, Babson AL. 1970. A colorimetric serum glucose determination using hexokinase and glucose-6-phosphate dehydrogenase. *Biochemical Medicine* **4**: 171–180.

Cathala N, Conejero G, Gojon A, Passama L, Robin P. 1992. Determination of the distribution of three nitrate reductase isoforms in soybean seedlings by chromatography and a simple method based on assay conditions. *Physiologia Plantarum* **85**: 541–548.

Cedergreen N. 2002. Nitrogen uptake by the floating macrophyte *Lemna minor*. *New Phytologist* **155**: 285–292.

Cedergreen N. 2003. Nitrate reductase activity in roots and shoots of aquatic macrophytes. *Aquatic Botany* **76**: 203–212.

Cheng C, Acedo GN, Gristinsin M, Conkling MA. 1992. Sucrose mimics the light induction of *Arabidopsis* nitrate reductase gene transcription. *Proceedings of the National Academy of Sciences, USA* **89**: 1861–1864.

Cooper HD, Clarkson DT. 1989. Cycling of amino-nitrogen and other nutrients between shoots and roots in cereals – a possible mechanism integrating shoot and root in the regulation of nutrient-uptake. *Journal of Experimental Botany* **40**: 753–762.

Delhon P, Gojon A, Tillard P, Passama L. 1996. Diurnal regulation of NO_3^- uptake in soybean plants IV. Dependence on current photosynthesis and sugar availability to the roots. *Journal of Experimental Botany* **47**: 893–900.

Forde BG. 2000. Nitrate transporters in plants: structure, function and regulation. *Biochimica et Biophysica Acta* **1465**: 219–235.

Forde BG. 2002. Local and long-range signalling pathways regulating plant responses to nitrate. *Annual Review of Plant Biology* **53**: 203–224.

Forde BG, Clarkson DT. 1999. Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. *Advances in Botanical Research* **30**: 1–90.

Foyer CH, Parry MAJ, Noctor G. 2003. Markers and signals associated with nitrogen assimilation in higher plants. *Journal of Experimental Botany* **54**: 585–593.

Gojon A, Dapoigny L, Lejay L, Tillard P, Rufty TW. 1998. Effect of genetic modification of nitrate reductase expression on $^{15}NO_3^-$ uptake and reduction in *Nicotiana* plants. *Plant, Cell & Environment* **21**: 43–53.

Gojon A, Plassard C, Bussi C. 1994. Root/ shoot distribution of NO_3^- assimilation in herbaceous and woody species. In: Roy J, Garnier E, eds. *A whole plant perspective on carbon–nitrogen interactions*. The Hague, The Netherlands: SPB. Academic Publishing, 131–148.

Hillman WS. 1961. The Lemnaceae, or duckweeds. A review of the descriptive and experimental literature. *Botanical Review* **27**: 221–287.

Hufton CA, Besford RT, Wellburn AR. 1996. Effects of NO ($+NO_2$) pollution on growth, nitrate reductase activities and associated protein content in glasshouse lettuce grown hydroponically in winter with CO_2 enrichment. *New Phytologist* **133**: 495–501.

Imsande J, Touraine B. 1994. N demand and the regulation of nitrate uptake. *Plant Physiology* **105**: 3–7.

Ingemarsson B. 1987. Nitrogen utilization in *Lemna* I. Relations between net nitrate flux, nitrate reduction, and in vitro activity and stability of nitrate reductase. *Plant Physiology* **85**: 856–859.

Kaiser WM, Huber SC. 2001. Post-translational regulation of nitrate reductase: mechanism, physiological relevance and environmental triggers. *Journal of Experimental Botany* **52**: 1981–1989.

Kaiser WM, Kandlbinder A, Stoimenova M, Glaab J. 2000. Discrepancy between nitrate reduction rates in intact leaves and nitrate reductase activity in leaf extracts: what limits nitrate reduction in situ? *Planta* **210**: 801–807.

Klein D, Morcuende R, Stitt M, Krapp A. 2000. Regulation of nitrate reductase expression in leaves by nitrate and nitrogen metabolism is completely overridden when sugars fall below a critical level. *Plant, Cell & Environment* **23**: 863–871.

Kristiansen S, Farbrot T, Kuosa H, Myklestad S, Quillfeldt C, Hv. 1998. Nitrogen uptake in the infiltration community, an ice algal community in Antarctic pack-ice. *Polar Biology* **19**: 307–315.

Landolt E, Kandeler R. 1987. Physiological characteristics. In: Landolt E, Kandeler R, eds. *The family of Lemnaceae – a monographic study*. Zürich, Switzerland: Stiftung Rübel, 54–113.

Lang A, Thorpe MR. 1989. Xylem, phloem and transpiration flows in a grape: application of a technique for measuring the volume of attached fruits to high resolution using Archimedes' principle. *Journal of Experimental Botany* **40**: 1069–1078.

Li X-Z, Oaks A. 1993. Induction and turnover of nitrate reductase in *Zea mays*. *Plant Physiology* **102**: 1251–1257.

Li X-Z, Oaks A. 1995. The effect of light on the nitrate and nitrite reductase in *Zea mays*. *Plant Science* **109**: 115–118.

Lillo C, Provan F, Appenroth KJ. 1998. Photoreceptors involved in the regulation of nitrate reductase in *Spirodela polyrhiza*. *Journal of Photochemistry and Photobiology* **44**: 205–210.

MacKintosh C, Douglas P, Lillo C. 1995. Identification of a protein that inhibits the phosphorylated form of nitrate reductase from spinach (*Spinacia oleracea*) leaves. *Plant Physiology* **107**: 451–457.

McDonald AJS, Ericsson A, Lohammer T. 1986. Dependence of starch storage on nutrient availability and photon flux density in small birch *Betula pendula* (Roth). *Plant, Cell & Environment* **9**: 433–438.

Minotta G, Pinzauti S. 1996. Effects of light and soil fertility on growth, leaf chlorophyll content and nutrient use efficiency of beech (*Fagus sylvatica* L.) seedlings. *Forest Ecology and Management* **86**: 61–71.

Muggli DL, Smith WO. 1993. Regulation of nitrate and ammonium uptake in the Greenland Sea. *Marine Biology* **115**: 199–208.

Orsel M, Filleur S, Fraisier V, Daniel-Vedele F. 2002. Nitrate transport in plants: which gene and which control? *Journal of Experimental Botany* **53**: 825–833.

Oscarson P, Ingemarsson B, Ugglas M, Larsson CM. 1988. Characteristics of NO_3^- uptake in *Lemna* and *Pisum*. *Plant and Soil* **111**: 203–205.

Pedersen O, Sand-Jensen K. 1993. Water transport in submerged macrophytes. *Aquatic Botany* **44**: 385–406.

Peterson AG, Ball JT, Lue Y, Field CB, Curtis PS, Griffin KL, Gunderson CA, Norby RJ, Tissue DT, Forstreuter M, Rey A, Vogel CS, CMEAL Participants. 1999. Quantifying the response of photosynthesis to changes in leaf nitrogen content and leaf mass per area in plants grown under atmospheric CO_2 enrichment. *Plant, Cell & Environment* **22**: 1109–1119.

Peuke AD, Jeschke WD. 1998. The effects of light on induction, timecourses, and kinetic patterns of net nitrate uptake in barley. *Plant, Cell & Environment* **21**: 765–774.

Priscu JC, Lizotte MP, Cota GF, Palmisano AC, Sullivan CW. 1991. Comparison of the irradiance response of photosynthesis and nitrogen uptake by sea ice microalgae. *Marine Ecology Progress Series* **70**: 201–210.

Raven JA. 1985. Regulation of pH and generation of osmolarity in vascular plants: a cost–benefit analysis in relation to efficiency of use of energy, nitrogen and water. *New Phytologist* **101**: 25–77.

Reich PB, Tjoelker MG, Walters MB, Vanderklein DW, Buschena C. 1998. Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Functional Ecology* **12**: 327–338.

Rufty TW, Huber SC, Volk RJ. 1988. Alternations in leaf carbohydrate metabolism in response to nitrogen stress. *Plant Physiology* **88**: 725–730.

Sakuratani T, Aoe T, Higuchi H. 1999. Reverse flow in roots of *Sesbania rostrata* measured using the constant power heat balance method. *Plant, Cell & Environment* **22**: 1153–1160.

Samuelson M, Ohlén E, Lind M, Larsson CM. 1995. Nitrate regulation of nitrate uptake and nitrate reductase expression in barley grown at different nitrate: ammonium ratios at constant relative nitrogen addition rates. *Physiologia Plantarum* **94**: 254–260.

Scheible WR, Gonzáles-Fontes A, Morcuende R, Lauerer M, Geiger M, Glaab J, Gojon A, Schulze E, Stitt M. 1997. Tobacco mutants with a decreased number of functional *nia* genes compensate by modifying the diurnal regulation of transcription, post-translational modification and turnover of nitrate reductase. *Planta* **203**: 304–319.

Sivasankar S, Oaks A. 1996. Nitrate assimilation in higher plants: The effect of metabolites and light. *Plant Physiology and Biochemistry* **34**: 609–620.

Skillman JB, Osmond CB. 1998. Influence of nitrogen supply and growth irradiance on photoinhibition and recovery in *Heuchera americana* (Saxifragaceae). *Physiologia Plantarum* **103**: 567–573.

Stitt M, Müller C, Matt P, Gibon Y, Carillo P, Morcuende R, Scheible WR, Krapp A. 2002. Steps towards and integrated view of nitrogen metabolism. *Journal of Experimental Botany* **53**: 959–970.

Thursby GB, Harlin MM. 1984. Interaction of leaves and roots of *Ruppia maritima* in the uptake of phosphate, ammonia and nitrate. *Marine Biology* **83**: 61–67.

Walch-liu P, Neumann G, Bangerth F, Engels C. 2000. Rapid effect of nitrogen form on leaf morphogenesis in tobacco. *Journal of Experimental Botany* **51**: 227–237.

Wintermans JFGM, De Mots A. 1965. Spectrophotometric characteristics of chlorophylls *a* and *b* and their phenophytins in ethanol. *Biochimica et Biophysica Acta* **109**: 448–453.