Nitrogen uptake by the floating macrophyte *Lemna minor*

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Summary

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• Both roots and leaves of free-floating plants can potentially take up nutrients. In this study, the ability and relative contribution of roots and fronds for N uptake by the floating macrophyte *Lemna minor* was investigated.

• The NH_4^+ and NO_3^- uptake kinetics of roots and fronds were measured on plants acclimated to three different NH_4NO_3 concentrations.

• *Lemna* had the capacity to take up NH_4^+ and NO_3^- through both roots and fronds; uptake kinetics for the two tissue types were comparable on an area basis. The overall contribution of root and frond to whole-plant uptake, estimated from measured kinetic characteristics, varied depending on plant N status (the root contribution increased from 32 to 73% for N-satiated and N-depleted plants, respectively).

• The shift in the balance between root and frond contribution to whole-plant uptake resulted from a 1.5–38 times greater increase in the area-specific uptake capacity and affinity of roots relative to fronds, combined with a larger decrease in the minimum concentration for uptake (C_{min}) for roots than fronds. At the morphological level, root–frond surface area increased with declining N supply, which might be beneficial to the plants since the area return per unit biomass invested was nine times greater for roots than for fronds.

Key words: acclimation, floating macrophyte, growth, *Lemna minor*, nitrogen uptake kinetics, root vs shoot uptake.

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Introduction

Among floating-leaved macrophytes, the free-floating species constitute a widely distributed group that flourish in mesotrophic and eutrophic lakes (Sculthorpe, 1967), where nutrient availability in the bulk water is sufficient to satisfy the nutrient requirement of the plants. A few of the free-floating species, such as the Wolffioideae of the family Lemnaceae, do not develop roots and have to take up all nutrients needed for growth thought the leaf or frond surface. Other species, such as the globally widely distributed genera *Lemna* and *Spirodela*, develop roots and should potentially have the ability to take up nutrients through both leaves and roots. However, the quantitative importance of roots in nutrient uptake by these species seems negligible based on the few reports available (Hillman, 1961; Muhonen *et al.*, 1983; Ice & Couch, 1987; Landolt *et al.*, 1987), although more circumstantial evidence suggests that roots might play a significant role (Landolt *et al.*, 1987; Oscarson *et al.*, 1988).

Structurally, the roots of *Lemna* and *Spirodela* appear to be similar to other monocotyledons, with well-developed vascular tissues (Landolt *et al.*, 1987), providing a transport pathway for ions from root to frond. In addition, observed morphological response patterns to nutrient availability, such as increased root : frond dry weight ratio and root length in response to nutrient deficiency (Landolt *et al.*, 1987; Oscarson *et al.*, 1988), indicate a role for roots in nutrient uptake. These response patterns are similar to those observed for terrestrial plants and have been suggested to be compensatory responses that enhance nutrient uptake efficiency in situations where nutrients are in short supply (Robinson, 1986).

For submerged rooted macrophytes, which do possess the ability to take up nutrients by both roots and shoots (Barko *et al.*, 1991), studies have shown that the contribution by roots to total phosphorus uptake might be dependent on nutrient availability in the sediment relative to the availability in the bulk water (Carignan, 1982), and it has been suggested

that the same applies to N uptake (Barko *et al.*, 1991). Floating macrophytes are unique in the sense that both roots and leaves are exposed to the same nutrient source. The relative contribution of roots and leaves to total nutrient uptake will therefore depend on: (1) the division of biomass between roots and leaves; (2) the specific surface area ratio of the two plant parts; and (3) differences in uptake kinetics between the two tissue-types.

For the free-floating plants, optimization of nutrient uptake in response to availability might be obtained through physiologically based changes in uptake kinetics. Acclimative changes at the morphological level could be expected because of the difference in construction costs between root and frond tissue. In this context, it might be more cost-efficient for floating-leaved plants such as *Lemna* and *Spirodela* to invest in root tissue rather than in fronds if roots have a greater surface area to biomass ratio relative to fronds, and thereby a greater area return per unit biomass invested in roots than in leaves. This could apply even if the frond has better uptake kinetic characteristics than the root.

The aim of this study was first to test whether roots of *Lemna minor* contribute significantly to N uptake and second to evaluate the potential for morphological and physiological acclimation by *Lemna* roots and fronds to nutrient availability with focus on NH_4^+ and NO_3^- uptake, and finally, to assess the relative importance of morphological and physiological acclimation by *Lemna* to nutrient stress.

Materials and Methods

Plant culture

Lemna minor L. was collected from a small, eutrophic pond in East Jutland, Denmark. The plants were brought to the laboratory, rinsed and placed in an artificial growth medium in 5 l tanks (30 cm long, 15 cm wide and 20 cm high). The tanks were kept in a growth cabinet at day/night temperatures of 20°C/15°C and light was provided by metal halide bulbs (Osram 250 W) at a photon flux density of 300 µmol m^{-2} s^{-1} (PAR) in a 16 h light/8 h dark cycle. The growth medium contained: 1.65 mol m^{-3} MgSO$_4$, 1.00 mol m^{-3} CaCl$_2$, 0.65 mol m^{-3} NaH$_2$PO$_4$, 0.50 mol m^{-3} K$_2$SO$_4$, 0.16 mol m^{-3} K$_2$CO$_3$, 27 mmol m^{-3} Fe-ethylenediaminetetraacetic acid (Fe-EDTA), 5.77 mmol m^{-3} H$_3$BO$_3$, 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$_2$MoO$_4$. Three N treatments were used: 10, 100 and 500 mmol N m^{-3} with N added as NH$_4$NO$_3$ (1 : 1 molar). The medium was changed every second day. Epiphytes were removed as necessary and always the day before N uptake kinetics were measured. The plants were allowed to grow for 2 wk under treatment conditions before morphology, growth and N uptake were measured. Plant densities were kept at about 200 g fresh weight (f. wt) m^{-2} by frequent harvesting.

Ammonium and nitrate uptake kinetics

Ammonium and nitrate uptake rates were measured under light and temperature conditions similar to growth conditions. Uptake kinetics were determined by incubating 0.5–1.5 g f. wt of plant material in a beaker with 100 ml N-free growth medium. A magnetic follower ensured adequate mixing of the solution. Root uptake was determined by placing the plants on a net fixed to a floating Perspex frame, keeping the lower surface of the frond above the water surface and leaving only the roots in the medium. Uptake by the lower frond surface was measured on floating fronds, which had had their roots removed 24 h prior to measurements.

After a preincubation period of 30 min, the NH$_4^+$ or NO$_3^-$ concentration of the incubation medium was increased in steps to a maximum concentration of 250 mmol N m^{-3} by adding aliquots of a N stock solution (growth medium with either (NH$_4$)$_2$SO$_4$ or KNO$_3$). After each addition, the N depletion was followed by withdrawing water samples (2 × 1 ml) at regular intervals and analysing for NH$_4^+$ or NO$_3^-$. At the end of the experiment, the plants were harvested and dry weight determined after 24 h at 85°C. Uptake rates were calculated from plant dry weight and changes in NH or NO$_3^-$ concentrations with time.

The uptake capacity (V_{max}) was determined as the mean of uptake rates obtained at saturating substrate concentrations. The initial slope of the uptake curve, α, was used as an affinity parameter and was determined by linear regression of uptake rate vs NH$_4^+$ or NO$_3^-$ concentration for low concentrations of the two ions. The concentration at which net uptake is zero, C_{min}, was determined by fitting the data to a modified Michaelis–Menten function (Barber, 1979):

$$V = V_{max}(S - C_{min})(K + (S - C_{min}))^{-1}$$

where V is uptake rate, $K + C_{min}$ is the Michaelis–Menten half-saturation constant, and S is the substrate concentration.

In order to assess longitudinal variation in ammonium uptake of *Lemna* roots, the relationship between root surface area and ammonium uptake capacity was determined for plants where the root length had been manipulated by cutting the roots to lengths of 1.0, 2.0, 3.5 and 5 cm 24 h before uptake measurements. Uptake was measured at 80 mmol NH$_4^+$ m^{-3} with the plants floating on the water surface.

Morphology and growth

Plant fresh weight and dry weight, frond surface area and weight, and root length and weight were determined at the end of the 2-wk growth period on five samples from each treatment. Each sample included *c.* 100 *Lemna* 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. Specific root area (SRA) and specific leaf area (SLA, one-sided) was calculated from root and frond surface area and dry weight. Root : frond weight ratio was determined by separating roots and fronds prior to drying at 85°C for 24 h.

Net population relative growth rate was measured as net dry weight increment of five samples from each treatment and calculated as $(\log_e W_2 - \log_e W_1)t^{-1}$, where 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 with the experimental material.

Chemical analysis

Ammonium concentration of water samples was measured spectrophotometrically using the salicylate method (Quikchem Method no. 10-107-06-3-A; Lachat Instruments, Milwaukee, WI, USA). Nitrate concentration was determined spectrophotometrically following the procedure of Oscarson *et al.* (1988). Tissue N concentration was measured on freeze-dried material using an NA 1500 CHN analyser (Fison, Rodano, Italy).

Statistical procedures

The kinetic parameters were analysed by a three-way analysis of variance (ANOVA) where plant part (root, frond), N species (NO_3^- or NH_4^+) and growth condition (low-, intermediate- and high-N treatment) 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 morphological and kinetic parameters tested by one-way ANOVA were tested by Fisher's LSD test ($P < 0.05$), and data given as mean ± SE. Homogeneity of variance was tested by Cochran's test.

Results

Population growth rate and tissue N concentration was higher for *Lemna* grown at high N than for plants grown at low N. Relative growth rate increased from 0.04 ± 0.01 d^{-1} at low-N to 0.20 ± 0.02 d^{-1} at intermediate-N, and 0.31 ± 0.02 d^{-1} at high-N, and the tissue N concentration increased from 0.40 ± 0.03 mmol N g^{-1} dry wt to 0.71 ± 0.05 mmol N g^{-1} dry wt and 1.95 ± 0.12 mmol N g^{-1} dry wt.

The N uptake measurements showed that *Lemna*, grown under the conditions used in this study, was able to take up NO_3^- and NH_4^+ through both roots and fronds (Fig. 1, Table 1). The relation between uptake rate and external N

Table 1 Uptake kinetic parameters for NO_3^- and NH_4^+ of *Lemna minor* grown for 2 wk at three concentrations of NH_4NO_3

Growth-N (mmol NH_4NO_3 m^{-3})	NO_3^- kinetic parameters			NH_4^+ kinetic parameters		
	V_{max} (μmol m^{-2} root or frond h^{-1})	α (μmol m^{-2} h^{-1} mmol^{-1} m^{-3})	C_{min} (mmol m^{-3})	V_{max} (μmol m^{-2} root or frond h^{-1})	α (μmol m^{-2} h^{-1} mmol^{-1} m^{-3})	C_{min} (mmol m^{-3})
5						
Root	173 ± 12b	10.6 ± 0.8de	13.3 ± 1.9a	790 ± 65b	36.7 ± 7.2bc	0 ± 0a
Frond	151 ± 18ab	3.0 ± 0.2c	22.6 ± 0.4b	1502 ± 42d	62.5 ± 2.7d	3.6 ± 0.8b
50						
Root	169 ± 14b	9.5 ± 0.7d	10.5 ± 1.0a	845 ± 18b	41.5 ± 4.8bc	0.5 ± 0.2a
Frond	112 ± 17a	1.8 ± 0.1b	16.8 ± 4.4ab	1216 ± 46c	53.2 ± 7.8cd	4.0 ± 0.3bc
250						
Root	218 ± 43bc	1.1 ± 0.1a	66.6 ± 62c	161 ± 4a*	6.4 ± 1.4a*	10.1 ± 1.2d*
Frond	290 ± 8c	14.9 ± 2.8e	12.3 ± 1.4a	760 ± 6b	34.8 ± 4.5b	7.2 ± 2.3cd

Data are means ± SE of uptake capacity (V_{max}), initial slope of the uptake curve (α) and the concentration at which net uptake is zero (C_{min}). Identical letters within columns indicate means with no statistically significant difference (Fisher's LSD-test, $P > 0.05$, $n = 3$). *Two replicas only.
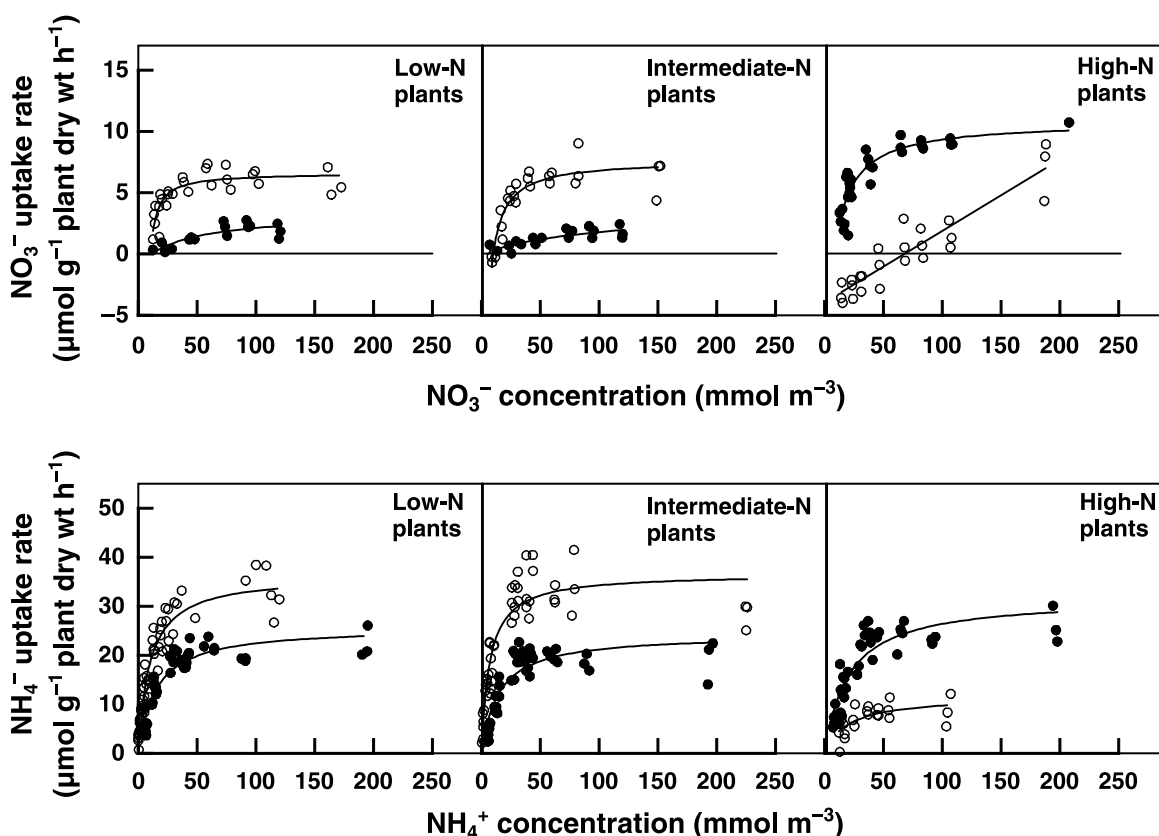
Fig. 1 Uptake of NO_3^- and NH_4^+ by roots (open circles) and fronds (closed circles) of *Lemna minor* grown for 2 wk at three concentrations of NH_4NO_3 (low-N, 5 mmol NH_4NO_3 m^{-3}; intermediate-N, 50 mmol NH_4NO_3 m^{-3}; and high-N, 250 mmol NH_4NO_3 m^{-3}). The uptake rates are expressed on whole-plant dry weight basis. Uptake data were fitted to a modified Michaelis–Menten model (Barber, 1979), except for root uptake of high-N grown plants, where linear regression was used.

concentrations followed saturation kinetics, except for root NO_3^- uptake by high-N grown plants, which was linearly coupled to the NO_3^- concentration within the range tested (Fig. 1). Assuming that the measured uptake rates of roots and fronds apply for intact plants, the data presented in Fig. 1 shows that the contribution of roots to plant NH_4^+ and NO_3^- uptake was higher for N-depleted plants (73%, no NO_3^- uptake due to a high C_{min}) than for plants grown at high N availability (32%) (Fig. 1).

The area-specific uptake capacity, V_{max}, differed significantly between roots and fronds, and between N species (NH_4^+ and NO_3^-) and was affected by growth condition (three-way ANOVA: $F_{1,23} = 122$, $F_{1,23} = 1093$, $F_{2,23} = 43$; $P < 0.01$ for root vs frond, N species and treatment; $F_{interactions}$, 19–137, $P < 0.01$). Irrespective, of growth conditions, however, the NH_4^+ uptake capacity of fronds was significantly greater than uptake capacity of roots and the capacity of both roots and fronds declined with increased N availability during growth (Table 1). For NO_3^- uptake, no difference between root and frond uptake capacity was observed, but there was a significant effect of growth conditions (Table 1, two-way ANOVA: $F_{1,12} = 0.56$, $P = 0.47$; $F_{2,12} = 11$, $P < 0.01$, no interaction)

with higher frond NO_3^- uptake capacity for high-N grown plants than for plants at low-N and intermediate-N. No effect of growth conditions on root NO_3^- uptake capacity was observed (Table 1). For both root and frond, the uptake capacity for NH_4^+ was 3–11 times higher than the capacity for NO_3^- uptake.

The initial slope of N uptake rate vs NO_3^- or NH_4^+ concentration provides an estimate of the affinity for the two N species. The affinity for NH_4^+, expressed on a surface area basis, declined with enhanced N availability during growth and was greater for fronds than for roots (Table 1, two-way ANOVA: $F_{1,11} = 22$, $F_{2,11} = 15$; $P < 0.01$). For NO_3^- uptake the response pattern to growth conditions was more complex. While the affinity was greater for roots than for fronds for low-N and intermediate-N grown plants (one-way ANOVA: $F_{1,4} = 101$, $F_{1,4} = 114$; $P < 0.01$), the opposite was observed for high-N plants (one-way ANOVA: $F_{1,4} = 15$, $P < 0.05$). In response to N availability, the affinity for NO_3^- declined with increasing availability for roots but increased for fronds, from 1.8 and 3.0 μmol N m^{-2} h^{-1} mmol^{-1} N m^{-3} at low availability to 14.9 μmol N m^{-2} h^{-1} mmol^{-1} N m^{-3} at high-N. The preference for NH_4^+ over NO_3^-, indicated by the higher

$r^2 = 0.90$
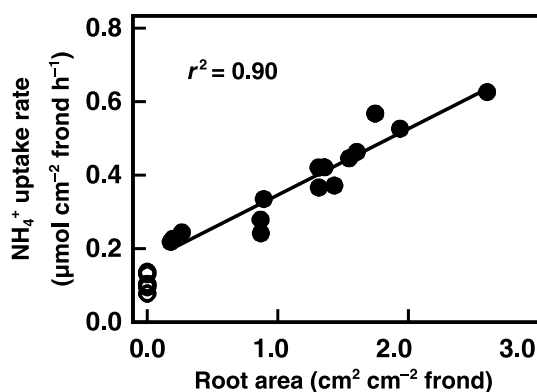
Fig. 2 The relationship between root area and NH_4^+ uptake by *Lemna minor*. Closed circles, uptake rates measured at 80 mmol NH_4^+ m^{-3}; open circles, uptake rates of fronds at concentrations ≥ 80 mmol NH_4^+ m^{-3}. Root area was manipulated by cutting roots 24 h prior to uptake measurements. All uptake rates are expressed on a frond-area basis.

uptake capacity for the former, was confirmed by the two to 30 times higher affinity for NH_4^+ than NO_3^-.

The kinetic parameters for root uptake reported above apply to intact roots. To assess the longitudinal variation in NH_4^+ uptake capacity, the plants were manipulated by cutting their roots to different lengths prior to measurements of uptake capacity. The results of these manipulative experiments showed that the NH_4^+ uptake capacity of *Lemna*, expressed on frond area basis, was linearly coupled to root area (Fig. 2, $r^2 = 0.90$, $n = 15$, $P < 0.01$), demonstrating that NH_4^+ was assimilated over the entire root surface. The estimated frond uptake (at zero root area) corresponded with the frond uptake rates reported above, indicating that no significant, short-term, up regulating of frond $NH4^+$ uptake capacity took place in response to removal of the root.

Plant morphology was significantly affected by N availability during growth (Table 2). The dry weight : fresh weight

ratio of entire plants, root : shoot dry weight ratio, frond dry weight, root dry weight and root length, decreased with increasing N availability during growth, whereas root diameter, root and frond surface area, specific root area (SRA) and specific leaf area (SLA) increased with increasing N availability (one-way ANOVA and Fisher's LSD test, $P < 0.05$). The root surface area, calculated from root diameter and root length, was high for low-N and high-N grown plants (0.146 cm^2 per plant and 0.171 cm^2 per plant, respectively) and lower (0.129 cm^2 per plant) for intermediate-N plants (Table 2). Root length was also shorter for intermediate N plants than for plants from the other treatments. Root surface area per unit frond area (one-sided) showed that root area was 2.5 and 2.3 times greater than frond area in plants acclimated to low and intermediate N availability, but only 1.7 times greater in plants acclimated to high N availability. This increase in root : frond area ratio of N-depleted plants was caused by an increased allocation of dry matter to roots, since the changes in SRA and SLA were parallel resulting in a fixed SRA : SLA ratio irrespective of growth conditions. Thus, as an average across treatments, investing one unit of biomass in root tissue returned a ninefold larger surface area than investing the same unit in frond tissue (linear regression: SRA = 9.11 × SLA + 0.26; $r^2 = 0.99$, $P < 0.001$).

Discussion

This study presents evidence showing that *Lemna minor* has the capacity to take up significant amounts of inorganic N through both roots and fronds. The rates of NH_4^+ and NO_3^- uptake measured were, both on weight and area basis, within the ranges reported for other aquatic species and for roots of terrestrial plants (Thursby & Harlin, 1982, 1984; Henriksen *et al.*, 1992; Lazof *et al.*, 1992; Rao *et al.*, 1993; Reidenbach & Horst, 1997; Gessler *et al.*, 1998). This contradicts the results of previous studies which, based on more indirect

Table 2 Morphology parameters for *Lemna minor* grown for 2 wk at three concentrations of NH_4NO_3

	N treatment (mmol NH_4NO_3 m^{-3})		
	5	50	250
Dry weight : fresh weight ratio	0.14 ± 0.00c	0.11 ± 0.00b	0.09 ± 0.00a
Root : frond biomass ratio	0.23 ± 0.00b	0.22 ± 0.02b	0.17 ± 0.00a
Root length (cm per plant)	3.3 ± 0.1c	2.6 ± 0.1a	3.1 ± 0.0b
Root diameter (µm)	140 ± 2a	156 ± 4b	176 ± 2c
Root surface area (cm^2 per plant)*	0.146 ± 0.003b	0.129 ± 0.004a	0.171 ± 0.002c
Root weight (mg dry wt per plant)	0.075 ± 0.002c	0.060 ± 0.002b	0.046 ± 0.002a
Specific root area (SRA) (cm^2 mg^{-1} dry wt)	1.959 ± 0.034a	2.140 ± 0.081a	3.730 ± 0.135b
Frond surface area (cm^2 per plant)†	0.059 ± 0.001a	0.056 ± 0.003a	0.104 ± 0.003b
Frond weight (mg dry wt per plant)	0.322 ± 0.007b	0.272 ± 0.017a	0.272 ± 0.08a
Specific leaf area (SLA) (cm^2 mg^{-1} dry wt)†	0.184 ± 0.001a	0.208 ± 0.003b	0.380 ± 0.002c

Data are means ± SE. Identical letters within rows indicate means with no statistically significant difference (Fisher's LSD-test: $P > 0.05$, $n = 5$; for root diameter $n = 20$).
*Calculated using mean root diameter and length. †One side.

measurements of nutrient uptake, have concluded that *Lemna* roots contribute only marginally to nutrient uptake (Hillman, 1961; Muhonen *et al.*, 1983; Ice & Couch, 1987; Landolt *et al.*, 1987). The roots of *Lemna* were shown to take up NH_4^+ at similar rates over the entire surface (Fig. 2), as has also been demonstrated for seedlings of some terrestrial plants (Lazof *et al.*, 1992; Henriksen *et al.*, 1992).

Although the primary role of roots is expected to be nutrient assimilation and the main role of foliage is inorganic carbon fixation, the area-specific N uptake kinetics of *Lemna* fronds were comparable to the uptake kinetics of roots. High nutrient uptake capabilities of leaves have also been reported for the submerged macrophytes *Ruppia maritima* and *Zostera marine*, where weight-specific uptake rates of phosphate and NH_4^+ by leaves was comparable to uptake rates by roots (Thursby & Harlin, 1982, 1984). The ability to take up nutrient ions by leaves is also known from terrestrial plants (Whitehead & Lockyer, 1987; Andersson, 1992; Wilson, 1992; Marschner, 1997; Wilson & Tiley, 1998), but will be of little quantitative significance under natural conditions as a result of the low nutrient availability in air and rainwater (Whitehead & Lockyer, 1987; Wilson & Tiley, 1998).

The contribution of roots to plant NH_4^+ and NO_3^- uptake was higher for N-depleted plants (73%, no NO_3^- uptake due to a high C_{min}) than for plants grown at high N availability (32%) (Fig. 1). This shift in importance of roots in N uptake was the result of a combination of physiological and morphological acclimations. Which of the two types of acclimation is the quantitatively more important depends on the basis for comparison. It is obvious from the data reported in Table 1 that the high C_{min} for NH_4^+ and NO_3^- of N-replete plants would prevent these plants from growing at low N concentrations without any physiological acclimation. Of the root contribution increase from 32% to 73% in N-depleted plants (a relative increase of about 130%), the increase in root surface area per unit frond area accounted for approximately 50% of the relative increase (from 1.7 to 2.5). The remaining 80% relative increase was then due to physiological acclimation. Hence, including the changes that did take place, both morphological and physiological acclimation contributed significantly to the increased root contribution of N-stressed plants.

Physiologically, the most important acclimation promoting increased N-contribution by roots of N-depleted plants was the greater increase in the area-specific uptake capacity and affinity of roots relative to fronds, and the decrease in the minimum concentration for uptake (C_{min}) of roots relative to fronds (Table 1). This difference between root and frond acclimation cannot be caused by differences in external N availability, since they are exposed to the same N source, and must therefore depend on internal factors not investigated in this study. Increased nutrient uptake capacity and affinity are responses typically observed for terrestrial plants in response to nutrient stress (Larsson, 1994; Ivashikina & Sokolov, 1997;

von Wiren *et al.*, 1997), and are believed to be caused by a relief of the feed back inhibition of uptake found in N-replete plants (von Wiren *et al.*, 1997; Forde & Clarkson, 1999), which may suggest that the changes in internal N concentrations in *Lemna* root and frond differ in response to changes in external N concentration.

The shift in the balance between root and frond uptake occurred for both NH_4^+ and NO_3^- uptake, but the physiological acclimation pattern contributing differed between the two ions. For NH_4^+, the most pronounced change in uptake kinetics was observed for roots with enhanced uptake capacity and lowered C_{min}. By contrast, NO_3^- kinetics changed for both roots and fronds, but both tissues appeared to be unable to maintain the ability to use NO_3^- at low N availability because of high C_{min}. Comparing the average daily N uptake of low- and medium-N plants, calculated from relative growth rate (RGR) and tissue N concentrations, however, gives a ratio of 1 : 10, parallel to the 1 : 10 ratio of N available in the growth medium. This indicates that NO_3^- was taken up by low-N plants during growth, and it is suggested that the high C_{min} measured for NO_3^- could be a result of handling stress, which is known to reduce net NO_3^- uptake in other plants (Delhon *et al.*, 1995). The preference for NH_4^+ agrees with observations by Ingemarsson *et al.* (1984), who showed that *Lemna gibba* growing on a mixed source of NH_4^+ and NO_3^- (1 : 2 molar) preferentially took up NH_4^+. Also, the more general differences between NH_4^+ and NO_3^- uptake kinetics found in our study, such as a higher uptake capacity and affinity, and a lower minimum concentration for uptake (C_{min}) for NH_4^+ compared with NO_3^- agree with the results of other studies (Ingemarsson *et al.*, 1984; Marschner *et al.*, 1991; Rao *et al.*, 1993; Gessler *et al.*, 1998).

At the morphological or developmental level, the greater contribution of root nutrient uptake by plants grown at low external N was consistent with a higher root biomass and a greater root : frond biomass ratio of these plants (0.25 and 0.23) relative to high-N grown plants (0.17). A higher root : shoot weight ratio is also commonly observed in N-deficient terrestrial plants (Reynolds & D'Antonio, 1996). By contrast to the response of *Lemna*, however, both total dry weight of the individual plant and root weight generally decrease with decreasing N supply in terrestrial plants (Reynolds & D'Antonio, 1996; Andrews *et al.*, 1999). The greater root : shoot ratio in N-depleted terrestrial plants is often interpreted as an enhanced investment in nutrient assimilation under nutrient stress (Robinson, 1986; Reynolds *et al.*, 1996) For a floating plant, however, where both leaves and roots participate in nutrient uptake and furthermore exploit the same nutrient pool, it could be hypothesized that the advantage associated with enhanced biomass allocation to root biomass under nutrient stress lies in the lower biomass, and thus carbon cost per unit surface area of roots compared with leaves. For *Lemna*, the specific root area was about nine times higher than the specific leaf area across N treatments

(Table 2), and the area return per unit biomass is therefore substantially higher when biomass is invested in roots rather than in fronds.

Despite the greater root biomass and the enhanced biomass allocation to roots in low-N grown plants, the absorbing root surface per plant was actually smaller for low-N compared with high-N grown plants. This was mainly due to the thinner roots of the former, since they were slightly longer than roots of high-N grown plants. Roots with a small diameter may be advantageous to terrestrial plants, allowing the root access to smaller soil pores in much the same way as mycorrhizal hyphae. However, for floating aquatic plants this does not apply: thin roots might be disadvantageous by restricting transport capacity if diameter and cross-sectional area of conducting tissue change in parallel to root diameter (Raven, 1999). However, thin roots might be more cost efficient that thick roots because of the greater surface area for nutrient uptake per unit root biomass. This was not the case for *Lemna*, however, as shown by the lower specific root area (cm^2 mg^{-1} dry wt) for low-N compared with high-N grown plants, indicating that tissue density was greater in thinner compared with thicker roots. In addition, roots with smaller diameters may have higher construction and maintenance costs (Eissenstat & Yanai, 1997).

In summary, this study shows that *L. minor* can acquire significant amount of inorganic N through both root and frond. *Lemna* acclimated morphologically and physiologically to N availability of the growth medium. At the morphological level, a greater root : shoot biomass ratio was observed for plants grown at low N. The advantage of this adjustment for a free-floating macrophyte, where both root and frond are exposed to the same medium, seems to be a much lower biomass investment per unit surface area for root than for fronds.

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