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# Nitrate reductase activity in roots and shoots of aquatic macrophytes

Nina Cedergreen*, Tom Vindbæk Madsen

*Department of Plant Ecology, University of Aarhus, Nordlandsvej 68, Risskov DK-8240, Denmark*

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**Abstract**

Aquatic macrophytes grow in an environment where nitrogen can be available at quantitatively significant concentrations around both roots and shoots. Because of the generally higher concentration of $NO_3^-$ in the bulk water compared to the sediment, and because of the energetic advantage of reducing $NO_3^-$ in shoots compared to roots, aquatic plants were expected primarily to reduce $NO_3^-$ in shoots. To test this hypothesis, nitrate reductase activity (NRA) of roots and shoots was measured in vitro on 18 aquatic macrophytes, representing 12 species. The plants were collected at seven locations with bulk water [$NO_3^-$] ranging from 0 to 550 μM and interstitial water [$NO_3^-$] ranging from 2 to 400 μM. All plants exhibited low NRA (<2 μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$) in both roots and shoots, except for the amphibious species, Cardamine amare, where shoot NRA reached 24 μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$. Higher NRA was however inducible, increasing 2–17 times in five selected species when induced with 500 μM $NO_3^-$. In 11 out of 17 plants with measurable NRA, the shoot:root NRA ratio was <1, showing that despite the proposed advantages of shoot $NO_3^-$ reduction, root reduction is apparently still of importance in aquatic plants.


*Keywords:* Nitrate reductase activity (NRA); Nitrogen nutrition; Ammonium inhibition; Shoot:root reduction

## 1. Introduction

Nitrate reductase, the primary nitrate reducing enzyme, can be present in both roots and shoots of terrestrial plants (Gojon et al., 1994). The balance of nitrate reduction between roots and shoots depends both on root:shoot biomass ratio and on the nitrate reductase activity of the two organs, and it varies with plant species, age and growth conditions

* Corresponding author. Present address: Department of Agricultural Sciences, The Royal Veterinary and Agricultural University (KVL), Højbakkegård Allé 9, DK-2630 Taastrup, Denmark. Tel.: +45-35-283-397; fax: +45-35-282-175.
*E-mail address:* ncf@kvl.dk (N. Cedergreen).

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(Andrews, 1986; Gojon et al., 1994). Studies on both $NO_3^-$ concentrations in xylem sap exudates and in vivo and in vitro nitrate reductase activity show that slow growing plant species, such as trees and woody scrubs, typically reduce most of their nitrate in roots (Andrews, 1986; Gojon et al., 1994). Fast growing crop species, on the other hand, tend to reduce a larger part of their $NO_3^-$ in the shoot, even when grown at similar external [$NO_3^-$] as the slow growing species (Andrews, 1986; Gojon et al., 1994; Claussen and Lenz, 1999). Common for a wide range of species is that shoot nitrate reductase activity (NRA) increase compared to root NRA as external $NO_3^-$ concentrations increase (Gojon et al., 1994; Samuelson et al., 1995). Hence, at low external $NO_3^-$ concentrations, $NO_3^-$ is primarily reduced in roots, and as $NO_3^-$ concentrations increase, root NRA reaches a plateau while shoot NRA keeps increasing (Gojon et al., 1994; Samuelson et al., 1995). It is therefore generally believed that the rate of $NO_3^-$ reduction in the shoot is determined by the flux of nitrate reaching the shoot (Gojon et al., 1994). This hypothesis is confirmed by studies on plants with $NO_3^-$ applied directly to leaves or grown in a $NO_x$ enriched atmosphere, showing a NRA increase in leaves with increased $NO_3^-$ or $NO_x$ supply rate (Hufton et al., 1996; Scheible et al., 1997b; Gojon et al., 1998). It is suggested that the species-specific difference in the relationship between NRA in root and shoot depends on plants $NO_3^-$ uptake rates (Gojon et al., 1994). Hence, slow growing plants with inherently low uptake capacity do not take up more $NO_3^-$ than can be reduced in the roots, compared to fast growing plant where high uptake rates exceed the reduction capacity of the roots (Gojon et al., 1994).

Unlike terrestrial plants, aquatic plants grow in an environment where nitrogen is present at quantitatively significant concentrations around both roots and shoots. Nitrate will usually only exist in high concentrations in the bulk water, since most sediments are anoxic with $NH_4^+$ being the dominant nitrogen form (Wetzel, 1983). An exception is oxidised sediments as might be found in streams with coarse substrate. Thus rooted macrophytes from lakes and slow flowing rivers can only take up significant quantities of nitrate by the shoot, whereas floating macrophytes and plants rooted in oxidised sediment have the possibility of taking up nitrate by both roots and shoots. Knowing that $NO_3^-$ uptake depends on external $NO_3^-$ concentrations (Marschner, 1995), NRA of roots and shoots was expected to be positively related to the external [$NO_3^-$]. This relationship however could be disturbed, if the presence of $NH_4^+$ affects the uptake rate of $NO_3^-$, as has been seen for several aquatic species (Nichols and Keeney, 1976; Ingemarsson et al., 1984; Thursby and Harlin, 1984). On the other hand, studies of N-uptake rates of roots and shoots of four submerged amphibious species generally showed substantial uptake rates of $NO_3^-$ even in the presence of 1–10 μM $NH_4^+$ (Nils Kongshøj, unpublished data). The suggested relationship between external $NO_3^-$ concentrations and NRA in roots and shoots, also implies that for plants growing in an anoxic sediment, shoot NRA will be higher than root NRA. The balance between NRA activity in roots and shoots of floating macrophytes and plants rooted in oxidised sediment, might depend more on root and shoot dry weight specific $NO_3^-$ uptake rates and on the availability of energy and carbon for nitrate reduction in the two tissues. From an energetic point of view, photosynthetic tissue would be a more advantageous location for nitrate reduction than respiratory root tissue when viewed on a whole plant basis (Raven, 1985). Thus, both the external availability of $NO_3^-$ and the physiological properties of roots and shoots seem to favour shoot $NO_3^-$ reduction over root $NO_3^-$ reduction for aquatic macrophytes.

In this study we test whether the activity of nitrate reductase of aquatic macrophytes are coupled to the availability of nitrate in the environment and how external availability affects induction and distribution of nitrate reductase between root and shoot tissue.

## 2. Materials and methods

### 2.1. Plant material

Submerged aquatic plants, of both submerged and amphibious species growing at depths < 50 cm and at temperatures of 9–15 °C, were collected in September 2000 from four lakes: Hald Sø (56°23′N, 9°22′E), Hampen Sø (56°01′N, 9°24′E), Slåen Sø (56°7′N, 9°38′E) and Torup Sø (56°01′N, 9°26′E) and from three streams: Dollerup Bæk (56°22′N, 9°18′E), Mattrup Å (55°58′N, 9°30′E) and Århus Å (56°11′N, 10°00′E). In total 12 species were collected, with two species being present at more than two locations. The collected plants were stored on ice when transported to the laboratory, where they were separated into roots and shoots, weighed and stored in liquid nitrogen for later analysis.

### 2.2. NRA induction

Shoots (5–10 cm) of four species, two from lakes: *Myriophyllum alterniflora*, *Elodea canadensis* and two from streams *Callitriche cophocarpa* and *Batrachium peltatum* were placed in nets in a N-free growth medium in an aquarium situated in a growth cabinet. *Lemna gibba* was left floating on N-free growth medium. The growth cabinet had a day/night temperature of 20 °C/15 °C 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 photoperiod. The N-free medium consisted of: 1.65 mM $MgSO_4$, 1.00 mM $CaCl_2$, 0.65 mM $NaH_2PO_4$, 0.50 mM $K_2SO_4$, 0.50 mM $KNO_3^-$, 0.16 mM $KCO_3$ and 27 μM Fe-EDTA, 5.77 μM $H_3BO_3$, 1.13 μM $MnCl_2$, 0.19 μM $ZnSO_4$, 0.08 μM $CuSO_4$ and 0.05 μM $Na_2MoO_4$, and was changed once a week. When roots had emerged (after 1–3 weeks), plants were incubated for 2 days in growth medium with 500 μM $KNO_3^-$, after which they were separated in roots and shoots, weighed, frozen in liquid nitrogen and used for NRA determination.

### 2.3. NRA measurements and chemical analysis

Nitrate reductase activity was measured on the stored plant material as maximal nitrate reductase activity (MacKintosh et al., 1995). The analysis was performed according to Scheible et al. (1997a) using an extraction buffer of 50 mM HEPES (pH 7.5), 5 mM MgAc, 1 mM EDTA, 2.5 mM DTT, 0.5% (w:v) BSA and 1% (w:v) PVP-40 and an assay-mix consisting of 30 mM phosphate buffer (pH 7.5), 10 mM $KNO_3$, 0.30 mM NADH and 5 mM EDTA. The $NO_2^-$ formed by nitrate reductase was determined by adding 300 μl sulphanilamide (1% (w:v) in 3N HCl) and 300 μl 155 μM *N*-1-Naphtylethylen-diamine to 700 μl incubated assay and measuring OD at 540 nm after 20 min. Dry weight/fresh weight ratio of the plants was measured on samples similar to those analysed for nitrate reductase activity.

On each site of plant collection, samples of the bulk water and 3–4 sediment cores were obtained. The upper 5 cm of each sediment core was removed, mixed and centrifuged at 7000 RPM for 5 min in a Sorvall Super Speed RC2-B centrifuge (CT, USA) and the supernatant removed and used for determination of pore water concentrations of $NO_3^-$ and $NH_4^+$. $NH_4^+$ analyses of bulk and pore water were performed immediately, using the salicylate method (Lachat Instruments, Quickchem Methods No. 10-107-06-3-A, Milwaukee, USA). Subsamples were acidified with HCl to a pH of ca. 2 and frozen for later analysis of $NO_3^-$, employing a Lachat flow injection analyser (QuickChem Method 10-107-04-1-C, Milwaukee, USA).

### 2.4. Statistical analysis

Differences between root and shoot NRA of plants from lakes and streams, and differences between $NO_3^-$ and $NH_4^+$ concentrations in lakes and streams and between interstitial water and bulk water were tested by two way analysis of variance (ANOVA) where tissue type (root versus shoot) or ion location (interstitial versus bulk water) constituted one factor and plant location (lake versus stream) constituted the other. If interactions between factors were significant ($P < 0.05$), data were split into two one-way ANOVA's. Homogeneity of variance was tested by Cochran's test, and data were log transformed if necessary.

## 3. Results

The nitrate reductase activity of all plants tested was low, being less than 2 μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ except for the amphibious species Cardamine amare which had root and shoot maximal nitrate reductase activities of $8.4 \pm 0.0$ μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ and $23.7 \pm 3.6$ μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ (Table 1). There was no relationship between NRA of roots and shoots and the external $NO_3^-$ concentration in the interstitial water and the bulk water ($r^2 = 0.00$, $P = 0.67$, $n = 74$ on log transformed data, omitting data from Cardamine amare) (Fig. 1). Knowing that large species-specific differences in the induction of NRA in response to external availability exist (Gojon et al., 1994), the effect of external $NO_3^-$ concentrations on NRA in roots and shoots was tested on *Elodea canadensis* and *Batrachium peltatum* collected at three locations where [$NO_3^-$] varied from 0 to 95 μM and from 4 to 551 μM. There was no correlation between external [$NO_3^-$] and NRA (*Elodea*: $r^2 = 0.10$, $P = 0.33$, $n = 12$; *Batrachium*: $r^2 = 0.02$, $P = 0.61$, $n = 12$), and there was no difference in NRA between tissue type (root versus shoot) or between growth habitat (lake versus stream) for the investigated species (two-way ANOVA: $F_{(\text{interaction})} = 0.0$, $P = 0.84$; $F_{(65,1)} = 0.8$, $P = 0.36$ for root versus shoot and $F_{(65,1)} = 2.6$, $P = 0.11$ for lake versus stream).

Comparing the nitrate reductase activity in roots and shoots using the shoot:root NRA ratio showed that root NRA was larger than shoot NRA in 11 out of 17 plants (shoot:root NRA ratio <1; expressed on weight basis) (Table 1). Difference in the shoot:root NRA ratio between growth habitats was not found (ANOVA: $F_{(16,1)} = 0.10$, $P = 0.76$).

The five plant species grown in the laboratory at 500 μM $NO_3^-$ showed a 2–17-fold increase in both root and shoot NRA compared to ambient NRA for all species, except for

Table 1
Nitrate reductase activity of roots and shoots of 18 aquatic macrophytes, representing 12 species collected at 7 locations: 4 lakes and 3 streams

| Locality | Species | Plant part | NRA | Shoot:root NRA |
|---|---|---|---|---|
| Lakes | | | | |
| Hald Sø | *Elodea canadensis* | Root | 0.33 ± 0.04 | 0.85 |
| | | Shoot | 0.28 ± 0.05 | |
| | *Lemna gibba* | Root | 2.08 ± 1.17 | 0.12 |
| | | Shoot | 0.26 ± 0.01 | |
| Hampen Sø | *Litorella uniflora* | Root | 0.12 ± 0.00 | 1.42 |
| | | Shoot | 0.17 ± 0.01 | |
| | *Myriophyllum alterniflora* | Root | 0.27 ± 0.00 | 1.35 |
| | | Shoot | 0.37 ± 0.03 | |
| Slåen Sø | *Nuphar lutea* | Root | NS | >1 |
| | | Shoot | 0.11 ± 0.01 | |
| | *Sparganium minimum* | Root | 0.30 ± 0.04 | 0.62 |
| | | Shoot | 0.19 ± 0.01 | |
| | *Litorella uniflora* | Root | NS | – |
| | | Shoot | NS | |
| | *Ceratophyllum demersum* | Shoot | NS | |
| Torup Sø | *Elodea canadensis* | Root | 0.47 | <1 |
| | | Shoot | NS | |
| Streams | | | | |
| Dollerup Bæk | *Carsamine amara* | Root | 8.42 ± 0.00 | 2.81 |
| | | Shoot | 23.69 ± 3.56 | |
| | *Berula erecta* | Root | 0.13 ± 0.01 | <1 |
| | | Shoot | NS | |
| | *Batrachium peltatum* | Root | 0.11 ± 0.01 | 3.33 |
| | | Shoot | 0.35 ± 0.06 | |
| | *Callitriche cophocarpa* | Root | 0.19 ± 0.03 | 0.84 |
| | | Shoot | 0.16 ± 0.00 | |
| Mattrup Å | *Elodea canadensis* | Root | 0.28 ± 0.02 | <1 |
| | | Shoot | NS | |
| | *Berulae erecta* | Root | 0.22 ± 0.00 | 0.61 |
| | | Shoot | 0.13 ± 0.01 | |
| | *Batrachium circinatum* | Root | 1.30 ± 0.04 | 0.45 |
| | | Shoot | 0.59 ± 0.18 | |
| | *Batrachium peltatum* | Root | 0.49 ± 0.01 | 1.48 |
| | | Shoot | 0.73 ± 0.03 | |
| Århus Å | *Batrachium peltatum* | Root | 0.20 ± 0.02 | <1 |
| | | Shoot | NS | |
| | *Sparganium erectum* | Root | 0.49 ± 0.05 | 0.60 |
| | | Shoot | 0.29 ± 0.01 | |

The nitrate reductase activity was expressed as $\mu mol\, NO_2^-\, g^{-1}\, DW\, h^{-1}$. The relationship between nitrate reductase activity in roots and shoots was given by the shoot:root ratio of NRA. Nitrate reductase activities are mean ± S.D. ($n = 2$–3), activities <0.10 are considered non-significant (NS).

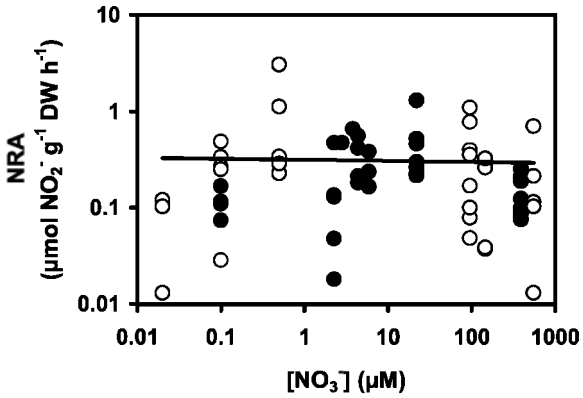


Fig. 1. Nitrate reductase activity of roots (closed circles) as a function of the $NO_3^-$ concentration of the interstitial water and shoots (open circles) as a function of bulk water $NO_3^-$ concentration of 18 aquatic macrophytes representing 12 species collected at 7 locations.

Elodea roots where NRA decreased (Fig. 2a and b). In the induced plants, root NRA was 1.3–7.2 µmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ larger than shoot NRA for *Callitriche*, *Batrachium* and *Lemna* while shoot NRA was 3.1 and 0.5 µmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ larger than root NRA for *Myriophyllum* and *Elodea* (Fig. 2).

The $NO_3^-$ and $NH_4^+$ concentration of interstitial water and bulk water is shown in Table 2. There was a significant difference between $NO_3^-$ concentrations in lakes and streams, but not between interstitial water and bulk water (two-way ANOVA: $F_{(\text{interaction})} = 12.4$, $P < 0.01$. One-way ANOVA: $F_{(25,1)} = 13.4$, $P < 0.01$; $F_{(23,1)} = 0.5$, $P = 0.50$ for lake versus stream and interstitial versus bulk water). For $NO_4^+$ there was no difference between lakes and streams, but a significant difference between interstitial and bulk water (two-way ANOVA: $F_{(\text{interaction})} = 0.1$, $P = 0.76$; $F_{(31,1)} = 0.0$, $P = 0.92$; $F_{(31,1)} = 8.3$, $P < 0.01$ for lake versus stream and interstitial versus bulk water).

Table 2
Nitrate and ammonium concentrations of interstitial water and bulk water

| Locality | Interstitial water | | Bulk water | |
|---|---|---|---|---|
| | $NO_3^-$ (µM) | $NH_4^+$ (µM) | $NO_3^-$ (µM) | $NH_4^+$ (µM) |
| Lakes | | | | |
| Hald Sø | 4 ± 3 | 172 ± 84 | 0 | 2 ± 0 |
| Hampen Sø | 6 ± 3 | 2 ± 3 | 0 | 1 ± 0 |
| Slåen Sø | 2 ± 1 | 18 ± 16 | 0 | 1 ± 1 |
| Torup Sø | 3 ±2 | 85 ± 10 | 0 | 3 ± 0 |
| Streams | | | | |
| Dollerup Bæk | 391 ± 187 | 7 ± 8 | 551 | 5 ± 5 |
| Mattrup Å | 22 ± 24 | 21 ± 12 | 95 | 1 ± 1 |
| Århus Å | 4 ± 3 | 31 ± 11 | 146 | 4 ± 1 |

Data are mean ± S.D. ($n = 2$–4), $n = 1$ for bulk water nitrate.

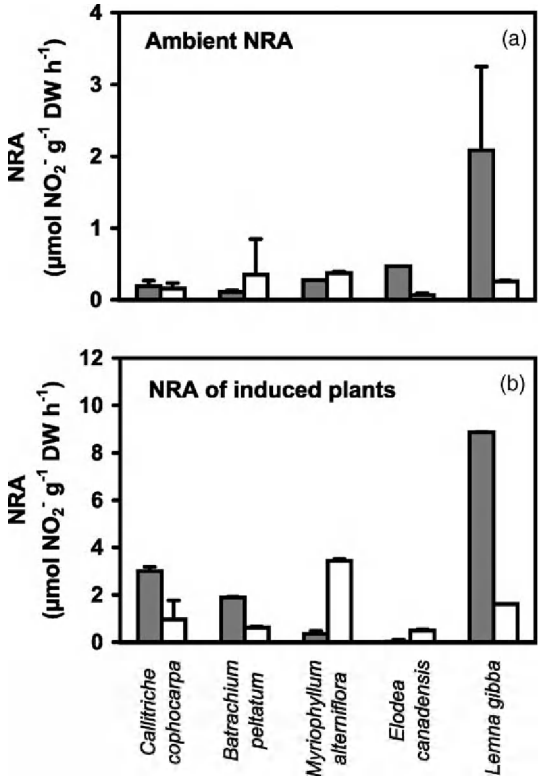


Fig. 2. Nitrate reductase activity of roots (filled bars) and shoots (open bars) of five macrophyte species grown under (a) ambient conditions and (b) under controlled conditions in N-free medium until new roots and shoots were formed after which they were induced for 2 days with 500 μM $NO_3^-$.

## 4. Discussion

The ambient nitrate reductase activity of roots and shoots, being less than 2 μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ for all the aquatic macrophyte species tested, except Cardamine amare, was similar to the in vivo NRA reported for herbs and scrubs from heathlands, mire, swamp forests and arctic habitats when grown in their natural habitat or with $NH_4^+$ as the main nitrogen source (Högbom and Ohlson, 1991; Atkin and Cummins, 1994; Troelstra et al., 1995a, b; Atkin, 1996). Care must be taken when comparing values of nitrate reductase activity measured in vivo, where plant tissue is intact but NRA often substrate limited, to those measured in vitro, where plants are homogenised but substrates exist at saturating concentrations. Usually in vitro NRA is higher than in vivo NRA (Fredeen et al., 1991; Cruz et al., 1991; Gojon et al., 1994). The in vitro NRA of this study, however, are still low compared to the in vivo NRA of roots and leaves of crop plants and of leaves of more than 100 British ruderal and woodland species, being in the rage of 0.3–52.6 μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ with the average of the British species being 6.0 ± 5.3 μmol $NO_2^-$ $g^{-1}$ DW $h^{-1}$ ($n$ = 102)

(Gharbi and Hipkin, 1984; Gojon et al., 1994) (All fresh weight data was recalculated using a dry to fresh weight ratio of 0.38 (Roderick et al., 1999)).

The lack of correlation between ambient NRA and external $NO_3^-$ concentrations in interstitial water and bulk water, even at $NO_3^-$ concentrations of 391–551 μM as in Dollerup bæk, indicate that factors other than $NO_3^-$ influence ambient NRA. One factor considered to be of importance, is the presence of $NH_4^+$, which is known to be preferred over nitrate by a variety of aquatic species (Nichols and Keeney, 1976; Ingemarsson et al., 1984; Thursby and Harlin, 1984). Thus, studies on *Myriophyllum spicatum* showed that $NH_4^+$ concentrations in the bulk water above 2 μM impeded the uptake of bulk water $NO_3^-$ (24 μM) (Nichols and Keeney, 1976), and in *Lemna* $NH_4^+$ concentrations had to be $<10$ μM before $NO_3^-$ uptake could be initiated (Ingemarsson et al., 1984). In *Ruppia maritima* leaf $NO_3^-$ uptake decreased by 50% when 40 μM $NH_4^+$ was applied to either leaves or roots and a total suppression of root $NO_3^-$ uptake was found when roots were exposed to 40 μM $NH_4^+$ (Thursby and Harlin, 1984). Thus, the low NRA found in the aquatic species tested in this study might be explained by $NH_4^+$ suppression of nitrate reductase. This hypothesis was strengthened by the >15 times increase in root NRA of *Callitriche* and *Batrachium* when grown under controlled conditions without $NH_4^+$, but at $NO_3^-$ concentrations comparable to ambient [$NO_3^-$]. Other factors such as temperature, light and carbon availability might also have influenced the NRA of induced plants, with an approximate doubling of temperature considered the most important difference. A doubling of root zone temperature in selected arctic plants from 10 to 20 °C, however, maximally resulted in a doubling of NRA. Thus a difference in temperature is not believed to have cause the large increase in NRA observed in induced plants compared to plants grown at ambient conditions. The high nitrate reductase activity of Cardamine amare was comparable to the activity of terrestrial herbs and grasses (Gharbi and Hipkin, 1984; Gojon et al., 1994). Contrary to the studies on aquatic plants showing a preference for $NH_4^+$ over $NO_3^-$, some amphibious and terrestrial plants are shown to take up $NO_3^-$ (Deane-Drummond and Glass, 1983; Samuelson et al., 1995; Ourry et al., 1996, Niels Kongshøj, unpublished data) and induce nitrate reductase in the presence of $NH_4^+$ (Samuelson et al., 1995). Thus, Cardamine amare might, in terms of nitrogen uptake, resemble a terrestrial herb more than an aquatic macrophyte, and therefore might possess the ability to take up $NO_3^-$ and induce nitrate reductase even in the presence of $NH_4^+$.

For the species tested, there were no consistent indications that NRA was higher in shoots compared to roots, as was expected from an energetic viewpoint (Raven, 1985). On the contrary root NRA exceeded shoot NRA in 11 out of 17 specimen. In terrestrial plants, root $NO_3^-$ reduction typically dominate in plants from habitats where $NH_4^+$ or $N_2$ is the major N-source (Raven, 1985; Claussen and Lenz, 1999). It has been suggested that assimilating $NO_3^-$ in roots might be advantageous because the enzymes of primary, non-photorespiration related, $NH_4^+$ assimilation is located only in roots in these plants (Raven, 1985). If $NH_4^+$ is the primary nitrogen-ion taken up by aquatic macrophytes, this hypothesis could apply to aquatic plants as well, since $NH_4^+$ concentrations will usually be larger in the sediment than in the bulk water, as was also seen in the present study. In floating plants as *Lemna*, the dominating root $NO_3^-$ reduction is a more puzzling phenomenon, since both root and shoot is exposed to the same nitrogen source, leaving no obvious arguments for a predominant root $NO_3^-$ reduction. In *Lemna* minor there are indications that even $NO_3^-$ taken up by leaves is transported to the roots for reduction there (Cedergreen, 2001).

When considering the ecological importance of reducing $NO_3^-$ in roots compared to shoots, it must be remembered, that even if the NRA on a biomass basis is larger in roots than in shoots, this does not necessarily mean that root $NO_3^-$ reduction is predominant on a whole plant basis, since the root weight proportion of total plant biomass is usually small in aquatic macrophytes. Thus, for 14 aquatic macrophyte species grown under varying light temperature and sediment conditions, roots contributed less than 25% to total plant dry weight, except in rosette plants as *Valisneria americana* and *Litorella uniflora* where the root system can account for more than 50% of the weight (Barko and Smart, 1981a, b; Barko et al., 1982; Sand-Jensen and Madsen, 1991; Robe and Griffiths, 1998).

In summary, the results of this study indicate that $NO_3^-$ is probably of limited importance as a nitrogen source for aquatic macrophytes under natural late summer conditions, even when present in high concentrations. This was suggested to be due to a plant preference for $NH_4^+$. It was also shown that root NRA was still of considerable importance in plants, naturally expected to experience higher $NO_3^-$ concentrations around shoots than around roots. The relatively high root NRA activity was proposed to be due to roots being the main location of nitrogen assimilation in plants naturally taking up a majority of their nitrogen as $NH_4^+$ by roots.

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