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Nitrogen uptake and translocation by *Chara*

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Abstract

The potential for above-ground and below-ground uptake and subsequent internal translocation of ammonium (NH_4^+) and nitrate (NO_3^-) by the macroalga *Chara* spp. was investigated. In a two compartment experimental set-up separating above-ground and below-ground algal parts, the charophytes were exposed to various combinations of ^{15}N-labelled NH_4^+ and NO_3^-. Uptake in one compartment and translocation to the other were measured. *Chara* spp. was able to take up and translocate nitrogen between below-ground and above-ground parts. Uptake of $^{15}NH_4^+$ in rhizoids was two-fold higher than that of $^{15}NO_3^-$, indicating a preferential uptake of $^{15}NH_4^+$. Translocation after 5 days was always less in the direction from above-ground to below-ground parts (on average 2% of total ^{15}N uptake), than in the below-ground to above-ground direction (on average 29%). Translocation occurred when the ratio of ^{15}N-atomic percentage in the algal material in the exposed compartment roughly exceeded 2%, and was thus more determined by the internal gradient in the ^{15}N content than by the nature of the N source (either NH_4^+ or NO_3^-). Translocation of ^{15}N from the below-ground to the above-ground compartment also occurred when the charophytes were exposed to high concentrations of either NO_3^- or NH_4^+ in the above-ground compartment. The results of this study are supportive for a mechanism with preferential uptake of NH_4^+ over NO_3^-, and subsequent passive diffusion between cells as the dominant transport mechanism.

Keywords: *Chara*; Nitrogen uptake; Translocation; Nitrate; Ammonium

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1. Introduction

Submerged macrophytes are an important ecosystem component affecting nutrient cycling in lakes. The underlying mechanisms can be indirect, such as the stimulation of coupled nitrification–denitrification by oxygenation of the sediment (Rysgaard et al., 1994) and the supply of surfaces for the attachment of microorganisms involved in these processes (Eriksson and Weisner, 1997; Triska and Oremland, 1981). A direct effect of submerged macrophytes on nutrient cycling is the uptake of nutrients, thus competing with microorganisms for the available nutrients, as well as influencing other microbial processes. For example, nitrification was limited by ammonium (NH_4^+) uptake by *Potamogeton perfoliatus* L. and *Elodea nuttallii* (Planch) St. John (Caffrey and Kemp, 1990).

Rooted submerged macrophytes can take up nutrients both from the sediment pore water (Barko and Smart, 1980) and from the overlying water (Ozimek et al., 1993). As nutrient concentrations are usually much higher in the sediment than in the overlying water, the sediments are generally thought to be the main source of nutrients (Barko et al., 1991; Granéli and Solander, 1988). However, for rooted aquatic macrophytes a distinction should be made between higher (vascular) plants and macroalgae. Vascular macrophytes can internally transport nutrients taken up by the root through the vascular system (i.e. xylem and phloem). Prior to this transport, nitrogen is often assimilated into organic compounds (Hageman, 1979; Syrett, 1981). Macroalgae, in contrast, do not have such specific transport tissue. Translocation of nutrients can therefore only take place by cell-to-cell contact and cytoplasmic streaming (Raven, 1981; Bostrom and Walker, 1976; Littlefield and Forsberg, 1965). Because of the relatively high energy and oxygen requirements of such cell-to-cell transport (Raven, 1981) and the ability for nutrient uptake by above-ground plant parts (Viaroli et al., 1996), it has been questioned if the rhizoids of macroalgae have a function for nutrient uptake similar to that of the roots of vascular plants. This may have implications for the role of macroalgae in releasing nutrients from the sediment by transportation from their below-ground to their above-ground algal parts (translocation), with the translocated nutrients thus becoming available for recycling by grazers.

The capability of rhizoids for the uptake and assimilation of phosphorus (P) has been shown in several (^{32}P-isotope) studies (Littlefield and Forsberg, 1965; Box, 1986; Andrews, 1987). However, studies on N uptake and assimilation in macroalgae have focused on whole plants (e.g. Henricsson, 1976), rootless seaweeds (e.g. Stimson and Larned, 2000) or excised internodal cells (e.g. Reid et al., 2000; Ryan and Walker, 1994), and the role of rhizoids has received less attention.

Although no explicit literature data were found to confirm this, macroalgae are expected to take up nitrogen from the sediment as well (Box, 1986). To our knowledge, the uptake of N by rhizoids of macroalgae and subsequent internal translocation has not been demonstrated previously.

In this study the potential for above-ground and below-ground uptake and subsequent internal translocation of ammonium (NH_4^+) and nitrate (NO_3^-) by the macroalga *Chara* spp. was investigated. In a two compartment experimental set-up separating above-ground and below-ground parts, charophytes were exposed to various combinations of ^{15}N-labelled NH_4^+ and NO_3^-. Uptake from one compartment and internal translocation to the other were measured.

2. Materials and methods

Chara spp. macroalgae were sampled from a continuous growth culture maintained in the laboratory. The charophytes originated from Lake Wolderwijd, a shallow freshwater lake situated in the centre of The Netherlands (52°20′N, 5°35′E). During recent years, charophytes have become the dominant submerged macrophyte in this lake, and their coverage has increased substantially (Meijer and Hosper, 1997).

The charophytes were carefully sampled from the growth culture to minimise damage to either above-ground or below-ground parts and to assure the charophytes to be intact. Adhering sediment particles were carefully washed from the rhizoids, and the charophytes were temporarily stored in water to prevent their drying-out.

The charophytes were placed in two-compartment plastic boxes. These boxes consisted of a clear PVC plate with small opaque PVC walls on all sides, and another opaque wall with small slots in the middle. These slots were filled with lanolin, and after the charophytes were put into the slot once again sealed with lanolin, to ensure a watertight separation between the compartments. Immediately after the charophytes (approximately 50 in each set-up) were positioned, the water in the compartments was removed by the side-tubing. A known volume (typically between 500 and 600 ml) of incubation medium was added to each compartment. The incubation medium used was NH_4^+ and NO_3^- free artificial lake water, with a composition resembling the water of Lake Wolderwijd, containing 3.49 mM Na^+, 2.66 mM Ca^{2+}, 1.94 mM Mg^{2+}, 0.25 mM K^+, 6.37 mM Cl^-, 2.85 mM SO_4^{2-} and 1.79 mM HCO_3^-, and with pH 8.2. ^{15}N-labelled nitrogen was added to a concentration of 0.20 mg N l^{-1}, either as $^{15}NH_4Cl$ (98 at.% ^{15}N; Fluka-Chemie, Germany), or as $Na^{15}NO_3$ (99.5 at.% ^{15}N; Isotec Inc., Miamisburg, OH, USA).

The set-ups were incubated horizontally for 5 days at ambient laboratory temperature (20 °C) underneath daylight TL lamp sets, irradiating the charophytes with approximately 60 μmol photons m^{-2} s^{-1}. On days 0, 1, 4 and 5 water samples were withdrawn by syringe from each compartment (50 ml). After each sampling a known volume of fresh incubation medium with nitrogen-addition according to the treatment was carefully added to maintain the water level which was reduced by the sampling and by evaporation.

After the last sampling, the remaining water was removed from each compartment and its volume was determined. All water samples were filtered (0.45 μm, Schleicher und Schuell, RC55) and stored at −20 °C, before analysis of NO_3^- and NH_4^+ on a Skalar SA40 auto-analyser.

The net amount of N taken up by the charophytes in each compartment was calculated for each time interval between two samplings from a mass balance based on the sum of the measured concentrations of NO_3^- and NH_4^+ and the calculated volumes of the incubation medium, at the beginning and end of each time interval:

$$\text{net uptake in interval } i\ (\mu\text{g N}) = (\text{volume} \times \text{NO}_3^- + \text{NH}_4^+ \text{ concentration})_{\text{start of interval } i} - (\text{volume} \times \text{NO}_3^- + \text{NH}_4^+ \text{ concentration})_{\text{end of interval } i}$$

The volumes were calculated from the measured initial water volume, the volumes of previously removed samples, the replacements after each sampling, and from the evaporation rate, which was assumed constant in time and was calculated as:

$$\text{evaporation rate (ml h}^{-1}) = \frac{\text{initial volume} - \text{end volume} - \text{sum of sampled volumes} + \text{sum of replacement volumes}}{\text{total incubation time}}$$

Eight different treatments were tested in duplicate, using incubation medium:

1. without N-addition in the above-ground compartment, and with 0.204 mg $N l^{-1}$ added as $^{15}NH_4^+$ in the below-ground compartment, referred to as control over $^{15}NH_4^+$;
2. with 0.41 mg $N l^{-1}$ added as $^{15}NH_4^+$ in the above-ground compartment and without N-addition in the below-ground compartment, referred to as $^{15}NH_4^+$ over control; unfortunately, the above-ground compartment in both duplicates was accidentally incubated at a concentration of 0.41 mg $^{15}NH_4$–$N l^{-1}$ instead of the intended 0.20 mg $N l^{-1}$;
3. without N-addition in the above-ground compartment and with 0.213 mg $N l^{-1}$ added as $^{15}NO_3^-$ in the below-ground compartment, referred to as control over $^{15}NO_3^-$;
4. with 0.213 mg $N l^{-1}$ added as $^{15}NO_3^-$ in the above-ground compartment and without N-addition in the below-ground compartment, referred to as $^{15}NO_3^-$ over control;
5. with 1.65 mg $N l^{-1}$ in the above-ground compartment added as NH_4^+, and with 0.204 mg $N l^{-1}$ added as $^{15}NH_4^+$ in the below-ground compartment, referred to as NH_4^+-surplus over $^{15}NH_4^+$;
6. with 2.25 mg $N l^{-1}$ added as NO_3^- in the above-ground compartment, and with 0.213 mg $N l^{-1}$ added as $^{15}NO_3^-$ in the below-ground compartment, referred to as NO_3^--surplus over $^{15}NO_3^-$;
7. with 2.25 mg $N l^{-1}$ added as NO_3^- in the above-ground compartment, and with 0.204 mg $N l^{-1}$ added as $^{15}NH_4^+$ in the below-ground compartment, referred to as NO_3^--surplus over $^{15}NH_4^+$;
8. with 1.65 mg $N l^{-1}$ added as NH_4 in the above-ground compartment, and with 0.213 mg $N l^{-1}$ added as $^{15}NO_3^-$ in the below-ground compartment, referred to as NH_4^+-surplus over $^{15}NO_3^-$.

These treatments were chosen to answer questions relating to:

- The relative potential for uptake by above-ground and below-ground algal parts of NH_4–N (compare treatment with $^{15}NH_4^+$ over control with treatment with control over $^{15}NH_4^+$) and, likewise, of NO_3–N (compare treatment with $^{15}NO_3^-$ over control with treatment with control over $^{15}NO_3^-$), and quantification of the translocation of N in either direction for these combinations.
- Preferential uptake by charophytes of NH_4–N over NO_3–N in either the above-ground compartment (compare treatment with $^{15}NH_4^+$ over control with treatment with $^{15}NO_3^-$ over control) or below-ground compartment (compare treatment with control over $^{15}NH_4^+$ with treatment with control over $^{15}NO_3^-$).
- The inhibition of below-ground uptake and subsequent translocation of either NH_4–N or NO_3–N by the presence of high concentrations of either NH_4–N or NO_3–N in the above-ground compartment: for inhibition of NH_4^+ uptake and translocation compare treatments with NO_3^--surplus over $^{15}NH_4^+$ and NH_4^+-surplus over $^{15}NH_4^+$ with

treatment with control over $^{15}NH_4^+$, and for inhibition of NO_3^- uptake and translocation compare treatments with NO_3^--surplus over $^{15}NO_3^-$ and NH_4^+-surplus over $^{15}NO_3^-$ with treatment with control over $^{15}NO_3^-$.

The *t*-test was used to test the significance of differences between treatments.

At the end of the incubation, the charophytes were cut loose from the slots, divided in above-ground and below-ground parts, while avoiding any smear of lanolin, and washed with demineralised water to remove any adhering incubation medium. The plant samples were dried for 2 days at 100 °C, weighed for dry weight (DW) and ground by mortar, and analysed for ^{15}N-atomic percentage ((^{15}N/total N) × 100) by combustion (NA 1500 Carlo Erba N-analyser) and subsequent mass spectrometry (Europe Scientific 20-20 MS). The ^{15}N% was also determined in three replicates of untreated above-ground and below-ground samples (controls).

The amount of ^{15}N in the plants after the incubation was calculated as the product of the measured ^{15}N-atomic percentage, the measured total N content per dry weight and the measured amounts of dry weight in each compartment. The total N content per dry weight after incubation was determined by acid digestion and subsequent analysis of resulting NH_4^+. To get an indication of the nutritional status of the charophytes, the initial total N content per dry weight was also determined in controls ($n = 4$) at 13.3 ± 4.5 mg N [g DW]$^{-1}$ for above-ground and at 15.4 ± 4.3 mg N [g DW]$^{-1}$ for below-ground parts.

3. Results

3.1. Net uptake of ^{15}N

The measured cumulative net uptake of $^{15}NO_3^-$ or $^{15}NH_4^+$ during the 5 days of incubation (Fig. 1) shows that net uptake occurred in all cases where the charophytes were exposed to N in one compartment. However, in the treatments with $^{15}NH_4^+$ over control and $^{15}NO_3^-$ over control, the concentrations of NH_4^+ and NO_3^-, respectively, in the above-ground compartment dropped to such low levels that uptake was likely to be limited by these low concentrations. The measured uptake of N in these treatments therefore corresponds to a lower boundary for the potential uptake. Nevertheless, comparison of treatments with control over $^{15}NH_4^+$ with $^{15}NH_4^+$ over control and comparison of control over $^{15}NO_3^-$ with $^{15}NO_3^-$ over control shows that for both NH_4^+ and NO_3^- the above-ground uptake was significantly higher than below-ground uptake ($P = 0.018$ for both comparisons). Comparison of measured uptakes from control over $^{15}NH_4^+$ with control over $^{15}NO_3^-$ shows that below-ground net uptake of NH_4^+ after 5 days was significantly higher ($P = 0.044$) than that of NO_3^-, and on average more than two-fold as high. From comparison of $^{15}NH_4^+$ over control with $^{15}NO_3^-$ over control unfortunately no conclusions can be drawn on the uptake of NH_4^+ from the above-ground compartment compared to that of NO_3^-, due to depletion of the concentrations in the incubation medium.

Below-ground uptake of $^{15}NH_4$–N was not hampered in all treatments with exposure to high concentrations of either NO_3^- or NH_4 in the above-ground compartment (Fig. 2):

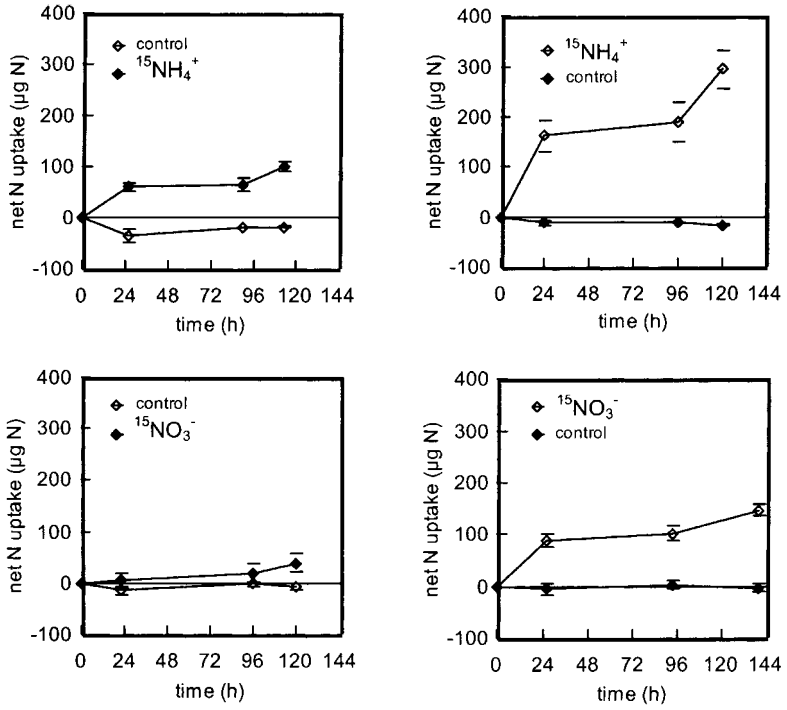

Fig. 1. Average cumulative N uptake (µg N) of duplicates during 5 days incubations in above-ground (open symbols) and below-ground (solid symbols) compartments incubated in nitrogen-free artificial lake water (control) or artificial lake water with addition of $^{15}NH_4^+$ or $^{15}NO_3^-$. Horizontal bars indicate the variation between duplicates.

net below-ground uptake after 5 days in treatments with NH_4^+-surplus over $^{15}NH_4^+$ and NO_3^--surplus over $^{15}NH_4^+$ did not differ significantly from that in treatment with control over $^{15}NH_4^+$ ($P = 0.33$ and 0.37, respectively). Likewise, below-ground uptake of $^{15}NO_3^-$ was not hampered by exposure to high NO_3^- concentrations in the above-ground compartment, and net below-ground uptake after 5 days in NO_3^--surplus over $^{15}NO_3^-$ did not differ significantly from that in control over $^{15}NO_3^-$ ($P = 0.28$), although the variability between duplicates is high in both treatments. The negative net uptake in treatment with NH_4^+-surplus over $^{15}NO_3^-$ suggests a net release to the below-ground compartment of NO_3^-, which was similar in both duplicates. This could indicate NH_4^+ excretion followed by nitrification.

Comparison of treatments with NH_4^+-surplus over $^{15}NH_4^+$ and NO_3^--surplus over $^{15}NH_4^+$ with treatments with NO_3^--surplus over $^{15}NO_3^-$ and NH_4^+-surplus over $^{15}NO_3^-$ shows a consistency with the results from treatments with control over $^{15}NH_4^+$ and control over $^{15}NO_3^-$, namely that net below-ground uptake of NH_4^+ is higher than that of NO_3^- ($P < 0.01$), now also when above-ground algal parts are exposed to high concentrations of either NO_3^- or NH_4^+.

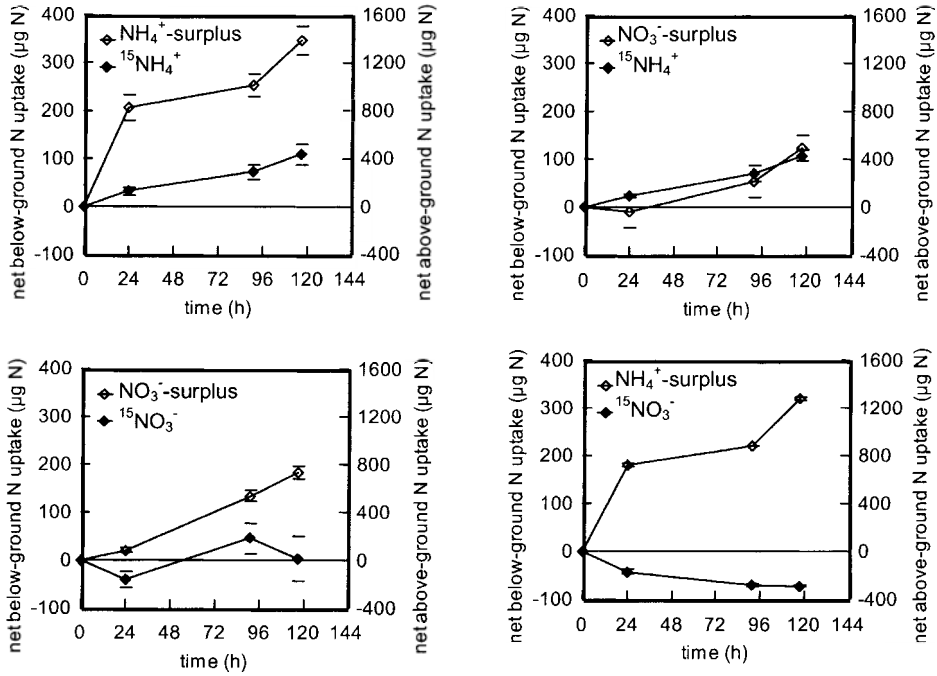

Fig. 2. Average cumulative N uptake (µg N) of duplicates during 5 days incubations in above-ground (open symbols) and below-ground (solid symbols) compartments incubated in artificial lake water with high concentrations of NH_4^+ or NO_3^- (surplus) in the above-ground compartment and artificial lake water with addition of $^{15}NH_4^+$ or $^{15}NO_3^-$ in the below-ground compartment. Horizontal bars indicate the variation between duplicates.

3.2. ^{15}N recovery in plant material

The background atomic percentage of ^{15}N of total N in dry weight was determined at $0.371 \pm 0.001\%$ ($n = 8$) for untreated plant material, which is in close agreement with the general value of 0.366% given by Lide (1997). Also, there was no difference between above-ground and below-ground parts. In all eight treatments the ^{15}N-atomic percentage of the algal material increased in the exposed parts compared to the background value (all $P < 0.001$), showing that ^{15}N-labelled nitrogen that was depleted from the incubation medium in both the above-ground and below-ground compartment was indeed taken up by the charophytes (Fig. 3).

The total amounts of ^{15}N recovered in the plant biomass in the above-ground and below-ground compartment in each treatment were generally in good agreement with the net uptake of N calculated from the concentrations of the incubation medium (Fig. 4). This suggests that release to the medium in the opposite compartment of translocated ^{15}N during the experiments was generally small. However, in all treatments where the charophytes were exposed to $^{15}NH_4$ in the below-ground compartment the recovered amount of ^{15}N was significantly smaller than the calculated net uptake of ^{15}N from the below-ground compartment ($P = 0.023$, 0.008 and 0.009 for treatments with control over $^{15}NH_4^+$,

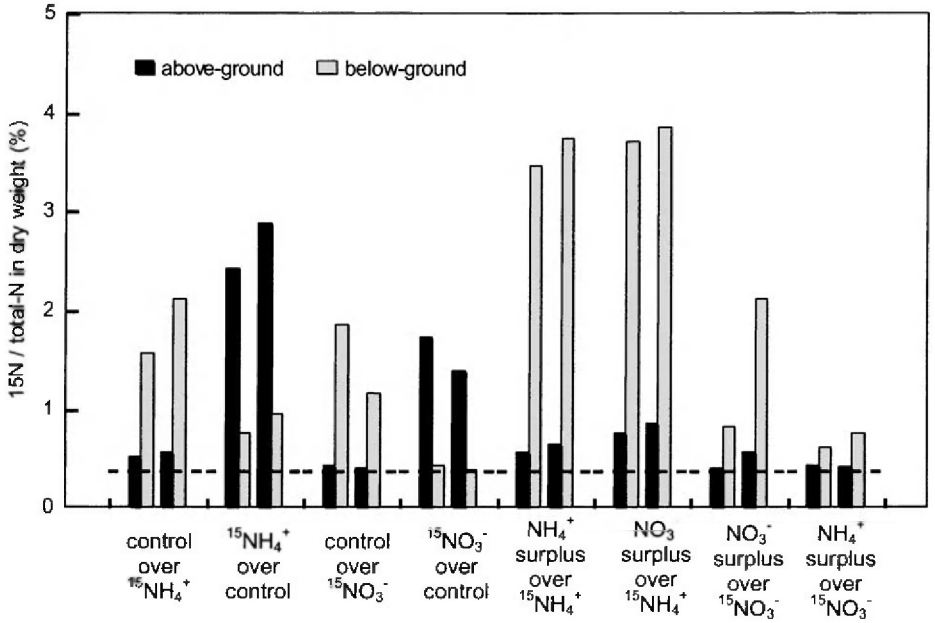

Fig. 3. ^{15}N-atomic percentage in the above-ground and below-ground algal parts after 5 days exposure to ^{15}N-labelled NH_4^+ or NO_3^- for the different treatments. The broken line represents the background value of 0.37% determined from controls of the original algal material. For each treatment both duplicates are shown.

NH_4^+-surplus over $^{15}NH_4^+$ and NO_3^--surplus over $^{15}NH_4^+$, respectively). This may indicate a release of ^{15}N in the above-ground compartment. For example, in treatment with control over $^{15}NH_4^+$ a negative net uptake (release) of on average 18 μg N after 5 days in the above-ground compartment was measured (Fig. 1), which partly explains the difference of on average 60 μg N between the calculated uptake in the below-ground compartment and the recovered amount of ^{15}N in the plant material. This may indicate an additional loss of $^{15}NH_4$ through other processes, possibly nitrification followed by denitrification.

3.3. *Translocation of* ^{15}N

In the treatments exposing the charophytes to $^{15}NO_3^-$ in one compartment (control over $^{15}NO_3^-$, $^{15}NO_3^-$ over control, NO_3^--surplus over $^{15}NO_3^-$ and NH_4^+-surplus over $^{15}NO_3^-$), the ^{15}N-atomic percentage in the plant material in the opposite compartment was only slightly (but significantly, all $P < 0.02$) elevated above the natural ^{15}N-atomic percentage background value, except for one of the duplicates of NO_3^--surplus over $^{15}NO_3^-$, where also the ^{15}N-atomic percentage in the exposed compartment was relatively high (Fig. 3). This suggests that translocation of NO_3^- is small, both from the below-ground to the above-ground compartment (treatments with control over $^{15}NO_3^-$, NO_3^--surplus

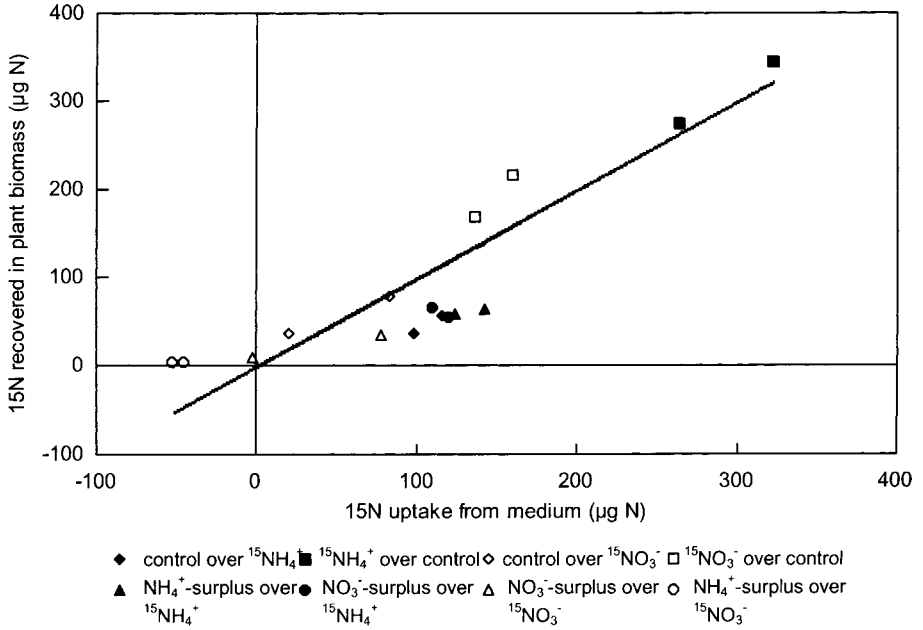

Fig. 4. Comparison of amounts of ^{15}N recovered from above-ground and below-ground plant material with calculated uptake from the incubation medium. The solid line represents the 1:1 slope.

over $^{15}NO_3^-$ and NH_4^+-surplus over $^{15}NO_3^-$) as in the opposite direction (treatment with $^{15}NO_3^-$ over control).

On the other hand, in all treatments with exposure to $^{15}NH_4$ (treatments with control over $^{15}NH_4^+$, $^{15}NH_4^+$ over control, NH_4^+-surplus over $^{15}NH_4^+$ and NO_3^--surplus over $^{15}NH_4^+$) the ^{15}N-atomic percentage in the plant material in the opposite compartment was always significantly ($P < 0.001$) higher than the background value, showing considerable translocation of ^{15}N in both directions. Translocation of $^{15}NH_4^+$ from the below-ground to the above-ground compartment thus also occurred when the above-ground compartment was exposed to high concentrations of either NO_3^- or NH_4^+ (treatments with NH_4^+-surplus over $^{15}NH_4^+$ and NO_3^--surplus over $^{15}NH_4^+$).

Expressed as the fraction of total ^{15}N uptake, translocation after 5 days was always smaller in the above-ground to below-ground direction (on average 2.0% ranging from 0.0 to 4.0% of total uptake), than in the below-ground to above-ground direction (on average 29%, ranging from 12 to 68% of total uptake).

Plotting of ^{15}N-atomic percentage in the opposite compartment against that in the compartment where uptake occurred (Fig. 5) suggests that a considerable increase in ^{15}N-atomic percentage in the opposing compartment by internal translocation occurs when the ^{15}N-atomic percentage in the exposed compartment roughly exceeds 2%. Although there is only a small overlap in the ranges of ^{15}N-atomic percentage in the exposed side, the graph does not reveal a substantial difference between $^{15}NO_3^-$ and $^{15}NH_4^+$ as the source.

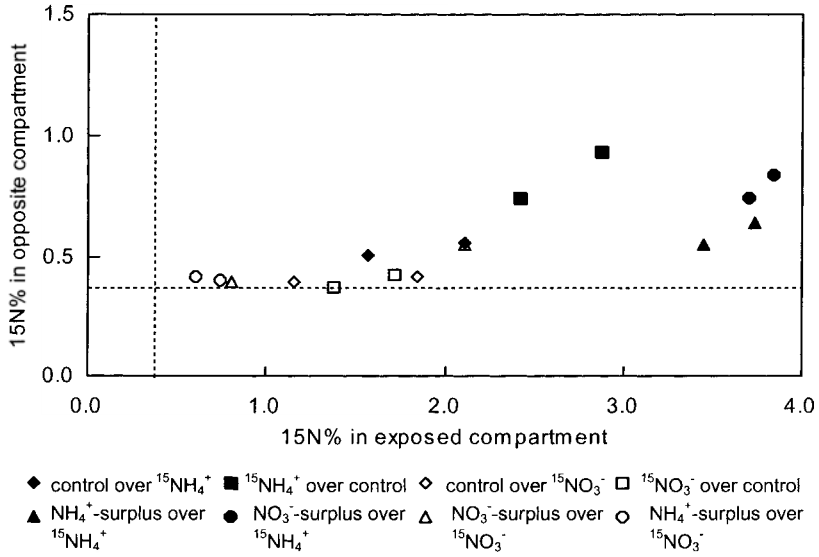

Fig. 5. ^{15}N-atomic percentage in plant material after 5 days incubation in opposite compartment compared to that in the compartment exposed to ^{15}N. Solid symbols are treatments with $^{15}NH_4^+$, open symbols are those with $^{15}NO_3^-$ as the nitrogen source.

4. Discussion

We measured uptake and internal translocation of N by charophytes. For the determination of internal translocation, the exclusion of leakage between compartments is crucial. Therefore an experimental set-up with horizontal batch incubations was preferred over a vertical incubation with continuous flow-through. Although the latter is a more realistic representation of field conditions, it was rejected based on practical complications encountered in pre-experiments. Batch experiments however have some disadvantages, such as a time variable concentration. Effects of this were minimised by the addition of fresh incubation medium with added nitrogen according to each treatment after each sampling, but still the N-concentration time course showed somewhat of a zigzag-pattern. As long as the concentration does not decrease below a level where uptake is seriously limited by the N-concentration in the incubation medium, the uptake rate is close to its maximum. This was unfortunately not the case in the treatments NH_4^+ over control and NO_3^- over control. For these experiments the measured uptake therefore corresponds to a lower boundary. Nevertheless, the uptake by the charophytes resulted in depletion of the available NH_4^+.

Uptake of NH_4^+ and NO_3^- can occur through either passive diffusion, or by active uptake. At the uptake of NH_4^+ a simultaneous efflux of H^+ takes place, by an H^+-ATPase (Ryan and Walker, 1994). Therefore, affinity for NH_4^+ is expected to decrease with decreasing pH in the incubation medium, as was shown for *Typha latifolia* by Dyhr-Jensen and Brix (1996). The influx of NO_3^- occurs by simultaneous import of H^+, after which the excess H^+ produced by assimilation is excreted (Raven and De Michaelis, 1979). As H^+ plays an

important role in the uptake of both NH_4^+ and NO_3^-, the uptake is sensitive to pH (Ryan and Walker, 1994). The pH of the incubation medium was always kept close to 8.2, and observed differences in uptake between treatments cannot be attributed to differences in pH.

The observed below-ground uptake of $^{15}NH_4^+$ was higher than that of $^{15}NO_3^-$. Also for other macroalgae a preference for NH_4^+ over NO_3^- has been reported (Ozimek et al., 1993). NH_4^+ uptake is energetically more efficient than that of NO_3^-, as the incorporation of NH_4^+ into amino acids requires less free energy than that of NO_3^- (Syrett, 1981). Although no experiments were performed with both NH_4^+ and NO_3^- present in the same compartment, the uptake of either may be limited when NH_4^+ and NO_3^- are both present. Usually, NO_3^- had little or no effect on NH_4^+ uptake, but data on the adverse effect of NH_4^+ on NO_3^- uptake are ambiguous. In some experiments no effect was found (Haines and Wheeler, 1978; Topinka, 1978; Henriksen et al., 1990), while presence of NH_4^+ decreased or even suppressed NO_3^- uptake in others (Haines and Wheeler, 1978; Macklon et al., 1990; Korb and Gerard, 2000).

The results show that ^{15}N-labelled NH_4^+ or NO_3^- is indeed taken up and translocated through the charophyte cells in either direction. This is consistent with observed translocation of phosphorus in *Chara globularis* Thuill. by Littlefield and Forsberg (1965), who showed uptake and translocation of phosphorus to be similar for both above-ground and below-ground algal parts. The measured higher translocation of $^{15}NH_4^+$ compared to that of $^{15}NO_3^-$ is more likely to be due to a higher uptake and subsequently a larger internal gradient in the ^{15}N content of the cells, than to a difference in internal transport rate. It may suggest that internal N transport has a pathway similar to that in vascular plants and seaweeds, where N is translocated in organic form (Hill-Cottingham and Lloyd-Jones, 1979), and NO_3^- is transformed via NO_2^- into NH_4^+ prior to assimilation into organic compounds like amino-groups (Hageman, 1979; Syrett, 1981). Translocation of N from below-ground to above-ground algal parts also occurred against a strong gradient in the internal N content of the cells when the charophytes were exposed to high concentrations of either NH_4^+ or NO_3^- in the above-ground compartment. This is supportive for a hypothesis that translocation in charophytes is mainly determined by passive diffusion, and that the difference in translocation between NH_4^+ and NO_3^- is determined by uptake and not internal transport.

Separate measurements of uptake of NH_4^+ and NO_3^- by below-ground and above-ground algal parts and subsequent translocation in either direction provide insight into the role of charophytes in nitrogen cycling in lakes. However, various factors affect nitrogen uptake in field conditions.

The relationship of the uptake rate with the *concentrations of N compounds* is generally considered non-linear, with uptake rate approaching a maximum at high concentrations. In the field the concentrations to which the charophytes are exposed are highly variable. In summer, porewater N-concentrations are much higher than those in the overlying water, while in spring concentrations in the overlying water of especially NO_3^- are relatively high. Uptake also depends on the *nutritional status of the cells with respect to N*. The initial N contents of the charophytes in this study were 13.3 ± 4.5 mg N [g DW]$^{-1}$ for above-ground and 15.4 ± 4.3 mg N [g DW]$^{-1}$ for below-ground algal parts, which was well in the range between 8.3 and 24.6 mg N [g DW]$^{-1}$ reported for various species of *Chara* (Kufel and Kufel, 2002, and references therein). *Other N-sources* not considered here, like various

forms of dissolved organic nitrogen, can contribute to N uptake in the field, and uptake differs between younger fast-growing and older slower-growing plant parts (Topinka, 1978). The effect of *redox conditions* are not clear. As the below-ground compartments were open to the atmosphere during the incubation, the experimental results represent the potential uptake by rhizoids under aerobic conditions. Natural sediments are often anaerobic and N uptake might be hampered (Raven, 1981; Box, 1986). However, in studies with *Phalaris* and *Glyceria* oxygen-depletion did not reduce NH_4^+ uptake (Brix et al., 1994). Also, downward transport of oxygen by plants may oxidise the immediate vicinity of the rhizoids and roots. Under natural conditions, above-ground plant parts experience *water flow*, by which nutrients are replenished and the diffusive boundary layer is reduced. This enhances above-ground uptake rates. Without stirring NH_4^+ uptake fell to undetectable levels in seaweed *Fucus spiralis* (Topinka, 1978). Also, in seagrass communities the water flow velocity positively affected NH_4 uptake rates (Thomas et al., 2000). For rhizoids on the other hand, uptake is limited by porewater diffusion in the rhizosphere (Steingrobe et al., 2000). Other factors that may affect uptake and translocation are irradiance (Box, 1986) and temperature (Topinka, 1978).

Due to variation in the field of all these factors that affect N uptake, extrapolation of laboratory measured N uptake to field conditions is virtually impossible. However, the occurrence of below-ground uptake and upward translocation of NH_4^+, even at high N-concentrations in the above-ground compartment, indicates that NH_4^+-withdrawal from the sediment is likely to be competitive to the N-demand by other organisms in the sediment. As N in the water column in lakes is mainly available as NO_3^-, and N in the sediment pore water mainly as NH_4^+, a net translocation from below-ground to above-ground algal parts is expected. We thus expect charophytes to serve as a pump releasing nitrogen from the sediments to the water column.

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