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SHORT COMMUNICATION

Ammonium and Nitrate Uptake by the Floating Plant *Landoltia punctata*

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• *Background and Aims* Plants from the family Lemnaceae are widely used in ecological engineering projects to purify wastewater and eutrophic water bodies. However, the biology of nutrient uptake mechanisms in plants of this family is still poorly understood. There is controversy over whether Lemnaceae roots are involved in nutrient uptake. No information is available on nitrogen (N) preferences and capacity of *Landoltia punctata* (dotted duckweed), one of the best prospective species in Lemnaceae for phytomelioration and biomass production. The aim of this study was to assess *L. punctata* plants for their ability to take up NH_4^+ and NO_3^- by both roots and fronds.
• *Methods* NO_3^- and NH_4^+ fluxes were estimated by a non-invasive ion-selective microelectrode technique. This technique allows direct measurements of ion fluxes across the root or frond surface of an intact plant.
• *Key Results Landoltia punctata* plants took up NH_4^+ and NO_3^- by both fronds and roots. Spatial distribution of NH_4^+ and NO_3^- fluxes demonstrated that, although ion fluxes at the most distal parts of the root were uneven, the mature part of the root was involved in N uptake. Despite the absolute flux values for NH_4^+ and NO_3^- being lower in roots than at the frond surface, the overall capacity of roots to take up ions was similar to that of fronds because the surface area of roots was larger. *L. punctata* plants preferred to take up NH_4^+ over NO_3^- when both N sources were available.
• *Conclusions Landoltia punctata* plants take up nitrogen by both roots and fronds. When both sources of N are available, plants prefer to take up NH_4^+, but will take up NO_3^- when it is the only N source.

Key words: Ammonium, eutrophication, ion fluxes, *Landoltia punctata*, nitrate, nitrogen uptake.

INTRODUCTION

Cultivation of wetland plant species in wastewater serves two purposes. First, these plants purify water in a simple, cheap and energy-efficient method. Second, the biomass produced by the plants can be used as fodder for cattle.

Among wetland species, plants from the family Lemnaceae are of primary importance for water cleansing because they have high biomass production and can grow in sewage with high concentrations of nitrogen (N) (Cheng *et al.*, 2002). During recent studies on secondary treatment of swine wastewater, several Lemnaceae genotypes were selected for high biomass production (Bergmann *et al.*, 2000). The most productive genotype belonged to *Landoltia punctata* (former *Spirodela punctata*), a 'dotted duckweed'; this is a small floating plant with two fronds (leaves) attached together and two descending roots. However, to date, no N uptake studies have been performed on *L. punctata*, which has genetic traits and metabolism distinct from its close relatives (Frick, 1994; Les and Crawford, 1999). Moreover, the only N uptake studies on Lemnaceae species, such as *Lemna minor*, were those with plants exposed to N concentrations close to their growing optimum at 50–400 μM NH_4NO_3 (Oscarson *et al.*, 1988; Cedergreen and Madsen, 2002), which is lower than N concentrations found in wastewater.

Both roots and fronds of Lemnaceae plants are exposed to the surrounding media and, in theory, are able to take up nutrients (including NH_4^+ and NO_3^-). However, there is long-standing controversy about root involvement in nutrient uptake in Lemnaceae species (cf. Cedergreen and Madsen, 2002). Only recent studies on *Lemna minor* have demonstrated that roots are involved in N uptake and that plants can regulate NO_3^- uptake via fronds or roots depending on light intensity (Cedergreen and Madsen, 2002, 2003). However, the technique used in the studies mentioned above required mechanical separation of the organs: to separate root contribution from the overall uptake, roots were removed. This approach, although apparently successful, could modify root–frond ion transport and alter plant requirements for N supply.

The ion-selective microelectrode technique (MIFE) allows non-invasive, simultaneous measurement of fluxes of three specific ions at the surface of an intact plant. It has high temporal and spatial resolutions (Newman, 2001). Ion-selective microelectrodes have been used to study NH_4^+ and NO_3^- uptake by crops and trees. The spatial dynamics of fluxes of these ions, the relationship with the proton flux, comparison of root capacity to absorb NO_3^- and NH_4^+, and preferences for N sources have been established for a range of terrestrial plant species (Henriksen *et al.*, 1992; Colmer and Bloom, 1998; Taylor and Bloom, 1998; Garnett *et al.*, 2001). Recent MIFE measurements on canola roots demonstrated an NO_3^- effect on NH_4^+ transport dynamics (Babourina *et al.*, 2006).

In the present study, the MIFE technique was used to determine: (1) the N uptake capacity of fronds and roots

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of intact *L. punctata* plants at three different concentrations of NH_4NO_3 comparable with N concentrations in wastewater; (2) the spatial distribution of NH_4^+ and NO_3^- fluxes along the root; and (3) plant preference for NH_4^+ or NO_3^- when supplied with different N sources.

MATERIALS AND METHODS

Plant materials

The free-floating macrophyte plant *Landoltia punctata* (G. Meyer) Les & D.J. Crawford was acquired from Lotus Blossom Garden, Perth, Western Australia (WA), and cultivated in a glasshouse at the University of WA.

The plants were cultured in 50-L opaque plastic containers on a modified Hoagland nutrient solution before treatment. The nutrient solution contained (in mM): NH_4^+, 0·143; NO_3^-, 0·143; Ca^{2+}, 0·05; Mg^{2+}, 0·025; Na^+, 0·56; K^+, 0·025; $H_2PO_4^-$, 0·032; Cl^-, 0·125; SO_4^{2-}, 0·05; and CO_3^-, 0·2.

For ion flux measurements, plants in Petri dishes were placed on an inverted optical microscope. The position of the root cap and the meristem, and elongation and mature zones of the root were determined for each plant measured. Because cells in the meristem zone were the smallest, this zone was darker in colour. Cells in the elongation zone gradually increased in size and in the mature zone they reached their maximal size. Root radius (R) was also measured under an optical microscope. To estimate the average size of fronds and root length (L), plants were scanned, and the surface area was measured by using SigmaScan Pro software (SPSS Inc., Chicago, IL, USA), $n = 106$. Root surface area was calculated as a cylinder surface ($2\pi R \times L$) where R is radius and L root length.

Ion flux measurements

Ion fluxes were measured non-invasively using the MIFE® system (University of Tasmania, Hobart, Australia) generally as described by Newman (2001). Electrodes were pulled from borosilicate glass capillaries (GC150–10, Harvard Apparatus, Kent, UK), dried at 230 °C for about 5 h, and silanized with tributylchlorosilane (#90765, Fluka Chemicals, Buchs, Switzerland). The tips of dried and cooled electrode blanks were broken to a diameter of 1–10 μm, depending on the ionophore, and then back-filled with appropriate solutions. Back-filling solutions were 15 mM NaCl and 40 mM KH_2PO_4 for the hydrogen electrode, 500 mM NH_4Cl for the NH_4^+ electrode, and 500 mM KNO_3 and 100 mM KCl for the NO_3^- electrode. Immediately after back-filling, the electrode tips were front-filled with commercially available ionophore cocktails for measuring H^+ (#95297, Fluka) and NH_4^+ (#09882, Fluka). The nitrate sensor contained 0·5 % (w/v) methyltridodecyl nitrate (MTDDA NO_3^-), 0·084 % (w/v) methyltriphenylphosphonium bromide (MTPPB) and 99·4 % (v/v) *n*-phenyloctyl ether (NPOE) (Zhen *et al*., 1992; Plassard *et al*., 2002). A reference electrode was fabricated in a similar way from a borosilicate glass capillary and filled with 0·1 M KCl in 1 % (w/v) agar. Electrodes were calibrated against a range of standards (pH from 5·3 to 7·2, NH_4^+ and NO_3^- from 1 to 10 mM). Electrodes with responses of less than 50 mV per decade were discarded.

Experimental procedures

Thirty minutes before ion flux measurements, plants were placed in the bathing solution containing (in mM): NH_4NO_3, 1·0; $CaCl_2$, 0·05; $MgSO_4$, 0·025; NaCl, 0·56; K_2SO_4, 0·025; NaH_2PO_4, 0·032; $NaHCO_3$, 0·2. Ion fluxes were measured 60–120 μm from the root surface and 200 μm from the frond surface. In mapping experiments, 4–5 plants were measured and averaged. Experiments were performed at 20–22 °C under standard laboratory lighting. Fluxes of H^+, NH_4^+ and NO_3^- were measured simultaneously.

For estimation of preferences of roots and fronds for NH_4^+ or NO_3^- supply, measurements were made at the border of the elongation and mature zones of roots and in the middle of the frond after 2 h exposure to the solution containing 0·5, 1·0 or 2·5 mM NH_4 or NO_3^- in addition to other nutrients (in mM): $CaCl_2$, 0·05; $MgSO_4$, 0·025; NaCl, 0·56; K_2SO_4, 0·025; NaH_2PO_4, 0·032; $NaHCO_3$, 0·2. Prior to measurements, the Petri dish was flushed with fresh solution containing the appropriate N source at the same concentration as in the pre-treatment solution. Flux measurements were performed 30 min after the transfer to a fresh solution to allow ion flux adjustment.

Statistical analysis

All treatments were performed in random order. To analyse the difference in ion fluxes in different zones of the root and at different NH_4NO_3 concentrations, the ion fluxes were evaluated by the Student's t-test.

RESULTS

The average surface area (one side, ± s.e.) of a single frond in our experiments was 0·028 ± 0·002 cm^2, whereas the surface area of a single root was 0·054 ± 0·003 cm^2. Therefore, despite their small diameter of 0·018 ± 0·001 cm, roots generally had a larger surface area than fronds to take up nutrients from the media.

Root morphology was similar to other wetland plant species (Landolt, 1986) with a large root cap covering the root apex, the meristem and the elongation zones. As with other members of the family Lemnaceae, *Landoltia punctata* does not have root hairs.

Mapping of ion fluxes along roots

As *L. punctata* is a floating plant and can potentially take up N by both roots and fronds (Cedergreen and Madsen, 2002), ion fluxes for both N forms at roots and fronds were compared. However, because the spatial distribution of ion fluxes along the plant root is uneven (cf. Newman, 2001), and there is no available information regarding NH_4^+ and NO_3^- flux distribution in *L. punctata* or its close relatives, it was necessary to map ion fluxes along the root.

Exposure to different concentrations of NH_4NO_3 had some impact on H^+ flux profile along *L. punctata* roots (Fig. 1A). The H^+ influx zone, which is usually linked to the meristem zone, was more pronounced at higher NH_4NO_3 concentrations. The root tip had higher influx than the adjoining meristem zone. The mature zone had higher influx than the elongation zone. The fluctuations in H^+ profiles were higher at higher concentrations of NH_4NO_3 (the largest fluctuations were found at 2·5 mM NH_4NO_3) than at the mid-range concentration of 1 mM NH_4NO_3.

NH_4^+ flux mapping along roots indicated that the root tip had the highest influx ($P < 0{\cdot}05$), which decreased at the distal elongation zone (Fig. 1B). The net NH_4^+ influx at the root tip was lowest at the lowest concentration studied (0·5 mM NH_4NO_3); however, at the proximal elongation and mature zones, NH_4^+ fluxes were similar at all concentrations studied.

As with NH_4^+ fluxes, the root tip had higher NO_3^- influx than the neighbouring zones ($P < 0{\cdot}05$). The difference in NO_3^- flux values between different root zones was highest at 2·5 mM NH_4NO_3, with the smallest influx in the middle of the elongation zone (Fig. 1C).

Comparison of NH_4^+ and NO_3^- uptake by fronds and roots

Generally, NH_4^+ and NO_3^- fluxes were more stable at the end of the proximal elongation zone and at the mature zone of the root. The border between the elongation

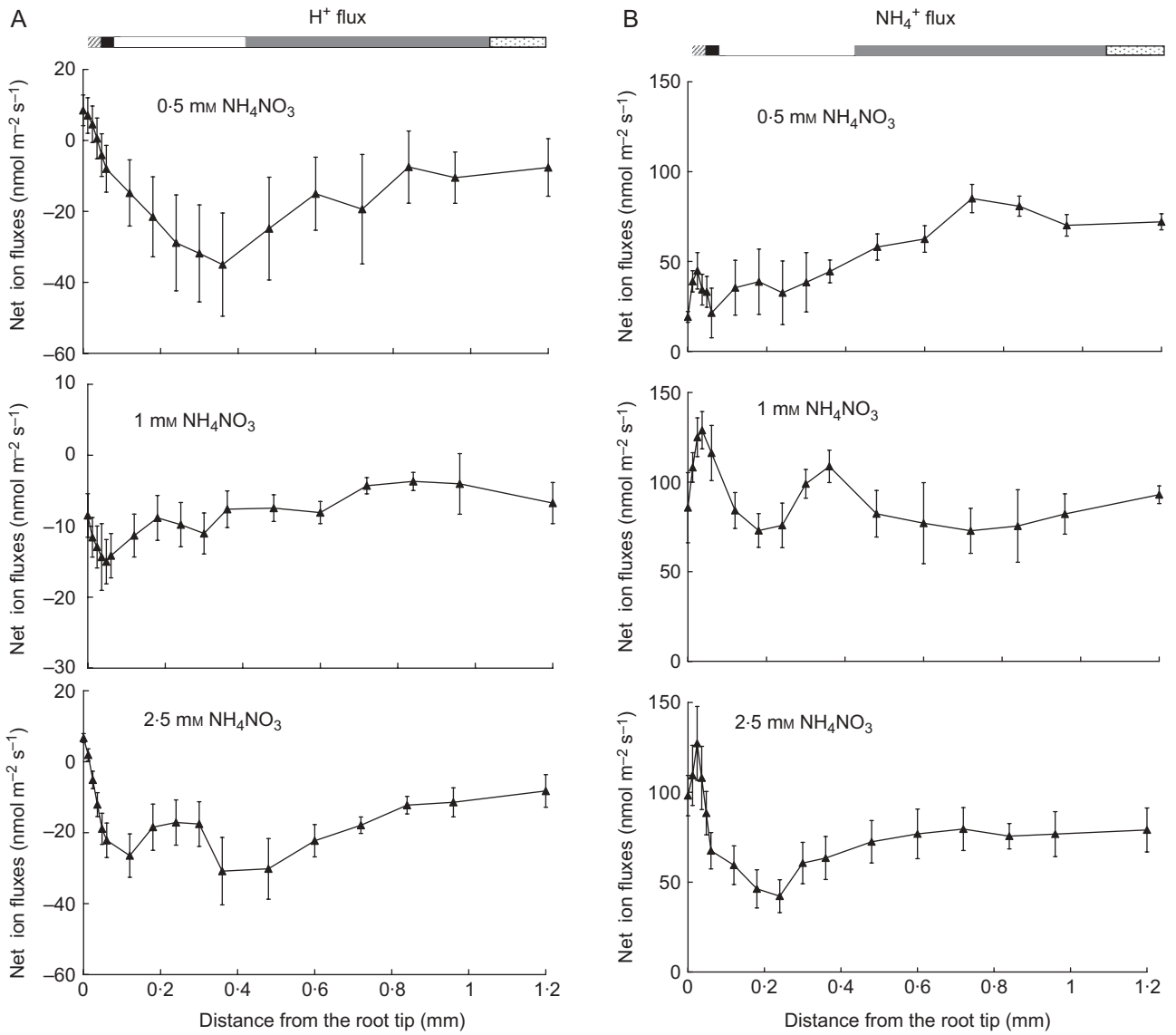

FIG. 1. Net ion fluxes along the root length of *Landoltia punctata* as noted in the bars at the top: root cap (hatched), meristem zone (black), distal elongation zone (white), proximal elongation zone (grey) and mature zone (speckled). (A) H^+, (B) NH_4^+, (C) NO_3^-. Numbers indicate the initial flux values. Flux measurements were taken while roots were exposed to the basal solution containing (in mM): NH_4NO_3, 0·5, 1·0 or 2·5; $CaCl_2$, 0·05; $MgSO_4$, 0·025; NaCl, 0·56; K_2SO_4, 0·025; NaH_2PO_4, 0·032; and $NaHCO_3$, 0·2.

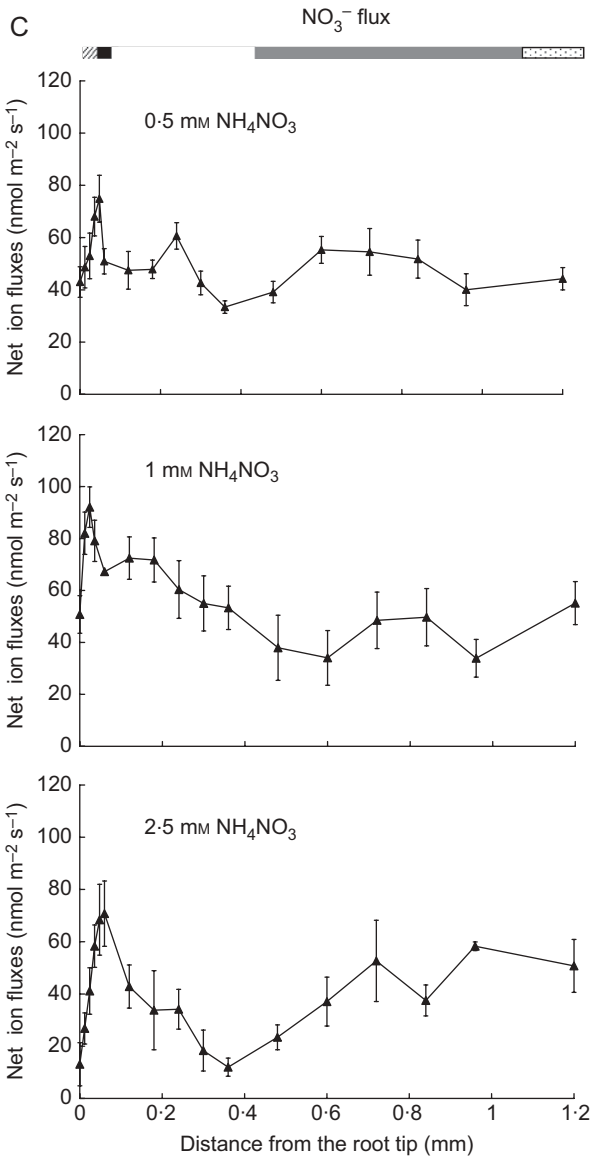

FIG. 1. Continued.

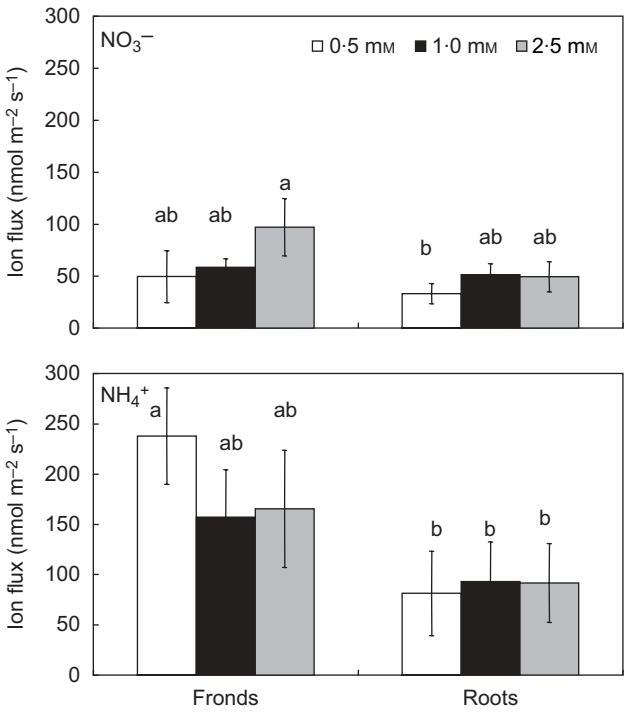

FIG. 2. Net fluxes (nmol m^{-2} s^{-1}) of NH_4^+ and NO_3^- at roots (the mature zone, 1·2 mm from the root tip) and fronds of *Landoltia punctata* exposed to different media (0·5, 1·0 or 2·5 mM NH_4NO_3). Values are means ± s.e. ($n = 5$). Different lower-case letters indicate significant differences ($P \leq 0.05$).

and the mature zones was chosen for further ion flux measurements to reduce variability between plants at different stages of their development. In fronds, ion fluxes were uniform when the microelectrodes were located at a relatively long distance (200 μm) from the frond surface.

At all NH_4NO_3 concentrations studied, both roots and fronds took up NH_4^+ at a higher rate than NO_3^-. The concentration increase did not affect root uptake of NH_4^+ and NO_3^- whereas fronds took up NO_3^- at a higher rate at 2·5 mM NH_4NO_3, although this was statistically insignificant (Fig. 2).

In comparisons of N uptake by roots and fronds, uptake rate of NH_4^+ and NO_3^- by roots was generally lower, although not statistically so. The highest flux values were observed for NH_4^+ in fronds at 0·5 mM NH_4NO_3.

Overall, ion fluxes at the root surface were lower than at the frond surface. However, because the surface area of the single root was almost twice that of the frond, as calculated above, the total capacity of roots to take up nutrients was almost equal to that of the fronds. On average, the ratio of fronds/roots for N uptake was 1·09 ± 0·20 (± s.e.) for NH_4^+ and 0·79 ± 0·12 for NO_3^-.

NH_4^+ and NO_3^- flux in different N media

Net NO_3^- and NH_4^+ fluxes were measured at three positions along *L. punctata* roots, including the root tip (0 mm), the distal elongation zone (0·6 mm) and the mature zone (1·2 mm), in three different N media (1·0 mM NH_4Cl, KNO_3 or NH_4NO_3) (Table 1). NO_3^- flux in 1·0 mM KNO_3 was larger than NH_4^+ flux in 1·0 mM NH_4Cl. However, NH_4^+ uptake tended to be higher than NO_3 uptake when NH_4^+ and NO_3^- were supplied together, especially at the root tip.

In 1·0 mM NH_4Cl media, the root tip and the distal elongation zone showed three-fold higher NH_4^+ influx than in the mature zone. In 1·0 mM KNO_3 media, the distal elongation zone and the mature zone showed two-fold higher NO_3^- uptake than the root tip.

When NO_3^- was the only source of N, the highest N influx was observed at the distal elongation zone and at the mature zone.

TABLE 1. *Net fluxes (nmol m^{-2} s^{-1}) of NH_4^+ and NO_3^- at three specific points on the roots of* Landoltia punctata *exposed to different media (1·0 mM NH_4Cl, KNO_3 or NH_4NO_3)*

Distance from tip (mm)	1 mM NH_4Cl NH_4^+ flux	1 mM KNO_3 NO_3^- flux	1 mM NH_4NO_3 NH_4^+ flux	NO_3^- flux
0	90 ± 10^{bc}	130 ± 10^{b}	140 ± 10^{b}	50 ± 10^{d}
0·6	80 ± 30^{bc}	260 ± 40^{a}	80 ± 10^{c}	30 ± 40^{d}
1·2	30 ± 10^{d}	270 ± 50^{a}	90 ± 30^{bc}	65 ± 20^{cd}

The same plants were measured in all three bathing solutions in random order. Values are means ± s.e.m. ($n = 4–5$).

Different letters indicate significant differences estimated by Student's *t*-test ($P \leq 0·05$).

DISCUSSION

Comparison of NH_4^+ and NO_3^- uptake by fronds and roots

Hillman (1961) suggested that roots of floating macrophytes function mostly as anchors, whereas fronds and leaves are the main organs involved in nutrient uptake. Subsequent studies, covering fronds and roots of *Spirodela polyrrhiza* and *Lemna minor* (Lemnaceae) with paraffin in one set of experiments, and removing roots in another set of experiments, led to conclusions that the roots of duckweeds played only a small role in nutrient uptake (Muhonen *et al*., 1983; Ice and Couch, 1987). However, the researchers based their conclusions on the observation that plants with excised roots multiplied more rapidly than the rooted plants, and obviously roots were present in new generations of the plants (Muhonen *et al.*, 1983). In earlier experiments, Gorham (1941) demonstrated that covering the undersides of fronds with lanolin decreased growth of duckweed plants, although he noticed that root length was increased. Recently, it has been found that increased ratio of the root surface to the frond surface led to increased NH_4^+ uptake rate in *Lemna minor* (Cedergreen and Madsen, 2002). These studies indicate that duckweed plants can regulate their life cycle, such as increased multiplication rate, and the surface area for nutrient absorption at the level of frond–root interactions.

The conclusion that roots are of low importance for N uptake in Lemnaceae was opposed by other studies, where it was shown that roots might have an important role in nutrient supply (Oscarson *et al*., 1988; Cedergreen and Madsen, 2002, 2003). Moreover, in *Lemna minor*, roots had a higher rate of uptake of both NH_4^+ and NO_3^- at low external concentration (5 μM NH_4NO_3) than fronds, whereas higher NH_4NO_3 supply (250 μM) reduced root uptake rates for both ions. This decreased uptake rate in roots at high NH_4NO_3 supply was compensated for by higher uptake rates in fronds (Cedergreen and Madsen, 2002). However, in all studies mentioned above, the researchers used mechanical approaches to separate the organs: covering fronds or roots with paraffin or lanolin, and removing roots. In the present study, intact plants were used to assess the relative contribution of fronds or roots to nutrient uptake by Lemnaceae plants, so that interactions between roots and fronds were not affected.

From the current study, roots of *L. punctata* contributed to N uptake at the same level as fronds. Even though the magnitude of ion fluxes in roots was lower than in fronds, the root surface area was two-fold greater than the frond surface area, and the ratio of fronds/roots in N uptake was close to 1 : 1·09 for NH_4^+ and 0·79 for NO_3^-. Therefore, plants have equal capacity to use fronds and roots for NO_3^- and NH_4^+ uptake.

Landoltia punctata *preferences for different N sources*

Different species have different preference for N forms. *Picea glauca* preferred NO_3^- (Kronzucker *et al.*, 1995), whereas NH_4^+ uptake by maize roots was twice that of NO_3^- uptake when both N forms were present (Taylor and Bloom, 1998). Plant preference for different forms of N is affected by temperature, pH and element composition of the solution as well as plant growth stage (Ikeda, 1991).

Although Lemnaceae (*Lemna minor*) have been shown to prefer NH_4^+ as a nitrogen source (Porath and Pollock, 1982; Cedergreen and Madsen, 2002), high concentrations of NH_4^+ can inhibit their growth (Oron *et al.*, 1985; Korner *et al.*, 2001). A rise in external concentration from 50 to 250 μM NH_4NO_3 significantly decreased uptake of both N forms by roots and increased uptake of NO_3^- by fronds (Cedergreen and Madsen, 2002). In the present study, the capacity to take up NH_4^+ was two-fold higher than NO_3^- in both fronds and roots when both ions were supplied (Fig. 2) at external concentrations of NH_4NO_3 much higher than in the study of Cedergreen and Madsen (2002). These findings indicate that: (1) differences in NH_4^+ uptake might depend on Lemnaceae species requirements and (2) differences in NH_4^+ uptake may be found between an intact plant and a plant with the root removed.

Spatial distribution of ion fluxes in the root of Landoltia punctata

High H^+ excretion at the root apex, which includes the apical initials, meristem and distal elongation zones, has been shown for many terrestrial plants (Taylor and Bloom, 1998; Newman, 2001). Lemnaceae plants have a large root cap covering the whole root apex, the full elongation zone and part of the mature zone, which makes them different from many other plants, including some wetland species (Landolt, 1986). In the current study, it was demonstrated that the root cap does not interfere with ion fluxes at the root surface: the H^+ flux profile of the root of *L. punctata* is similar to other plants studied (cf. Newman, 2001). Higher NH_4^+ uptake at the root apex was established for rice adventitious roots and primary maize seminal roots (Colmer and Bloom, 1998; Taylor and Bloom, 1998). Using a higher spatial resolution (0·2 mm), the highest NH_4^+ influx at the root apex was found to be associated with the meristem zone rather than with the whole apex (Fig. 1B). Likewise, increased NO_3^- influx was noted at the root meristem zone, similar to observations made by Colmer and Bloom (1998) on maize primary seminal roots.

CONCLUSIONS

Landoltia punctata plants prefer to take up NH_4^+ when both N sources are available. Although the absolute flux values for NH_4^+ and NO_3^- in roots are lower than those at the frond surface, the overall capacity of roots to accumulate ions equalled that of fronds because of the greater surface area of roots compared with fronds.

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