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# Growth and membrane permeability of two submersed macrophytes in response to ammonium enrichment

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ABSTRACT: Increased ammonium ($NH_4^+$) concentrations are reported to be an important cause of decline in submersed macrophytes in eutrophic lakes. In this study, a subacute experiment was conducted to examine the effects of various $NH_4^+$ concentrations on growth and membrane permeability of 2 submersed macrophytes, *Myriophyllum spicatum* and *Ceratophyllum demersum*. Apical shoots of the plants were incubated in modified Hoagland solution with 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 mg $l^{-1}$ $NH_4^+$-N for 4 d. The plants were then collected for examination of cell membrane permeability and the solutions were used to determine the concentrations of $NH_4^+$, sodium ($Na^+$), potassium ($K^+$), magnesium ($Mg^{2+}$) and calcium ($Ca^{2+}$). The results indicate that high $NH_4^+$ concentrations had significant adverse effects (e.g. reduced growth and leaf chlorosis) on the macrophytes and increased membrane permeability, leading to net leakage of $NH_4^+$ and the cations. Compared to *M. spicatum*, *C. demersum* had higher membrane permeability, which might enhance $NH_4^+$ transportation across the membrane and thus increase its tolerance to $NH_4^+$ stress. Significant increases in the membrane permeability of *M. spicatum* and *C. demersum* were observed in the treatments with external $NH_4^+$-N concentrations of ≥2 mg $l^{-1}$, which fall within the range of $NH_4^+$ concentrations in many eutrophic lakes.

KEY WORDS: $NH_4^+$ stress · Membrane permeability · Cations · Submersed macrophyte

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## INTRODUCTION

Submersed macrophytes are primary producers and play key functional roles in shallow lake ecosystems (Klaine & Lewis 1995). Decline of submersed macrophytes has occurred in many eutrophic lakes worldwide, due to direct and indirect effects of nitrogen (N) and phosphorus (P) overloading in the waters (Scheffer et al. 1993). As high ammonium ($NH_4^+$) concentrations are toxic to both animals and higher plants, this has been repeatedly reported as a causal factor for the decline of submersed macrophytes in eutrophic lakes, where $NH_4^+$-N concentrations in the water column can reach 10 mg $l^{-1}$ and surpass the $NH_4^+$ tolerance thresholds for many submersed macrophytes (van Katwijk et al. 1997, Cao et al. 2007).

$NH_4^+$ plays an important role as a source of N for plants (Kronzucker et al. 1997); however, excessive $NH_4^+$ has a range of negative effects, e.g. uncoupling of photophosphorylation, inhibiting photosynthesis, triggering oxidative stress, causing internal carbon–nitrogen imbalance (Gibbs & Calo 1959, Rudolph & Voigt 1986, Cao et al. 2004, Nimptsch & Pflugmacher 2007, Wang et al. 2008) and changing cation–anion relationships in the plants (Kirkby & Mengel 1967, Santa-María et al. 2000, Szczerba et al. 2006). $NH_4^+$-fed plants have been shown to have lower sodium ($Na^+$), potassium ($K^+$), magnesium ($Mg^{2+}$) and calcium ($Ca^{2+}$) contents (Kirkby & Mengel 1967, Langelaan &

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Troelstra 1992, Smart & Bloom 1993), and higher chloride, sulfate and phosphate contents (Gloser & Gloser 2000) compared with $NO_3^-$-fed plants. This can affect plant performance as a result of a cation–anion imbalance (Kirkby & Mengel 1967), as appropriate concentrations of these ions are required to maintain the stability and function of enzymes and cell membranes (Davenport et al. 1997, Wei et al. 2003).

The cell membrane is a barrier system that maintains intracellular ion equilibrium with external ion dynamics. Thus, plant cell membrane permeability is sensitive to external ion concentrations and closely associated with ion flux between the plant and its environment (Llamas et al. 2000, Suwalsky et al. 2009). High $NH_4^+$ concentrations are reported to affect the growth and mineral composition of submersed macrophytes (Kirkby & Mengel 1967, Vale et al. 1987, 1988, Gerendas et al. 1997, Gloser & Gloser 2000), but the underlying mechanisms of $NH_4^+$ toxicity are far from clear, especially due to the lack of data concerning possible changes in membrane permeability under $NH_4^+$ stress. *Myriophyllum spicatum* and *Ceratophyllum demersum* are submersed macrophytes dwelling in eutrophic waters worldwide (Nichols & Shaw 1986, Royer & Minshall 1997). Both species display a preference for $NH_4^+$ over $NO_3^-$ when both are available at equivalent concentrations (Jorga & Weise 1981). In the present study, growth and membrane permeability of the 2 macrophytes were examined in relation to external $NH_4^+$ enrichment, with the aim of testing whether (1) *M. spicatum* and *C. demersum* encounter $NH_4^+$ stress at high $NH_4^+$ concentrations, and (2) $NH_4^+$ enrichment changes membrane permeability and disturbs the cation balance of the 2 species.

## MATERIALS AND METHODS

### Culture and treatment

The experiments were carried out at the Donghu Experimental Station of Lake Ecosystems (30° 33′ N, 114° 21′ E), China. *Myriophyllum spicatum* and *Ceratophyllum demersum* were cultured outdoors with sediment collected from the eutrophic Tanglinhu area of Lake Donghu (total phosphorus [TP] = 1.57 mg $g^{-1}$ dry weight [DW], total nitrogen [TN] = 4.23 mg $g^{-1}$ DW) and tap water ($PO_4^{3-}$-P: 0.013 mg $l^{-1}$, $NO_3^-$-N: 1.18 mg $l^{-1}$, $NH_4^+$-N: undetectable) for more than 3 mo before the plant shoots were collected for the $NH_4^+$ treatments. The sediment and tap water supplied adequate nutrients for growth of the plants (Cao et al. 2011).

In the pre-treatments, 20 cm long apical shoots were collected and incubated in a modified Hoagland solution (1652 mg $l^{-1}$ $Ca(NO_3)_2{\cdot}4H_2O$, 506 mg $l^{-1}$ $KNO_3$, 272 mg $l^{-1}$ $KH_2PO_4$, 493 mg $l^{-1}$ $MgSO_4{\cdot}7H_2O$, 1.8 mg $l^{-1}$ $MnCl_2{\cdot}4H_2O$, 0.2 mg $l^{-1}$ $ZnSO_4{\cdot}7H_2O$, 0.1 mg $l^{-1}$ $CuSO_4{\cdot}5H_2O$, 2.8 mg $l^{-1}$ $H_3BO_3$, 0.025 mg $l^{-1}$ $Na_2MoO_4$, and 18.2 mg $l^{-1}$ Fe-EDTA) for 7 d, with an irradiance of 50 µmol $m^{-2}$ $s^{-1}$ and a 10 h light:14 h dark cycle at 25°C.

For the $NH_4^+$ treatments, ammonium stock solution was prepared with $(NH_4)_2CO_3$. Each of the collected shoots was incubated separately in a 200 ml Erlenmeyer flask containing the modified Hoagland solution (without sediment) plus $NH_4^+$-N at 0 (control), 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 mg $l^{-1}$. Each treatment had 6 replicates. The $NH_4^+$ treatment lasted for 4 d and the incubating solutions were renewed each day. The replaced solutions were pooled and stored at –80°C for further analysis of cations. At the end of the treatments, the plants shoots were collected, rinsed 3 times with distilled water and used for determination of growth and membrane permeability.

### Examination of growth and chlorophyll content

The fresh weight of each shoot was determined at the beginning and the end of the 4 d $NH_4^+$ treatments. Relative growth rate (RGR) was calculated by the formula:

$$RGR = (\ln M_2 - \ln M_1) / (t_2 - t_1) \quad (1)$$

where $M_1$ is the shoot fresh weight after the 7 d pretreatment period but at the beginning ($t_1$) of the $NH_4^+$ treatments and $M_2$ is the shoot fresh weight at the end ($t_2$) of the $NH_4^+$ treatments. At the end of the $NH_4^+$ treatments, a leaf sample was collected from each shoot for analysis of chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) contents following the method of Lichtenthaler (1987). The development of chlorosis in the leaves was checked every day by carefully inspecting the leaves and comparing with the control.

### Determination of cations and cell membrane permeability

Cell membrane permeability of each plant shoot was determined according to the method described by McCann & Solomon (2000) with a little modification. A sample (circa 0.5 g) of the plant shoot was rinsed 3 times with deionized water to remove electrolytes from the shoot surface and incision area, and then placed into a 250 ml glass bottle containing

200 ml of deionized water and left to exosmose for 24 h at 25°C. Conductivity of the water within the glass bottles was determined using a TEMP/COND/TURB sensor coupled with a Water Quality Monitoring System (W-23XD, HORIBA). Thereafter, the glass bottles containing the shoot samples were immersed in boiling water for 20 min and then left to exosmose for a further 24 h at 25°C. The aim of the boiling was to ensure full leakage of ions. After boiling, the conductivity of the water within the glass bottles was determined. All conductivity readings were zeroed by subtracting the average of 3 blank samples that contained only deionized water and had undergone identical manipulations to the samples. Membrane permeability is expressed as a percentage of total ion leakage (TIL) and was calculated by the formula:

$$\% \text{ TIL} = (C_i / C_o) \times 100 \quad (2)$$

where $C_i$ and $C_o$ are conductivity of water within the glass bottles measured before and after heating, respectively.

Cation concentrations in the $NH_4^+$-treatment solutions were determined by a DX-100 ion chromatograph (Dionex) equipped with an IonPac CS12A analytical column (250 × 4 mm). Methanesulfonic acid solution (20 mM) was used as eluent with a flow rate of 1.0 ml $min^{-1}$ and the total analytical time lasted for 10 min. The cation measurements were carried out in triplicate for the $NH_4^+$-treatment solutions and the blank solution (deionized water). All reagents were analytical grade and dissolved in deionized water.

### Statistical analysis

Differences between the effects of $NH_4^+$ treatments were analyzed by 1-way ANOVA using SPSS. The pairwise comparison was performed with the Student-Newman-Keuls method. Curve estimation was used to further visualize the effects of $NH_4^+$ treatments on membrane permeability. A linear regression analysis (stepwise) was applied to obtain the regression equation between those indices with high Pearson's correlation coefficients. Difference was considered significant if $p \leq 0.05$.

## RESULTS

### Growth and chlorophyll content

Growth of *Myriophyllum spicatum* and *Ceratophyllum demersum* was significantly affected by the $NH_4^+$ treatments, with an $NH_4^+$-N concentration of 1 mg $l^{-1}$ slightly stimulating the growth of *M. spicatum* while $NH_4^+$-N concentrations above 8 mg $l^{-1}$ inhibited the growth of both species ($p < 0.05$; Fig. 1). Moreover, negative growth was observed for *M. spicatum* and *C. demersum* at $NH_4^+$-N concentrations ≥16 mg $l^{-1}$. The chl *a* and chl *b* contents of *M. spicatum* and *C. demersum* were significantly increased at 8 and 16 mg $l^{-1}$ $NH_4^+$-N, respectively ($p < 0.05$; Fig. 1). The chl *a* content of both species was decreased at >16 mg $l^{-1}$ $NH_4^+$-N. The ratio of chl *a* to chl *b* of *M. spicatum* was decreased at >4 mg $l^{-1}$ $NH_4^+$-N and that of *C. demersum* at >16 mg $l^{-1}$ $NH_4^+$-N, although the chl *b* content was unaffected (Fig. 1). Visual chlorosis was observed in the shoots of *M. spicatum* and *C. demersum* at >2 and >8 mg $l^{-1}$ $NH_4^+$-N, respectively.

### Membrane permeability and uptake/leakage of cations

Compared with the control, treatment with high $NH_4^+$ concentrations ($NH_4^+$-N ≥ 2 mg $l^{-1}$) significantly increased membrane permeability of *Myriophyllum spicatum* and *Ceratophyllum demersum* ($p \leq 0.001$; Fig. 2). The membrane permeability (expressed as percentage of TIL) showed a quadratic response to the external $NH_4^+$ enrichment ($p < 0.001$; Fig. 2). When the plants were incubated in 8 to 128 mg $l^{-1}$ $NH_4^+$-N, the membrane permeability of *M. spicatum* and *C. demersum* increased by 0.9 to 4.8 fold and 1.6 to 11.7 fold, respectively, as compared to the control. *C. demersum* had greater ion leakage than *M. spicatum* at the equivalent $NH_4^+$ treatments (Fig. 2).

The net changes in $NH_4^+$ concentration in the incubating solutions showed a concave curve response to the initial $NH_4^+$ concentration for *Myriophyllum spicatum* and *Ceratophyllum demersum*. *M. spicatum* had a slight net $NH_4^+$ leakage at an external $NH_4^+$-N concentration of ≤4 mg $l^{-1}$, a slight net $NH_4^+$ absorption at 8 to 16 mg $l^{-1}$, and a massive net $NH_4^+$ leakage at ≥32 mg $l^{-1}$ ($p < 0.001$; Fig. 3). *C. demersum* had a net $NH_4^+$ absorption at an external $NH_4^+$-N concentration of ≤64 mg $l^{-1}$, with maximum $NH_4^+$ absorption at 16 to 32 mg $l^{-1}$, and a massive net $NH_4^+$ leakage at >64 mg $l^{-1}$ ($p < 0.001$; Fig. 3).

For both *Myriophyllum spicatum* and *Ceratophyllum demersum*, the net changes in the cation ($Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$) concentrations in the incubating solutions were positively correlated with the $NH_4^+$ concentrations in the incubating solutions ($r > 0.6$ and $p < 0.001$ for all relationships; Fig. 4). The net

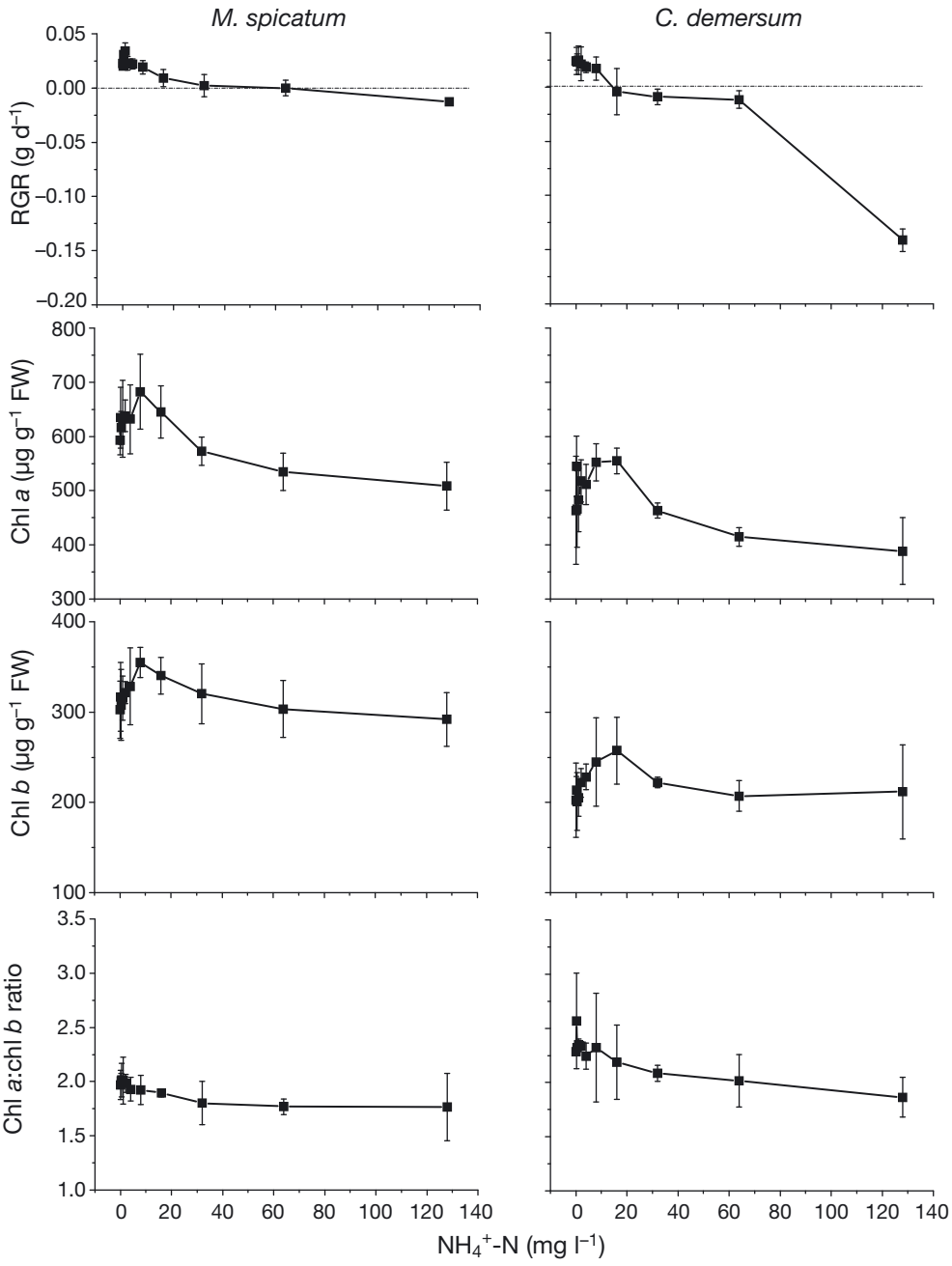


Fig. 1. *Myriophyllum spicatum* and *Ceratophyllum demersum*. Relative growth rate (RGR), chl *a* and chl *b* contents (in µg g$^{-1}$ fresh weight [FW]) and chl *a* to chl *b* ratios following growth in modified Hoagland solution with various $NH_4^+$-N concentrations for 4 d. Data represent means ±SD (n = 3)

changes in the cations were dependent on the type of cations, the plant species and the $NH_4^+$ concentration. For *M. spicatum*, net absorption of $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ occurred when the external $NH_4^+$-N concentration was not more than 2, 16, 4 and 16 mg l$^{-1}$, respectively, above which the $NH_4^+$ treatment induced net leakages of the cations. For *C. demersum*, net absorption of $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ occurred when the external $NH_4^+$-N concentration was not more than 2, 2, 2 and 8 mg l$^{-1}$, respectively, above which the $NH_4^+$ treatment induced net leakages of the cations. The threshold external $NH_4^+$ concentrations inducing cation net leakages were higher for *M. spicatum* than for *C. demersum*.

The net changes in the cation ($Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$) concentrations in the incubating solutions were positively correlated with the membrane permeabilities of *Myriophyllum spicatum* and *Ceratophyllum demersum* ($r > 0.7$ and $p < 0.001$ for all relationships; Fig. 5, Table 1), and responded in 2 phases. During the first phase, when the membrane permeability was lower than a critical value (7 to 8% TIL), they were negative and independent of the membrane permeability, and during the second phase, they

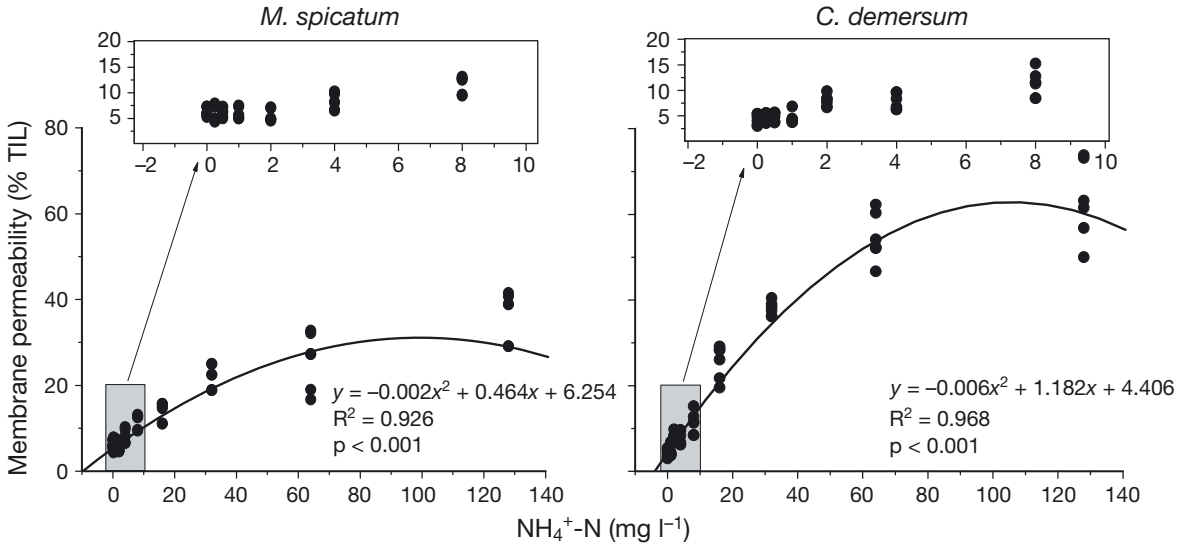


Fig. 2. *Myriophyllum spicatum* and *Ceratophyllum demersum*. Membrane permeability following culture in modified Hoagland solution with various $NH_4^+$-N concentrations for 4 d. Curves indicate quadratic responses of membrane permeability to $NH_4^+$-N concentrations. The insets show magnified sections of the curves. TIL: total ion leakage

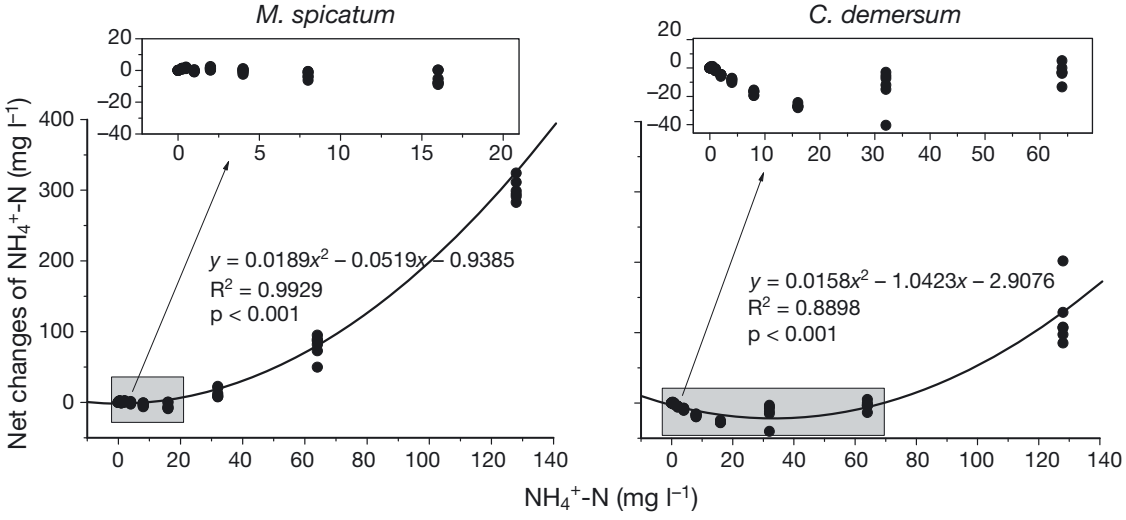


Fig. 3. *Myriophyllum spicatum* and *Ceratophyllum demersum*. Net changes in $NH_4^+$-N concentration induced by incubating in modified Hoagland solution with various initial $NH_4^+$-N concentrations for 4 d. Curves indicate quadratic responses of net changes in $NH_4^+$-N concentration to various initial $NH_4^+$-N concentrations. The insets show magnified sections of the curves

showed a gradual increase with increasing membrane permeability. The slopes for the linear relationships between the net changes in cation concentration and membrane permeability were steeper for *M. spicatum* than for *C. demersum* (Fig. 5).

The net changes in $NH_4^+$ concentration in the incubating solutions showed a quadratic curve response to the membrane permeability. Based on the fitted quadratic equations, maximum $NH_4^+$ absorption of *Myriophyllum spicatum* and *Ceratophyllum demersum* occurred at about 9 and 23% TIL, respectively ($p < 0.001$; Fig. 6).

## DISCUSSION

The results of our studies on the effects of $NH_4^+$ gradients on growth and membrane permeabilities clearly show that high $NH_4^+$ concentrations in the incubating solutions were stressful to *Myriophyllum spicatum* and *Ceratophyllum demersum*, as indicated by reduced growth, lower chlorophyll contents, lower chl *a*:chl *b* ratios, leaf chlorosis, and increased cation leakage due to increased membrane permeabilities. Based on leaf chlorosis, we suggest that 2 and 8 mg $l^{-1}$ $NH_4^+$-N in the solutions were toxic to

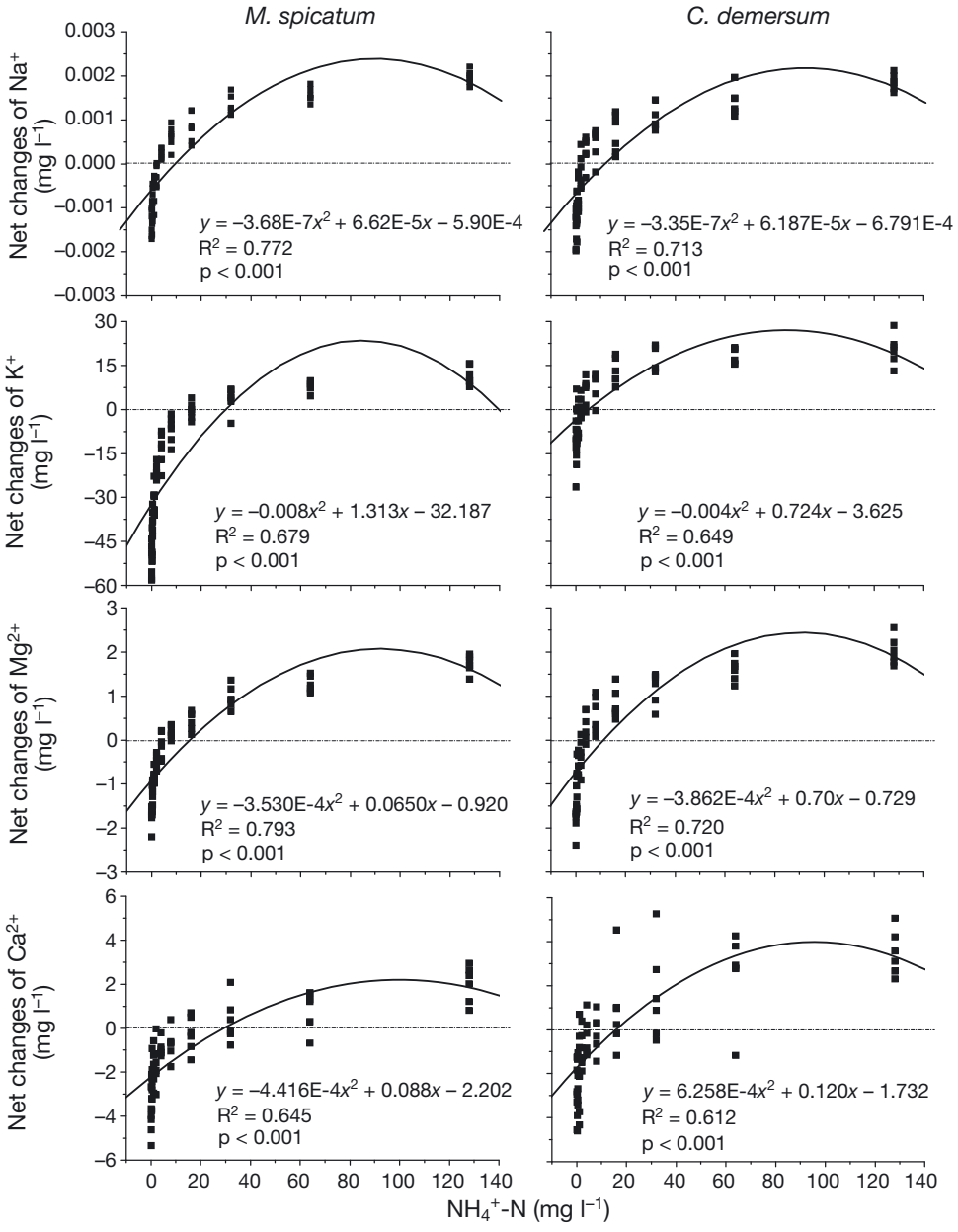


Fig. 4. *Myriophyllum spicatum* and *Ceratophyllum demersum*. Net changes in $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ concentrations induced by incubating in modified Hoagland solution with various initial $NH_4^+$-N concentrations for 4 d. Curves indicate quadratic responses of net changes in $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ concentrations to the various initial $NH_4^+$-N concentrations

*M. spicatum* and *C. demersum*, respectively. In our study (0 to 128 mg $l^{-1}$ $NH_4^+$-N), chlorosis was visible under high $NH_4^+$ concentrations and did not have to be precisely measured, e.g. with the method of Giesen (1990). The threshold $NH_4^+$ concentrations for *M. spicatum* and *C. demersum* were higher than those reported for other taxa, e.g. 0.35, 0.5, 0.56 and 1 mg $l^{-1}$ $NH_4^+$-N were toxic to the submersed macrophytes *Zostera marina*, *Potamogeton maackianus*, *Vallisneria natans* and *Elodea Canadensis*, respectively (Dendene et al. 1993, van Katwijk et al. 1997, Cao et al. 2007, Li et al. 2007), but still fall within the range of $NH_4^+$ concentrations of many eutrophic lakes (Jin 2003, Jin et al. 2005, Wu et al. 2006, Cao et al. 2007, Zhang et al. 2011). Rolando Quirós (2003) demonstrated that $NH_4^+$-N concentrations are closely related to the TN levels in lakes. From this perspective, the corresponding TN can be used as a reference for $NH_4^+$-N when evaluating the effects on macrophytes.

We found that $NH_4^+$ enrichment of the solutions increased membrane permeability of *Myriophyllum spicatum* and *Ceratophyllum demersum*, and consequently affected the flux of cations ($Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$ and $NH_4^+$) between the plants shoots and the solutions. Concentrations of 4 and 2 mg $l^{-1}$ $NH_4^+$-N were enough to increase the membrane permeability of *M. spicatum* and *C. demersum*, respectively, and

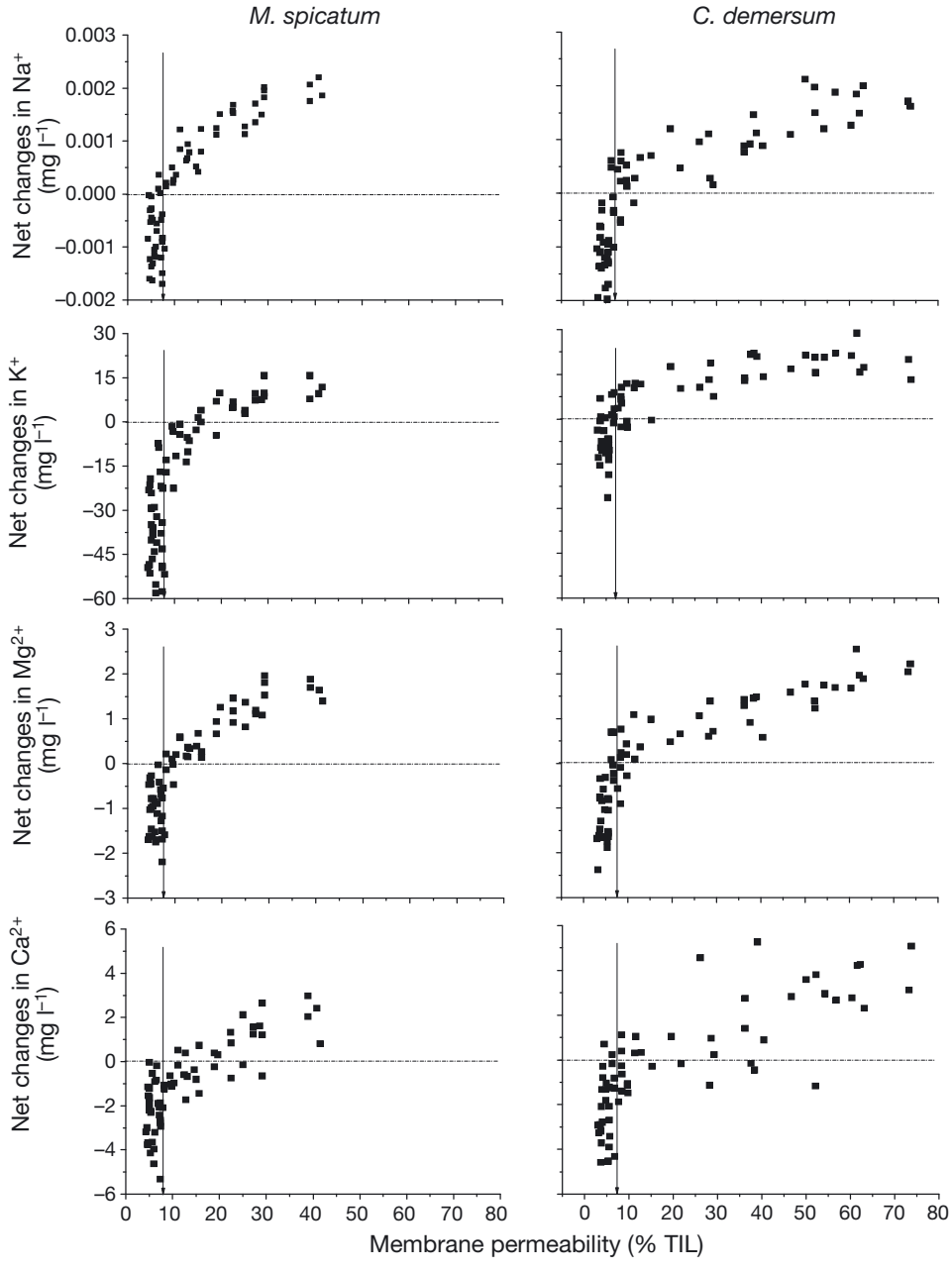


Fig. 5. *Myriophyllum spicatum* and *Ceratophyllum demersum*. Net changes in $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ concentrations in relation to membrane permeability (% total ion leakage, TIL) following incubation in modified Hoagland solution with various initial $NH_4^+$-N concentrations for 4 d. Vertical arrows indicate the membrane permeability threshold below which the net changes in the cation concentrations were not responsive to membrane permeability, and above which they showed a gradual increase with increasing membrane permeability

to induce cation leakage from the plants, although the threshold $NH_4^+$ concentrations differed between the specific cations. Therefore, the cation leakages induced by the $NH_4^+$ treatments in the present study were in agreement with the decreased contents of $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ in plant tissues that have been observed in many $NH_4^+$-fed plants (Salsac et al. 1987, Engels & Marschner 1993, Gloser & Gloser 2000). As the molecular radius of $NH_4^+$ is equivalent to that of $K^+$, $NH_4^+$ is presumed to compete for $K^+$ channels in plant cell membranes, a process explaining the lower $K^+$ contents in $NH_4^+$-fed plants (Szczerba et al. 2008, ten Hoopen et al. 2010). However, the occurrence of net absorption of $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ by *M. spicatum* and *C. demersum* induced by the low external $NH_4^+$ concentrations and the net leakages of the cations induced by the high external $NH_4^+$ concentrations in the present study imply that the increased membrane permeability of the plants resulted in the increased mobilization of a range of cations and was not specific to $K^+$. Many plants tend to accumulate N in excess of their requirements for growth when they grow in exclusive $NH_4^+$ environments. With the uptake of a large quantity of $NH_4^+$

Table 1. *Myriophyllum spicatum* and *Ceratophyllum demersum*. Relationships between membrane permeability and concentrations of $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$ in the incubating solutions after culture in the solutions for 4 d. All correlations are significant at the 0.001 level (2-tailed)

| | Permeability | $Na^+$ | $K^+$ | $Mg^{2+}$ |
|---|---|---|---|---|
| *M. spicatum* | | | | |
| $Na^+$ | 0.850 | | | |
| $K^+$ | 0.774 | 0.945 | | |
| $Mg^{2+}$ | 0.855 | 0.960 | 0.958 | |
| $Ca^{2+}$ | 0.785 | 0.878 | 0.878 | 0.897 |
| *C. demersum* | | | | |
| $Na^+$ | 0.823 | | | |
| $K^+$ | 0.770 | 0.872 | | |
| $Mg^{2+}$ | 0.840 | 0.906 | 0.895 | |
| $Ca^{2+}$ | 0.784 | 0.759 | 0.714 | 0.814 |

ions into the cell, there will be a change in the electrochemical gradient between the cytosol and medium, which needs to be counteracted by simultaneous absorption of anions and/or by extruding cations out of the cytosol. Therefore, uptake of $NH_4^+$ ions is generally accompanied by changes in the anion and cation content of the culture medium that depend on the cation–anion balance in the cytosol (Kirkby & Mengel 1967). This is probably why an increase in the supply of one ion species in the culture solution decreases the uptake of other ion species with a similar charge when there is no specific competition for a carrier site (Lohaus et al. 2000, Shi & Sheng 2005). Therefore, high $NH_4^+$ concentrations in eutrophic lakes have the potential to induce nutrition limitation of submersed macrophytes due to increased membrane permeability and cation leakage.

In the present study, we found that the net changes in the cations in the incubating solutions showed a 2-phase response to the membrane permeability of *Myriophyllum spicatum* and *Ceratophyllum demersum*. When the membrane permeability was below 7 to 8% TIL, the net change in the cation was negative and independent of the membrane permeability. When the membrane permeability was above 7 to 8% TIL, the net change in the cation showed a gradual increase with increasing membrane permeability. Considering that membrane permeability below 7 to 8% TIL did not respond to the external $NH_4^+$ concentration, the absorption of cations by *M. spicatum* and *C. demersum* in the first phase was possibly mediated by structural cation channels in the membrane. In the second phase, the leakage of cations from the plants was perhaps mediated by inducible cation channels in the membrane that were affected by the high external $NH_4^+$ concentrations. Slopes for the linear relationships between the net changes in the cation concentration and membrane permeability were steeper for *M. spicatum* than for *C. demersum*,

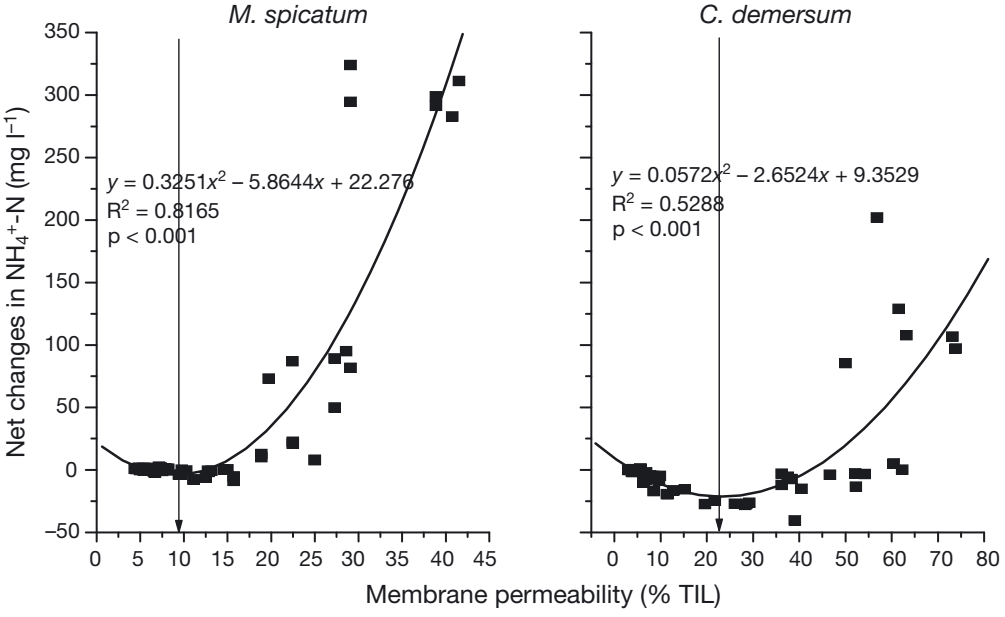


Fig. 6. *Myriophyllum spicatum* and *Ceratophyllum demersum*. Net changes in $NH_4^+$-N concentration in relation to membrane permeability (% total ion leakage, TIL) following incubation in modified Hoagland solution with various initial $NH_4^+$-N concentrations for 4 d. Curves indicate quadratic responses of net changes in $NH_4^+$-N concentration to membrane permeability. Vertical arrows indicate the membrane permeability that induced maximum net absorption of $NH_4^+$-N according to the quadratic curves

indicating that *M. spicatum* was more sensitive to $NH_4^+$ enrichment than *C. demersum*. The 2-phase response of the cation flux to membrane permeability in the present study is in agreement with the results of many studies showing that cation uptake by plants is mediated by structural cation channels in the cell membrane at low cation concentrations and by inducible cation channels at high cation concentrations (Epstein et al. 1963, Epstein 1976).

Our results reveal that the maximum net absorption of $NH_4^+$ by *Myriophyllum spicatum* and *Ceratophyllum demersum* occurred in the treatments with medium $NH_4^+$ concentrations and moderate membrane permeability, and net $NH_4^+$ leakage from the plants was observed in the treatments with high $NH_4^+$ concentrations. At a first glance, the net $NH_4^+$ leakage from *M. spicatum* and *C. demersum* in the present study seems inconsistent with the accumulation of $NH_4^+$ and free amino acids (FAA) in many submersed macrophytes under moderate $NH_4^+$ stress (Smolders et al. 1996, Cao et al. 2004, 2007, 2009, 2011, Zhang et al. 2011). However, it is possible that such contradictory processes could occur simultaneously because intracellular $NH_4^+$ and FAA can also be generated from degradation of proteins and transport of $NH_4^+$ out of the plant cell, which may happen when plants are under severe $NH_4^+$ stress (Jackson et al. 1993, Britto & Kronzucker 2002). Compared with *M. spicatum*, *C. demersum* had higher net $NH_4^+$ absorption at the medium $NH_4^+$ concentrations and lower net $NH_4^+$ leakage at the high $NH_4^+$ concentrations in the present study, implying that *C. demersum* was more effective in transporting $NH_4^+$ across the membrane. These results, together with the lower FAA accumulations of *C. demersum* under $NH_4^+$ stress (Cao et al. 2011), demonstrate that the higher membrane permeability of *C. demersum* allows greater transport of $NH_4^+$ out of the plant cell, which consequently decreases $NH_4^+$ and FAA accumulation. This supports the futile $NH_4^+$ cycling hypothesis of $NH_4^+$ toxicity proposed by Britto et al. (2001).

In summary, we found that high external $NH_4^+$ concentrations were stressful to *Myriophyllum spicatum* and *Ceratophyllum demersum*, and increased their membrane permeability. This affects a range of plant physiological functions, e.g. the net flux of $NH_4^+$ and cations in the plant cells and the effectiveness of $NH_4^+$ transport across the membrane. The higher membrane permeability of *C. demersum* might increase its tolerance to $NH_4^+$ stress due to more effective $NH_4^+$ cycling across the membrane as compared to *M. spicatum*.

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