Kinetics of ammonium, nitrate and phosphorus uptake by *Canna indica* and *Schoenoplectus validus*

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**Abstract**

*Canna indica* L. is an upright perennial rhizomatous herb, and *Schoenoplectus validus* (Vahl) A. Löve and D. Löve is a tall, perennial, herbaceous sedge. The nutrient uptake kinetics of *C. indica* and *S. validus* were investigated using the modified depletion method after plants were grown for 4 weeks in simulated secondary-treated wastewater. The maximum uptake rate (*I* \(_{\text{max}}\)) and Michaelis–Menten constant (*Km*) were estimated by iterative curve fitting. The *I* \(_{\text{max}}\) for NH\(_4\)-N (623 μmol g\(^{-1}\) dry root weight h\(^{-1}\)) was significantly higher than that for NO\(_3\)-N (338 μmol g\(^{-1}\) dry root weight h\(^{-1}\)) in *S. validus*. In contrast, no difference was observed in *C. indica*. The *I* \(_{\text{max}}\) values for NO\(_3\)-N and NH\(_4\)-N were higher in *S. validus* than in *C. indica*. A significantly lower *Km* was detected for NO\(_3\)-N uptake in *C. indica* (385 μmol L\(^{-1}\)) compared to that in *S. validus* (1908 μmol L\(^{-1}\)). The *I* \(_{\text{max}}\) for PO\(_4\)-P did not differ between the plant species. The *Km* for PO\(_4\)-P was significantly higher in *C. indica* (157 μmol L\(^{-1}\)) than in *S. validus* (60 μmol L\(^{-1}\)). In conclusion, we found that *S. validus* preferred NH\(_4\)-N over NO\(_3\)-N, had greater capacity for N uptake and higher affinity for PO\(_4\)-P, but *C. indica* had greater affinity for NO\(_3\)-N. Nutrient uptake capacity is likely related to habitat preference, and is influenced by the structure of roots and rhizomes.

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1. Introduction

Wetland angiosperm species may differ in their capacity to assimilate nutrients from wastewater (Kadlec and Knight, 1996). For instance, the nutrient uptake capacity of *Phragmites australis* (2.5 kg N ha\(^{-1}\) yr\(^{-1}\) and 120 g P ha\(^{-1}\) yr\(^{-1}\)) was higher than that of *Cyperus papyrus* (1.1 kg N ha\(^{-1}\) yr\(^{-1}\) and 50 g P ha\(^{-1}\) yr\(^{-1}\); Brix, 1994). Differences in nutrient accumulation between species are likely to reflect differences in developmental stages and efficiency of nutrient uptake and use (Tanner, 1996; Güsewell and Bollens, 2003).

Angiosperms generally assimilate nitrogen as either NH\(_4\)-N or NO\(_3\)-N, with some species showing a strong preference for one ionic form over the other (Kronzucker et al., 1997; Forde and Clarkson, 1999). Wetland soils are anoxic throughout much of the profile; hence, many wetland plants have adapted to taking up NH\(_4\)-N because NO\(_3\)-N availability is low (Sasakawa and Yamamoto, 1978). However, a widely grown variety of lowland rice was exceptionally efficient in absorbing and assimilating NO\(_3\)-N compared to other plant species (Kronzucker et al., 1999, 2000). A modelling study by Kirk and Kronzucker (2005) implied that wetland plants might be efficient in capturing NO\(_3\)-N formed in the rhizosphere. Fang et al. (2007a) have observed that some wetland species require NO\(_3\)-N as a source of N. Hence, NO\(_3\)-N uptake by wetland plants could be more important than previously thought (Kirk and Kronzucker, 2005).

The kinetics of nutrient uptake allow comparisons of uptake efficiency among species, provide insight into uptake mechanisms, and facilitate predictive modelling of nutrient uptake. Even though the knowledge of kinetics of nutrient uptake would be useful in clarifying the functions of aquatic macrophytes in constructed wetlands, there is a lack of information on the kinetics of N and P uptake for freshwater emergent macrophytes (e.g. Nelson et al., 1981; Brix et al., 1994; Dyhr-Jensen and Brix, 1996; Romero et al., 1999; Tylova-Munzarova et al., 2005).

*Canna indica* L. is an upright perennial rhizomatous herb native to tropical America, belonging to the family Cannaceae. *Schoenoplectus validus* (Vahl) A. Löve and D. Löve is tall, perennial, herbaceous sedge (family Cyperaceae). These two plant species grow naturally in disturbed land and along creeks, and is often grown in streets and parks as an ornamental plant, whereas *S. validus* grows along creeks, lakes and open swamps (in fresh and brackish water). Habitat differences imply that *S. validus* could be more tolerant of prolonged inundation compared to *C. indica*. 
Hence, it is possible that S. validus has adapted to NH4–N nutrition because nitrification is limited in anoxic conditions. Hence, the objective of this study was to compare the kinetics of NH4–N, NO3–N and P uptake by the two plant species, to verify the pattern suggested by habitat preferences.

2. Materials and methods

2.1. Plant material and initial conditions

The experiment was conducted in a controlled-environment room (20/15 °C day/night temperature, 75–85% relative humidity, 12-h photoperiod, irradiance of 375–490 μmol quanta m−2 s−1, PAR) at the University of Western Australia (31°58’S, 115°49’E). The seedlings of C. indica and S. validus were collected from the local nursery and transplanted into a solution culture system in 4-L plastic vessels. At the planting time, seedlings of C. indica were approximately 0.06 m tall with 1–2 leaves, and S. validus were approximately 0.07 m tall with 2–3 ramifications. The nutrient solution had N and P concentrations similar to the secondary-treated wastewater at the Subiaco Wastewater Treatment Plant in Perth, Western Australia, as described in Zhang et al. (2007a, b). The solution contained following macronutrients (mmol L−1): 1.25 N (with a 1:1 ratio of NH4–N and NO3–N) and 0.32 P (as KH2PO4), 0.64 K+, 0.25 Ca2+, 0.21 Mg2+ and 0.22 SO42−, and micronutrients (μmol L−1): 0.6 Zn2+, 0.15 Cu2+, 6.1 Fe3+, 0.9 Mn2+, 24 BO33− and 5 MoO42−. The pH was adjusted to 6.5 and the nutrient solutions were replaced twice weekly to avoid significant changes in pH and depletion of nutrients. Solutions were vigorously aerated. After 4 weeks of growth, the uptake kinetics of ammonium, nitrate and phosphorus by plants were measured separately.

2.2. Uptake kinetics measurements

A modified depletion method (Barber, 1984) was used to measure nutrient uptake kinetics. The measurements were conducted in the same controlled-environment room used for the plant establishment. Plants of uniform size were selected, rinsed with deionised water and placed into nutrient solutions as described above but without N or P for 2 days to elicit starvation-induced maximal uptake response during the subsequent measurements. After this starvation period, one C. indica plant or three S. validus plants were put into a jar containing 400 mL nutrient solution with different concentrations of N or P. The numbers of plants used was based on plant biomass (on average, 2.22 g dry weight per jar). The roots and rhizomes were submerged in the solution. The initial concentrations of N ([NH4]SO4 or KNO3) were (mmol L−1) 0.05, 0.1, 0.2, 0.4, 0.6, 1.0 and 2.0. The concentrations of P (KH2PO4) were (μmol L−1) 3, 5, 10, 30, 60, 120 and 240. The other nutrients were compensated for and kept at levels described above. Each treatment had four replicates.

Each jar was weighed at the beginning and at the end of the experiment to calculate the water loss through evapotranspiration. The solution was sampled and the plants were harvested after two hours of nutrient uptake. The solution samples were stored before N or P analysis. The roots were separated from the rhizomes. All plant samples were dried to a constant weight at 70 °C for 5 days in a forced-air cabinet and weighed.

Ammonium, nitrate and nitrite in solution were measured by an automated Skalar segmented-flow analyzer. The method for the determination of NH4–N, NO3–N and PO4–P uptake kinetics of S. validus was as a function of N concentrations. The lines were drawn by non-linear Michaelis–Menten curve fitting.

The uptake kinetics of NH4–N, NO3–N and PO4–P uptake by C. indica and S. validus was based on plant biomass (on average, 2.22 g dry weight per jar). The nutrient solution with different concentrations of N or P. The numbers of plants were estimated by fitting Michaelis–Menten equation using Statgraphics Centurion XV (StatPoint Technologies, Inc., Virginia, USA).

All statistical tests were performed using SPSS version 16.0 for Windows. Two-way ANOVA was used to determine the effect of N forms and plant species on the kinetic parameters for N uptake and the main effects of plant species and N forms (Table 1). In S. validus, the kinetic parameters for NH4–N uptake were significantly higher for NH4–N than NO3–N. By contrast, the kinetic parameters for NO3–N uptake were similar to those for NO3–N in C. indica. The kinetic parameters for both N forms were significantly lower in C. indica than S. validus. A significantly lower kinetic parameter was detected for NO3–N uptake in C. indica compared to S. validus, but no significant differences were observed for NH4–N uptake. The rates of PO4–P uptake by C. indica and S. validus as a function of PO4–P concentrations. The lines were drawn by non-linear Michaelis–Menten curve fitting.

3. Results

The uptake of NO3–N by C. indica was slightly higher than uptake of NH4–N. By contrast, S. validus took up more NO3–N than NH4–N. The uptake of NH4–N was higher by S. validus than C. indica, and the trends of NO3–N uptake were different between the two species (Fig. 1). The uptake of PO4–P of the two species was similar (Fig. 2).

![Fig. 1. The rates (means ± SE; n = 4) of NH4–N and NO3–N uptake by C. indica and S. validus as a function of N concentrations. The lines were drawn by non-linear Michaelis–Menten curve fitting.](image)

![Fig. 2. The rates (means ± SE; n = 4) of PO4–P uptake by C. indica and S. validus as a function of PO4–P concentrations. The lines were drawn by non-linear Michaelis–Menten curve fitting.](image)

Table 1

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Species</th>
<th>( I_{\text{max}} ) (μmol g⁻¹ dry root weight h⁻¹)</th>
<th>( K_m ) (μmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4–N</td>
<td>C. indica</td>
<td>238 ± 25</td>
<td>1434 ± 128</td>
</tr>
<tr>
<td></td>
<td>S. validus</td>
<td>623 ± 70</td>
<td>1505 ± 151</td>
</tr>
<tr>
<td>NO3–N</td>
<td>C. indica</td>
<td>167 ± 14</td>
<td>385 ± 27</td>
</tr>
<tr>
<td></td>
<td>S. validus</td>
<td>338 ± 19</td>
<td>1908 ± 120</td>
</tr>
<tr>
<td>PO4–P</td>
<td>C. indica</td>
<td>22 ± 3.8</td>
<td>157 ± 25</td>
</tr>
<tr>
<td></td>
<td>S. validus</td>
<td>18 ± 4.8</td>
<td>60 ± 9.0</td>
</tr>
</tbody>
</table>

Means (±SE, n = 4) with different letters within a column are significantly different (p < 0.05) based on Duncan’s new multiple range method with Bonferroni correction for N uptake and t-test for P uptake.
difference in the \( K_m \) for NH\(_4\)-N uptake was observed between the species. The \( K_m \) for PO\(_4\)-P uptake was significantly influenced by the plant species, but no significant difference in the \( I_{\text{max}} \) was detected (Table 1).

4. Discussion

The results of N kinetics revealed differences between \( C. \ indica \) and \( S. \ validus \) in the uptake of NH\(_4\)-N and NO\(_3\)-N. Both NH\(_4\)-N and NO\(_3\)-N are important nitrogen sources for plant growth, and the preferences of different species for particular N sources can have important ecological and practical implications (Forde and Clarkson, 1999). \( S. \ validus \) showed higher maximum uptake capacity (\( I_{\text{max}} \)) for NH\(_4\)-N than for NO\(_3\)-N. Indeed, NH\(_4\)-N is the preferred form of inorganic N for most wetland macrophytes (e.g. Brix et al., 2002; Tylova-Munzarova et al., 2005; Fang et al., 2007b; Jampeetong and Brix, 2009). In contrast, \( C. \ indica \) showed no preference for N forms. In a study of four wetland plants, Fang et al. (2007a) observed that two species (\( B. \ monnieri \) and \( A. \ )) had preference for NO\(_3\)-N, whereas both N forms were required by \( L. \ repens \).

As we postulated in the introduction, the differential preference for N forms between \( C. \ indica \) and \( S. \ validus \) may relate to habitat preference and relative tolerance to inundation. Habitat preference, however, can also relate to other factors, such as temperature, aeration, pH, sulfide and composition of nutrients in solution, water and salt stress, the plant growth stage and the capacity to form a symbiotic relationship with bacteria or fungi (Bradley and Morris, 1990; Brix et al., 1994, 1996). Differences in nutrient uptake between legumes and cereals. Compared to bacteria or fungi (Bradley and Morris, 1990; Brix et al., 1994, 1996). Differences in nutrient uptake between legumes and cereals. Compared to bacteria or fungi (Bradley and Morris, 1990; Brix et al., 1994, 1996).

Many studies on N uptake have been conducted on crop species. For example, Rao et al. (1993) observed that there were significant differences in N uptake between legumes and cereals. Compared to crops, fewer studies on N uptake kinetics have been reported on emergent wetland species, but the study by Tylova-Munzarova et al. (2005) found that \( P. \ australis \) had higher affinity for NH\(_4\)-N and NO\(_3\)-N compared to \( G. \ maxima \). The study presented here showed higher N uptake capacity (\( I_{\text{max}} \)) in \( S. \ validus \) compared to \( C. \ indica \), but \( C. \ indica \) had higher affinity for NO\(_3\)-N than \( S. \ validus \).

Reported \( I_{\text{max}} \) and \( K_m \) values are greatly variable with plant age, species or varieties and growth conditions, but also due to resolution of the measurement set-up (Bot et al., 1998). The \( I_{\text{max}} \) and \( K_m \) values in the present study were below the highest values reported in the literature. It is worth noting that the rhizome system of \( S. \ validus \), which is similar to \( C. \ indica \), might contribute to N uptake, because Brooker et al. (1999) reported that the rhizome system of \( C. \ indica \) bigelowii could act as a route for N uptake. The nutrient uptake by rhizome may result in the calculated uptake rate to be higher than the actual uptake by the roots of \( S. \ validus \). By contrast, \( C. \ indica \) also has rhizome, but its rhizome acts only as storage for nutrients (Chen et al., 2007).

In the present study, the \( I_{\text{max}} \) for PO\(_4\)-P in \( C. \ indica \) was slightly higher than in \( S. \ validus \), but the \( K_m \) was higher in \( C. \ indica \) than \( S. \ validus \). Therefore, the capacity for P uptake might be greater in \( C. \ indica \) than \( S. \ validus \) when P concentration in the substrate was relatively high, otherwise it might be higher in \( S. \ validus \) when P concentration in the substrate was relatively low. The difference in P uptake efficiency between \( C. \ indica \) and \( S. \ validus \) may relate to their root systems. In a study of eight wetland plant species, including four species (\( C. \ indica \), \( C. \ suportus \), \( P. \ ) and \( Z. \ ), with a fibrous root system and the remaining four (\( A. \ calamus \), \( H. \ ) and \( T. \ ) with a rhizomatic root system, Chen et al. (2007) have observed that root growth was significantly faster, and root surface area considerably larger, with fibrous than with rhizomatic root systems in wastewater culture systems. \( C. \ indica \) had the most developed root system compared to other species studied, with the highest number of roots and root surface area (Chen et al., 2007).

References


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