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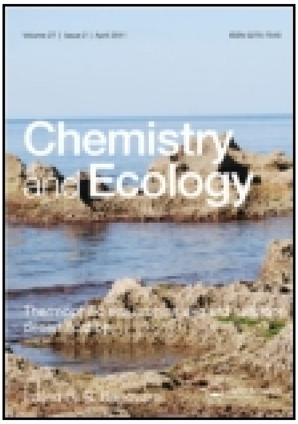

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A comparative study on the growth and nitrogen and phosphorus uptake characteristics of 15 wetland species

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A comparative study on the growth and nitrogen and phosphorus uptake characteristics of 15 wetland species

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The biomass, root morphology and nutrient uptake capacities of 15 species of local wetland plants were investigated in hydroponic culture. The wetland plants were exposed to $38.5\,\mathrm{mg{\cdot}L^{-1}}$ of NH_4^+-N, $132.8\,\mathrm{mg{\cdot}L^{-1}}$ of NO_3^--N and $10\,\mathrm{mg{\cdot}L^{-1}}$ of dissolved inorganic P for 28 days. Mean total biomass of the 15 species ranged from 1.2 to $21.6\,\mathrm{g\,plant^{-1}}$, with above/below ground ratios (AG:BG) in the range 1.7–5.5. Mean NH_4^+-N, NO_3^--N uptake rates ranged from 3.7 to $14.3\,\mathrm{mg\,N{\cdot}day^{-1}}$ (accounting for 8.0–49.4% of the NH_4^+-N supply) and 17.8 to $59.4\,\mathrm{mg\,N{\cdot}day^{-1}}$ (17.8–59.6% of the NO_3^--N supply). Mean P uptake rate ranged from 1.71 to $4.61\,\mathrm{mg\,P{\cdot}day^{-1}}$ (24.1–61.5% of the P supply). The N and P concentrations in plant tissues ranged from 28.2 to $606.1\,\mathrm{mg\,N{\cdot}plant^{-1}}$ and 4.1 to $53.1\,\mathrm{mg\,P{\cdot}plant^{-1}}$, with AG:BG ratio in the range 1.7–7.0 and 1.6–4.6, respectively. The accumulation of N and P in plant tissues was both significantly correlated with plant biomass and root surface area. Among the different species, *Canna generalis*, *Typha latifolia*, *Thalia dealbata* and *Lythrum salicaria* had greater above- and below-ground biomass, larger root surface area, and greater nutrient uptake and storage rates than the other plants. Our results suggest (or indicate) that the selection of plant species suitable for constructed wetlands can be based on plant biomass and root surface area.

Keywords: wetland plant; nutrient uptake; root morphological characteristics; biomass

1. Introduction

Eutrophication (excessive nutrient enrichment) in freshwaters has led to an increase in occurrences of cyanobacteria blooms worldwide [1]. In China, occurrences of cyanobacteria blooms and associated degradations of aquatic environments (e.g. reduced transparency of waters and malodour) have been documented since 1990 [2]. Effluent from municipal wastewater treatment plants has been identified as one of the major point sources of excessive nutrients. One way to remove excessive nutrients from wastewater is the use of constructed wetlands [3]. In many parts of China, constructed wetlands have been used as part of municipal wastewater treatment to reduce not only nutrients, but also organic matter, solids and pathogens under a wide range of loading conditions [4,5]. The advantages of constructed wetland in contrast to tradition wastewater treatment

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facilities are that they require less energy to operate and are efficient in the removal of nutrients and other pollutants, providing multiple ecological benefits [6]. In this regard, plants, as an integral part of wetland systems, play an important functional role [7–9], through turbulence reduction that encourages particle settling, facilitation of chemical and bacterial processes by changing rhizosphere properties, enhancement of nutrients removal through biomass accumulation, fixation of inorganic and organic particulates, and the creation of an oxidised rhizosphere [10,11]. In a constructed wetland system, plants could remove 50–80% of the nitrogen (N) and phosphorus (P) loads under different hydraulic retention times [12]. Dierberg et al. [13] reported that the mean total P concentration of 107 $\mu g \cdot L^{-1}$ in flows was decreased to 52, 29 and 23 $\mu g \cdot L^{-1}$ in effluents under hydraulic retention times of 1.5, 3.5 and 7.0 days, respectively. Nutrient removal rates by different plant species used in constructed wetland systems varied considerably across studies [12,14]. The nutrient removal rate of *Phragmites australis* (2.5 kg $N \cdot ha^{-1} \cdot year^{-1}$ and 120 g $P \cdot ha^{-1} \cdot year^{-1}$) was higher than that of *Cyperus papyrus* (1.1 kg $N \cdot ha^{-1} \cdot year^{-1}$ and 50 g $P \cdot ha^{-1} \cdot year^{-1}$) [15]. The removal of total N was enhanced by the presence of *Cyperus alternifolius,* compared with wetland plant communities without this species [16].

Many studies on constructed wetlands have focused on the effects of nutrient removal of wetland systems, in relation to wetland engineering design, operation mode and overall efficiency of nutrient removal by a few plant species. However, there have been very few studies on the species-specific ecological characteristics and performances of plants used in constructed wetlands. In China, approximately 45 species of plants have been used in constructed wetlands [17]. Of these, 15 plant species are representative of and most commonly used for constructed wetlands in the Tai Lake region of China [18–20]. In this study, we investigated the growth, morphological characteristics and nutrient uptake of these 15 plant species, with the aim of providing a scientific basis for selecting suitable plant species for constructed wetlands.

2. Materials and methods

2.1. *Plants tested*

Fifteen wetland species that are representative of and common for constructed wetland systems in the Tai Lake region of China were investigated (Table 1).

2.2. *Plant culture and treatment*

Two groups of seedlings were purchased from Hangzhou Nursery Garden. The seedings were cultivated to a height of 15–20 cm, and then carefully washed with tap water and acclimated in clean water for 3 days. Plants were selected for uniformity in vigour and were transferred to 3 L plastic containers ($d = 20$ cm, $h = 17$ cm) filled with nutrient solution. Three replicates for each species were grown in tanks and fixed by a piece of foam on polystyrene plates with four holes each plate. The composition of the nutrient solution followed Yoshida et al. [21] for rice cultivation. The concentrations of NH_4^+-N and NO_3^--N were 38.5 and 132.8 $mg \cdot L^{-1}$, respectively. Phosphorus (10 $mg \cdot L^{-1}$) was supplied in the form of $NaH_2PO_4 \cdot H_2O$. The culture solution was renewed every 4 days.

2.3. *Plant sampling*

After 28 days of culture in nutrient solution, plants were harvested, carefully washed with tap water, and rinsed with deionised water before sample preparation. One group of plants was

Table 1. The characteristics of the different wetland plants.

Name	Abbreviation	Characteristics
Cyperus nutans	CN	Type of perennial wetland plant, widespread in temperate regions
Juncus effusus	JE	Common plant, native in most temperate countries, usually found at water edges or in ditches
Cyperus alternifolius	CA	Grass-like plant in the large sedge family, cultivated worldwide and native to Madagascar
Zizania latifolia	ZL	Manchurian wild rice, a perennial native of China
Arund donax	AD	Giant reed, a tall perennial grass growing in fresh and moderately saline waters of temperate and subtropical regions
Canna generalis	CG	Widespread horticultural species, native to America
Typha latifolia	TL	Common cattail, a perennial herbaceous plant growing in the marshes of temperate and tropical regions in the northern hemisphere
Scirpus triangulatus	ST	Perennial wetland species, common in the Taihu lake region
Typha orientalis	TO	Raupo, a perennial herbaceous wetland species, common in the Taihu lake region
Reineckia carnea	RC	Rare evergreen perennial originating from the Himalayas, and cultivated in mild climates
Iris ensata	IE	Russian iris, a flowering species with a long hollow stem
Thalia dealbata	TD	Cultivated tropical perennial, native to America and Mexico
Alisma orientale	AO	Still water perennial species
Cladium mariscus	CM	Great-Fen sedge, widely distributed in Europe, Asia and Africa, characterised by leaves with sawtooth-like margins
Lythrum salicaria	LS	Purple-loosestrife, a flowering plant native to Europe, Asia, northwest Africa and southeast Australia

prepared for root oxidising capacity (α-naphthylamine) [22] and root morphological analysis (MIN MAV, STD1600$^+$, Epson, USA). Root analysis was carried out using WinRhizo software (MAC, STD1600$^+$, Canada). Another group was separated into shoots and roots, heated in an oven at 105 °C for 30 min and then dried at 70 °C for 48 h. Oven-dried shoots and roots were ground, weighed and put in digestion tubes to measure N and P concentration [23], there were three replicates for each analysis.

2.4. *Laboratory analysis*

Before the culture solution was renewed, water samples were taken and NH_4^+-N, NO_3^--N and total phosphorous (TP) measured. NH_4^+-N was determined by the Nessler method, NO_3^--N by ultraviolet spectrophotometry, and P by the ascorbic acid method [22]. The average daily uptake of nutrients by plants was calculated by from:

$$U = (C_0 - C_s) \cdot 3/4,$$

where U is the average daily uptake of the nutrients by the plants, C_0 is the original concentration of the nutrient solution and C_s is the concentration of the nutrient solution at sampling.

2.5. *Statistical analysis*

The mean and standard deviation of replicates were determined. A one-way analysis of variance (ANOVA) model was used to analyse the significance of biological and chemical parameters. All statistical analyses were performed using SPSS 11.0 software.

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3. Results

3.1. *Plant growth and root morphological characteristics*

All of the test species showed positive growth in the culture solution without obvious symptoms of nutrient deficiency. After 28 days of growth, the mean biomass of plants ranged between 1.2 and 21.6 g·plant^{-1} with mean above/below-ground (AG:BG) ratios of 1.7–5.5 (Figure 1). Biomass varied widely among the 15 species. The maximum plant above- and below-ground biomass was recorded for *Canna generalis* (15.5 and 6.1 g), *Thalia dealbata* (13.2 and 2.9 g), *Lythrum salicaria* (12.8 and 4.6 g) and *Typha latifolia* (10.8 and 3.0 g). The total biomass of the four plants were significantly higher than the other plants.

Mean root surface area ranged between 581.05 and 1683.21 cm^2, with highest root surface area records for *C. generalis*, *Th. dealbata* and *T. latifolia* (Table 2), the differences of the tree plants were significant to the others. Mean number of tips ranged from 6874 to 24023. *C. generalis*, *T. latifolia*, *Typha orientales* and *L. salicaria* produced more tips than the other species and were significantly higher than the other plants. Mean root oxidising capacity ranged from 68.17 to 180.27 μg·g^{-1}·h^{-1} with highest the oxidising capacity reached by *C. generalis*, *Th. dealbata*,

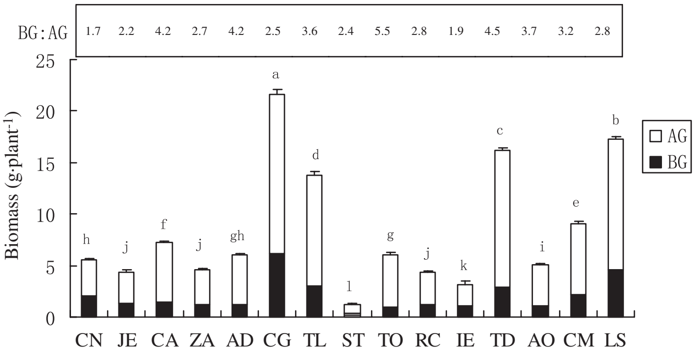

Figure 1. Mean above-ground (AG) and below-ground (BG) biomass of the 15 wetland species after 28 days of culture.

Table 2. Root morphological characteristics and oxidising capacities of the 15 different plant species.

Species	Surface area (cm^2)	Root oxidising capacity (ROC) (ug·g^{-1}·h^{-1})	Number of tips
Cyperus nutans	824.03 ef	83.48 de	8449.37 e
Juncus effusus	581.05 g	71.39 e	6874.24 ef
Cyperus alternifolius	934.68 de	76.74 e	7772.08 ef
Zizania latifolia	985.238 cd	107.93 cde	7514.05 ef
Arund donax	800.44 ef	80.72 e	6353.77 f
Canna generalis	1683.21 a	151.01 ab	17866.35 b
Typha latifolia	1439.83 b	98.62 cde	23873.41 a
Scirpus triangulatus	716.84 fg	68.17 e	7416.98 ef
Typha orientalis	1111.86 c	130.88 bc	24023.51 a
Reineckia carnea	807.34 ef	72.85 e	7199.33 ef
Iris ensata	715.43 fg	79.95 e	7827.09 ef
Thalia dealbata	1488.49 b	148.63 ab	12083.45 d
Alisma orientale	878.54 de	180.27 a	13884.36 c
Cladium mariscus	1133.956 c	123.58 bcd	11435.28 d
Lythrum salicaria	1104.28 c	175.93 a	17204.52 b

Note: Ducan's test (SSR), different letters in the same row (or column) indicate a significant difference at $p = 0.05$.

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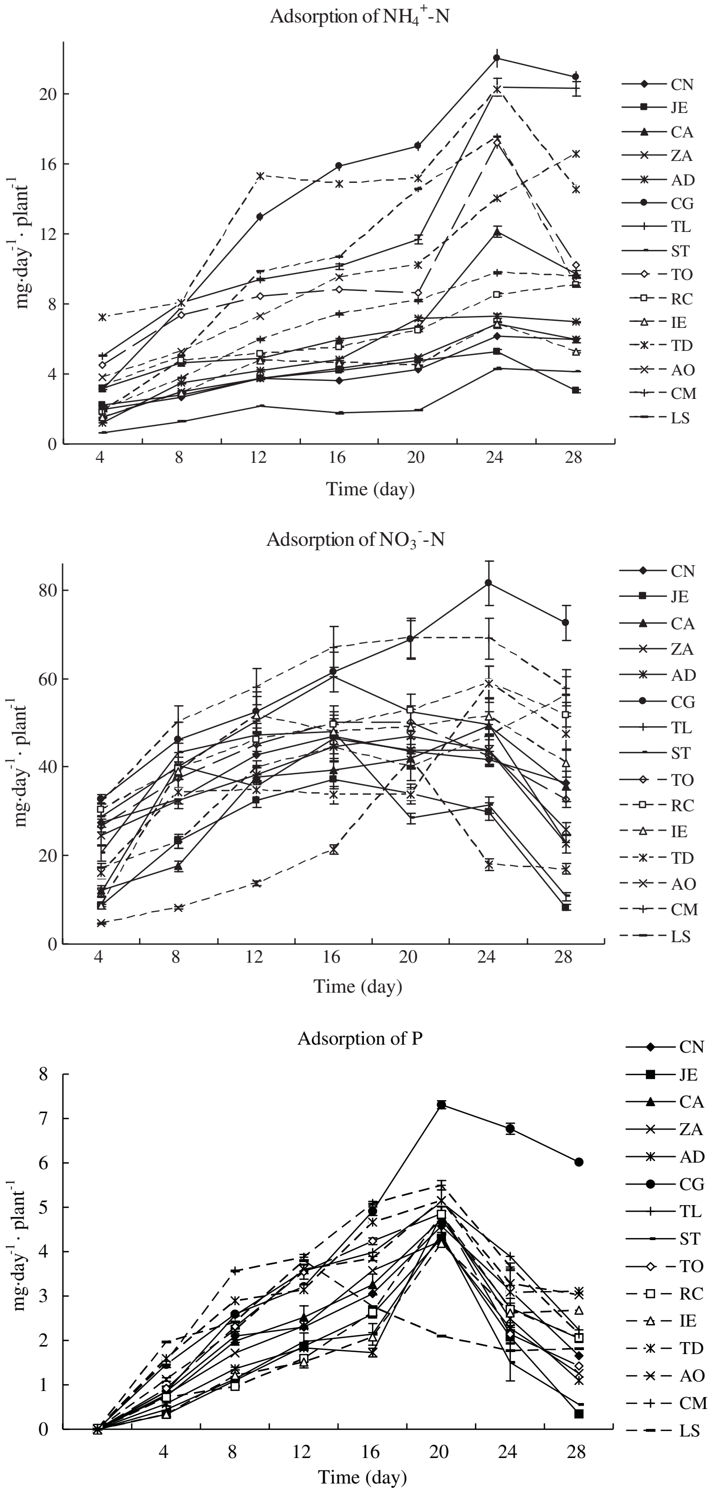

Figure 2. Mean daily NH_4^+-N, NO_3^--N and P uptake rates by the 15 kinds of wetland plants during the 28 days of culture.

Alisma orientale and *L. salicaria*. *C. generalis* showed higher root surface area, more tips and higher oxidising capacity compared with the other plants.

3.2. *Nitrogen and phosphorus uptake by the plants during the culture period*

N uptake differed among species and over time. At the beginning of the experiment, NH_4^+-N uptake ranged from 0.64 to 7.24 mg·day^{-1}. As time passed, the uptake of NH_4^+-N increased and reached a peak of 4.29 to 22.06 mg·day^{-1} during the days 20–24 of culture. After that, a slight decrease was noticed for most of the plants during the days 24–28 (Figure 2). All of the plants showed a gradual increase of NO_3^--N uptake from 4.65-32.75 mg·day^{-1} on day 4 to 21.31–67.04 mg·day^{-1} on days 12–16 day. After that, most of the plants showed a decline in the uptake of NO_3^--N, whereas others like *C. generalis* and *Reineckia carnea* continued to increase in NO_3^--N uptake until day 24 of culture. The initial uptake rate of P ranged from 0.34 to 1.96 mg·day^{-1} on day 4 of culture and gradually increased to 4.30 to 7.31 mg·day^{-1} on day 20 of culture for most of the plants except *L. salicaria*.

Differences among the plant species in N and P uptake became more obvious over time. Mean NH_4^+-N uptake was highest for *C. generalis* (14.3 mg·day^{-1}), *T. latifolia* (12.2 mg·day^{-1}), *Th. dealbata* (13.6 mg·day^{-1}) and *L. salicaria* (10.0 mg·day^{-1}). Mean NO_3^--N uptake rates were highest for *C. generalis* (59.4 mg·day^{-1}) and *Cladium mariscus* (57.6 mg·day^{-1}). Mean P uptake rates were highest for *C. generalis* (4.61 mg·day^{-1}), *T. latifolia* (3.17 mg·day^{-1}), *Th. dealbata* (3.38 mg·day^{-1}) and *Cl. mariscus* (3.62 mg·day^{-1}). Mean plant uptake accounted for 8.0–49.4% of NH_4^+-N, 17.8–59.6% of NO_3^--N and 24.1–61.5% of P supplied to the culture solution.

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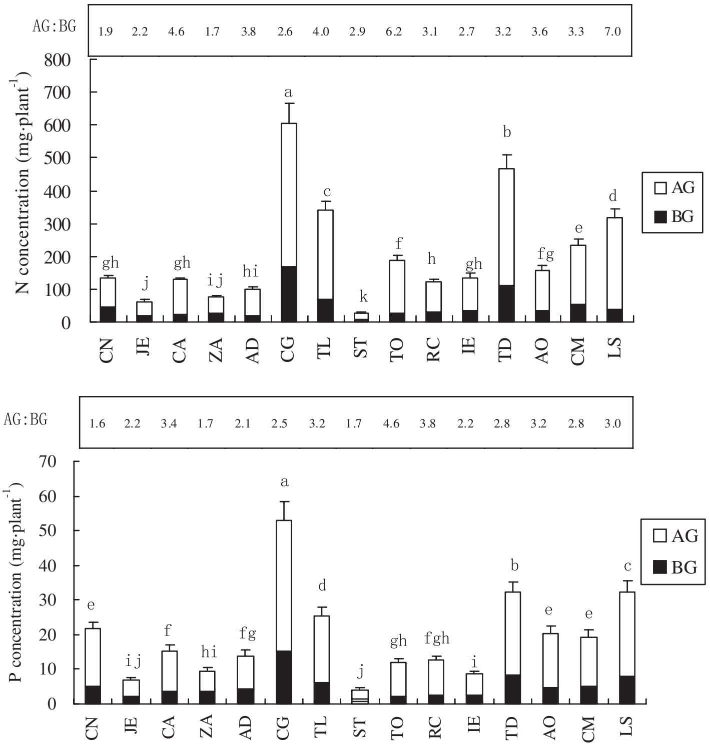

Figure 3. N and P concentration in the plant above-ground (AG) and below-ground (BG) tissues after 28 days of culture.

3.3. *Tissue nitrogen and phosphorus*

Plant tissue N and P concentrations ranged between 28.2 and 606.1 mg N·plant^{-1} and 4.1 and 53.1 mg P·plant^{-1} (Figure 3). Above-ground levels of N and P storage were 21.0–354.6 mg N·plant^{-1} and 2.6–38.0 mg P·plant^{-1}, respectively. Below-ground levels of N and P were much lower, 7.3–169.8 mg N·plant^{-1} and 1.5–15.1 mg P·plant^{-1} respectively, and the relative levels and allocation of nutrients showed wide variation between species. Highest storage of N and P was recorded by *C. generalis*, 606 mg N (28.9% of total N uptake) and 53 mg P (41.1% of total P uptake), respectively, followed by *Th. dealbata*, *L. salicaria* and *T. latifolia*. The lowest N and P storage was recorded by *Scirpus triangulates*, 28.2 mg N (2.9% of total N uptake) and 4.1 mg P (8.4% of total P uptake), respectively. The AG:BG ratio for P storage in the plant tissue

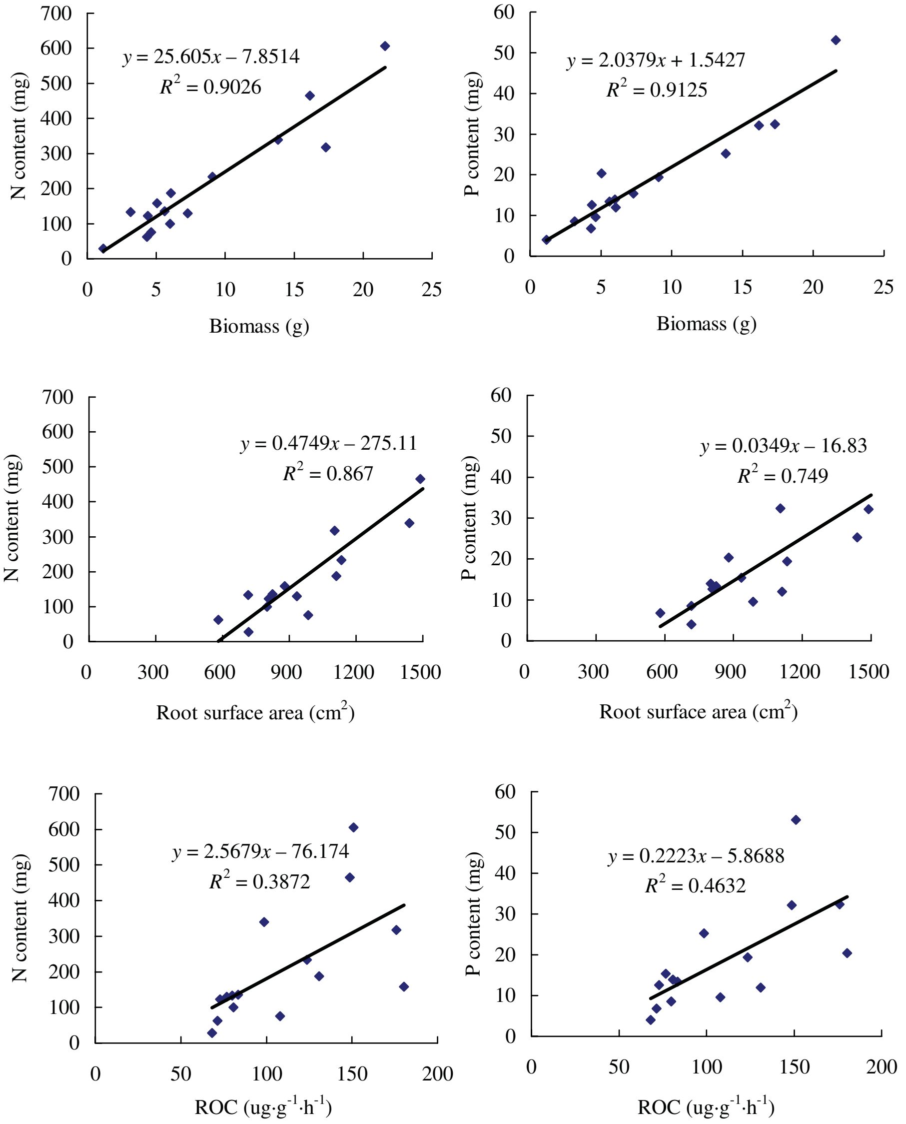

Figure 4. The correlation between P and N concentrations in the plant tissue and biomass, root surface area and root oxidising capacity (ROC).

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ranged from 1.7 to 7.0 and that for N storage ranged from 1.6 to 4.6. For *C. generalis*, *Th. dealbata*, *L. salicaria* and *T. latifoli*, both the N and P concentrations of plant tissue in above and below ground were significantly higher than the others.

Accumulations of N and P in above- and below-ground tissues largely reflected patterns of biomass allocation. The N and P accumulations in the plant tissues were significantly correlated with plant biomass ($r = 0.9501$ and 0.9552, $p < 0.01$, respectively; Figure 4), root surface area ($r = 0.9311$ and 0.8654, $p < 0.01$, respectively), and root oxidising capacity ($r = 0.6225$ and 0.6806, $p < 0.05$, respectively).

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4. Discussion

4.1. *Plant growth and root morphological characteristics*

The biomass of 15 plant species varied widely after 28 days of culture. Among the 15 tested plants, *C. generalis*, *T. latifolia*, *Th. dealbata* and *L. salicaria* produced higher above- and below-ground biomass than the other species. By studying above and below-ground biomass and plant growth characteristics of eight species of wetland plants, Tanner [9] showed that great differences in total biomass, above- and below-ground ratios among the wetland plants. Growth of *Baumea articulate* and *Juncus effusus* was relatively poor, whereas *Zizania latifoli* and *Glyceria maxima* showed the highest above-ground biomass values. Plants with high biomass could uptake and store more nutrients in their tissue, suggesting that these four species are more suitable for wetland application owing to higher pollutant removal capacities.

Higher oxidising capacity could influence the rhizosphere environment by increasing oxygen concentration, releasing exudates like carbohydrates and amino acids, and enhancing the growth and proliferation of microorganism [11]. Iron (Fe) oxyhydroxide plaque is often formed on the root surface of aquatic plants [24,25]. The formation of Fe plaque is caused by oxidation of ferrous to ferric iron and the precipitation of ferric oxide on the root surface, involving a radical oxygen loss from the plants [26]. Fe plaque has a great influence on the plant nutrient uptake [27,28]. Kyambadde et al. [29] reported that in a comparative study of wetlands using *Cyperus papyrus* and *Miscanthidium violaceum*, the former decreased N by 69.5% and P by 88.8%, whereas the latter decreased N by 15.8% and P by 30.7%. These differences were contributed to differences in their root surface area (208.6 vs 72.2 cm^2) respectively. In this study, among the 15 species, *C. generalis* had a higher root surface area, more tips and higher oxidising capacity than other plants, which might provide additional spaces for the precipitation and uptake of pollutants, facilitation of chemical and bacterial processes by changing rhizosphere properties through the creation of an oxidised rhizosphere. Therefore, *C. generalis* had greater ability to uptake N and P from wastewater.

4.2. *Nitrogen and phosphorus uptake by the plants*

Iamchaturapatr et al. [30] reported that about 80–90% of N could be removed by planted treatments whereas only 34–46% of N could be removed by an unplanted treatment. Plant uptake, microbial assimilation and denitrification are the primary processes that removed N from wastewater [31,32]. In this study, plant uptake of N ranged from 21 to 57% of the supplied N, less than the value reported by Iamchaturapatr et al. [30]. This may be because different plant species, substrate adsorption, mode of operation, wetland design and so on affect the removal of the nutrients in the constructed wetland. As to the different kinds of N, *C. generalis*, *T. latifolia*, *Th. dealbata* and *L. salicaria* showed maximum NH_4^+-N uptake rates, and *C. generalis* and *T. latifolia* were better for NO_3^--N uptake.

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Some studies have shown no difference in P removal between a planted treatment and an unplanted treatment because P is stored in the substrate [33,34]. Other studies showed considerable differences in P removal between constructed wetland with and without plants, although plant adsorption accounts only for a small part of the total P removed [30]. We also found that 24–62% of the P could be absorbed by plants. Meanwhile, *C. generalis, T. latifolia* and *Th. dealbata* also had higher P uptake rates in comparison with the other wetland species. Through regression analysis, both N and P uptake rates by the species were significantly correlated with biomass, which suggests that choosing plant species in wetland is important for N and P removal. This is contrary to previous studies showing that P removal was not correlated with plant species or biomass but N removal was [9]. This might be due to differences in the treatment conditions. In our experiment, the plant was the only impact factor in the simulation-constructed wetland compared with constructed wetland in which P removal was mainly vai the substrate instead of by plants.

4.3. *Nitrogen and phosphorus in the plants*

The translocation and storage of nutrients in plant tissues differed significantly among species. In this study, four plants, *C. generalis*, *Th. dealbata*, *L. salicaria* and *T. latifolia*, reached maximum levels of N and P storage in both above- and below-ground tissues. Among these four species, *C. generalis and Th. dealbata* absorbed and stored more N and P than *L. salicaria* and *T. latifolia*. However, the latter two were higher in terms of AG:BG ratio for N and P storage. Most of the plants have AG:BG ratios >1 for N and P storage, which means that the above-ground plant tissues stored more N and P than the below-ground tissues, and this might facilitate the eventual removal of N and P from a wetland system by harvesting. In our study, N and P accumulation in the plant tissues was significantly correlated with plant biomass, root surface area and root oxidising capacity; similar to reports in other studies [29–32].

5. Conclusions

In this study, 15 plant species showed significantly different nutrient uptake and storage rates under the same culture conditions. *C. generalis*, *T. latifolia*, *Th. dealbata* and *L. salicaria* had higher above- and below-ground biomass, nitrogen and phosphorus uptake than the other species. The accumulation of N and P in the plant tissues was significantly correlated with plant biomass, root surface area and root oxidising capacity.

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