

Removal of Nitrogen by Three Plant Species in Hydroponic Culture: Plant Uptake and Microbial Degradation

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Abstract Three macrophyte species, *Typha augus tifolia* (*T. augustifolia*), *Phragmites australis* (*P. australis*), and *Acorus calamus* L. (*A. calamus* L.), have been grown in hydroponic cultivation systems fed with synthetic wastewater. The experiment was designed as 3×2 factorial, with three species and two ratios of $\text{NH}_4^+/\text{NO}_3^-$ so as to investigate the nitrogen transformation and nitrogen removal capacity of each species. The nitrogen removal mechanism was further disclosed by comparing biomass production, nitrogen mass balance, and root exudates of the three plant species under different $\text{NH}_4^+/\text{NO}_3^-$ ratios. The results indicated there exists a linear relationship, with positive significance ($r = 0.946$, $p < 0.05$), between plant biomass and total nitrogen (TN) removal efficiency; in other words, biomass could best reflect plant ability to remove nitrogen. It is also found that $\text{NH}_4^+/\text{NO}_3^-$ ratio could influence plant biomass and root exudates significantly. Additionally, the hydrogen donor and source of energy in denitrification happened in this research were mainly organic acids and soluble sugars, accounting for approximately 50 % of the composition in root exudates.

Keywords Denitrification · Macrophyte species · $\text{NH}_4^+/\text{NO}_3^-$ ratio · Plant uptake · Root exudates

1 Introduction

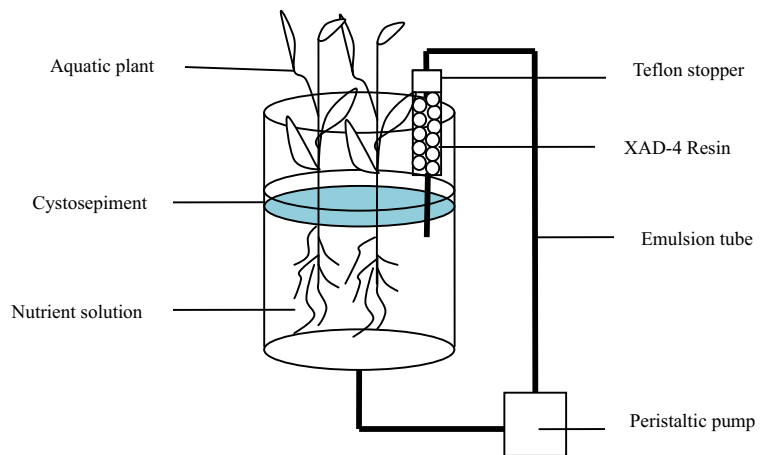
Given the widespread extent of water quality degradation associated with nutrient enrichment, eutrophication has and continues to pose a serious threat to water environment (Anderson et al. 2002). A variety of strategies and treatment methods have been taken and developed to minimize the effect (Lurling and van Oosterhout 2013; Sanchez-Carrillo and Alvarez-Cobelas 2001). Among them, constructed wetlands (CWs), as an emerging ecological engineering technology, have shown their attractions and been widely employed due to high nitrogen removal efficiency and low operation cost (Liu et al. 2009; Vymazal 2005). The nitrogen pollutants usually treated by CWs, different from the organic nitrogen present in amino acids and urea, are mainly in the form of ionic inorganic nitrogen, including ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) (Lee et al. 2009). Plant uptake and microbial denitrification are considered to be the two main mechanisms of nitrogen removal in wetlands (Lin et al. 2002; Tanner et al. 2002), while the interactions between plants, water, and microorganisms are little known.

In most cases, macrophytes with comparable size and growth form show significant differences in pollutant removal capacity. For example, Coleman et al. showed that under the same daily 0.22 g m^{-2} loading rate of total Kjeldahl nitrogen (TKN), *Typha latifolia* L. signi

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Fig. 1 Plants hydroponic cultivation system



ificantly outperformed *Juncus effusus* L. and *Scirpus validus* L. in growth and its ability to improve effluent quality (Coleman et al. 2001). Similarly, Akratos found that cattails removed TKN more efficiently than common reeds under daily organic loads ranging from 0.46 to 1.6 g m⁻² (Akratos and Tsihrintzis 2007). Nitrogen absorption rates by plants in wetlands have been reported to be in the range of 4–90 % (Tanner 2001; Jan Vymazal 2007; Borin and Salvato 2012). This high variability is caused by the growth characteristics of different plant species and their age, climatic conditions, type of applied wastewater, and other environmental conditions (Borin and Salvato 2012).

In terms of NH₄⁺/NO₃⁻ ratio which varies from different sources of wastewater, different plant species may have their specific tolerances and preferences to it. Some studies have demonstrated that the NH₄⁺/NO₃⁻ ratio in the nitrogen supply can affect the rate of plant growth and biomass allocation, and most species grow better and accumulate more nitrogen when supplied with a mixture of NO₃⁻ and NH₄⁺ (Brueck and Guo 2006; Guo et al. 2002; Ali et al. 2001).

The intensity of microbial nitrification and denitrification is also affected by plants, which release organic compounds and O₂ from their roots (Landi et al. 2006; Yin et al. 2013). In the process of nitrogen removal in CWs, root exudates initiate and modulate the interactions between the roots and soil microbes (Kumar et al. 2013). However, few studies have systematically investigated the behavior of the major components of macrophyte exudates.

This study focused on nitrogen degradation and transformation in a hydroponic cultivation system with

three macrophytes, *Typha augustifolia* (*T. augustifolia*), *Phragmites australis* (*P. australis*), and *Acorus calamus* L. (*A. calamus* L.). The predominant influencing factors on nitrogen removal were investigated through the comparison of nitrogen removal under different NH₄⁺/NO₃⁻ ratios. Meanwhile, the experiments were also designed to explore how plant exudates (organic acids and soluble sugars) affect denitrification. The results have implications for the screening of wetland plants and provide insight into the mechanisms of nitrogen removal in wetland systems.

2 Materials and Methods

2.1 Plant Materials

The three macrophyte species, *T. augustifolia*, *P. australis*, and *A. calamus* L., were collected from the Dengbeiqiao wetland, a simulated surface flow CWs located in Yunnan, China (26.01 N, 100.09 E). The wetland was built for purification of nitrogen-polluted wastewater. After soil was washed off from their roots rinsing in distilled water, the plants were divided into homogenous groups and cultivated for 14 days in Hoagland culture solution (Hoagland and Arnon 1950) in a greenhouse.

2.2 Experimental Setup

A hydroponic cultivation system (10 L, 25 cm Ø, 28 cm H, glass vessels) with 6 L of nutrient solution was used. An XAD-4 resin-packed column (50 mL, 2.6 cm Ø,

Table 1 HPLC analysis conditions

	Chromatographic column	Mobile phase constituent	Mobile phase velocity	Sample size	Detector
Organic acid HPLC	HC-C18 (analytical 4.6 * 250 mm, 5- μ m, Agilent, America)	25 mmol L ⁻¹ KH ₂ PO ₄ (pH 2.4) and methanol at ratio of 93:7	0.6 mL min ⁻¹	20 μ L	UV detector (wavelength 210 nm)
Soluble sugar HPLC	HPX-87H (Bio-Rad, USA), (column temperature 85 °C)	Ultrapure water	0.6 mL min ⁻¹	20 μ L	Differential refraction detector (temperature 40 °C)

16 cm H) was installed on top of the system with a Teflon stopper (Fig. 1), and culture medium was passed through the apparatus at a rate of 2 mL min⁻¹ during the experiment. The glass vessel exteriors were covered with aluminum foil to avoid photodegradation. Six to ten similar-sized plants of each species were selected and distributed at random between the treatments, with a coverage area of 200 cm² (approximately 40 % coverage). On the day of planting, the fresh mass of each plant species was measured; the average fresh weight was 46.7 \pm 2.1, 71.9 \pm 3.3, and 62.5 \pm 1.9 g (mean \pm SD) for *T. augustifolia*, *P. australis*, and *A. calamus* L., respectively.

The experiment was designed as 3 \times 2 factorial, with three species and two ratios of NH₄⁺/NO₃⁻ (1.85 \pm 0.05/9.44 \pm 0.24 mg L⁻¹, approximately equal to 1:5; and 7.4 \pm 0.11/4.0 \pm 0.09 mg L⁻¹, approximately equal to 2:1). The nutrient solution was prepared using (NH₄)₂SO₄ and KNO₃; other nutrients including Ca²⁺, Mg²⁺, Fe²⁺, and Na⁺ in the solution were referenced to Hoagland nutrient medium (Hoagland and Arnon 1950); the solution does not contain any organic matter; daily total nitrogen (TN) loading rate was 0.3 g m⁻²; solution pH was 7.0; each treatment had three replicates. In addition, a control experiment without plants was carried out. Considering evapotranspiration and sampling from the vessels, lost water was supplemented with distilled water every day. The greenhouse was programmed with a day/night cycle of 25:22 °C, 12:12 h light, and 70 % relative air humidity. The photon flux density at the base of the plants was approximately 400 μ mol m⁻² s⁻¹.

2.3 Sampling

Sampling of the culture solution in the vessels was conducted every 3 days during 1 month experiment. Culture solution from each vessel was collected and filtered through a 0.45- μ m pore size mixed cellulose

ester membrane filter (Sangon Biotech, China) and analyzed for NH₄⁺ and NO₃⁻ using a flow injection analysis instrument (QC8500, Hach, USA). At the end of the experiment, the resin column was eluted with 200 mL of distilled water, followed by another elution of 200 mL of methanol. After the elution, the residual methanol was removed by evaporating the column under dry vacuum on a rotary evaporator at 40 °C, and the dried exudates were dissolved in 10 mL of distilled water for high-performance liquid chromatography (HPLC) (Table 2, Waters, USA) and total organic carbon (TOC) analysis (multi 3100, Analytik Jena, Germany). All fractions were stored in a freezer at -20 °C. The total fresh plant mass, maximum root length, and maximum leaf length of individual plants were measured at days 0 and 30 to calculate fresh mass production and change in maximum root and leaf length.

2.4 Exudate Analysis

The following organic acids were targeted for analysis: oxalic acid, tartaric acid, malic acid, malonic acid, citric acid, acetic acid, succinic acid, and propionic acid (GR, Aladdin, China). The analyzed sugars included glucose, sucrose, fructose, xylose, galactose, and arabinose (GR, Aladdin, China). A detailed description of the HPLC procedures and the analytical materials is thoroughly described in Table 1.

2.5 Statistical Analysis

Statistical analyses were performed with SPSS software (version 22 for Windows). One-way ANOVA and post hoc Tukey's HSD tests were used to compare the plant growth parameters in the different species at the 5 % significance level. Pearson correlation analyses were used to evaluate the relationships between the biomass production and nitrogen removal efficiency, nitrogen

Table 2 Average fresh biomass production and change in maximum root length and maximum leaf length in three species of plant under two $\text{NH}_4^+/\text{NO}_3^-$ ratios after 30 days of the experiment

	Fresh mass production (g)		Maximum root length (cm)		Maximum leaf length (cm)	
	1:5	2:1	1:5	2:1	1:5	2:1
<i>T. augustifolia</i>	39.7 ± 0.3c	40.7 ± 1.5c	15 ± 1.0b	14 ± 1.0b	45 ± 3.5c	41 ± 2.0c
<i>P. australis</i>	15.8 ± 0.1a	17.3 ± 0.3a	8 ± 1.2a	8 ± 0.2a	10 ± 2.5a	8 ± 1.8a
<i>A. calamus L.</i>	34.4 ± 0.6b	38.1 ± 0.3b	10 ± 0.5a	11 ± 2.0a	28 ± 3b	25 ± 5b

Values are mean ± SD ($n = 3$)

Letters a, b, and c indicate significant difference among the three plant species ($p < 0.05$)

mass balance and nitrogen removal efficiency, and exudate concentration and nitrogen mass balance.

3 Results

3.1 Plant Growth

We selected two $\text{NH}_4^+/\text{NO}_3^-$ ratios for the experiment, and TN was approximately $11 \pm 0.34 \text{ mg L}^{-1}$. The

$\text{NH}_4^+/\text{NO}_3^-$ ratio of 1:5 simulated agricultural wastewater, which derived more NO_3^- from fertilizer. The $\text{NH}_4^+/\text{NO}_3^-$ ratio of 2:1 simulated wastewater from a sewage treatment system, which derived more NH_4^+ from domestic wastewater. The species showed different growth characteristics: biomass production was $P. australis < A. calamus L. < T. augustifolia$ under both two $\text{NH}_4^+/\text{NO}_3^-$ ratio conditions (Table 2). The individual physiological difference of plants significantly affected the growth location of fresh mass; in contrast,

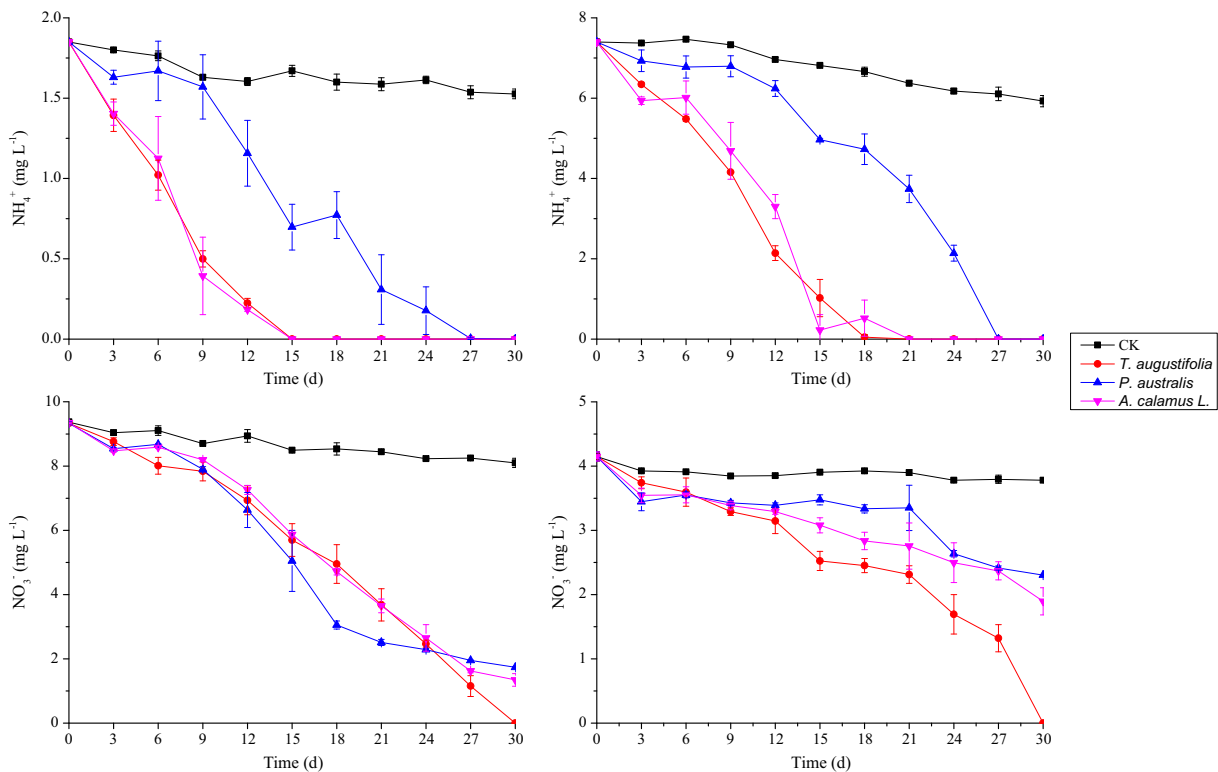


Fig. 2 Average (\pm SD) concentrations of NH_4^+ and NO_3^- in the culture solutions of the three plant species ($n = 3$) and two $\text{NH}_4^+/\text{NO}_3^-$ ($n = 3$) concentrations during the 30-day experiment (the

$\text{NH}_4^+/\text{NO}_3^-$ ratio was 1:5 for the figures on the left and 2:1 for those on the right)

Table 3 Average dry biomass production, plant nitrogen mass concentration, and plant nitrogen accumulation under two $\text{NH}_4^+/\text{NO}_3^-$ ratios during the 30 days of the experiment (mean \pm SD)

	Plant nitrogen mass concentration (mg/g)		Dry mass (g)		Plant nitrogen accumulation (mg)	
	1:5	2:1	1:5	2:1	1:5	2:1
<i>T. augustifolia</i>	5.4 \pm 0.3a	6 \pm 0.2a	8.5 \pm 0.1c	8.7 \pm 0.3c	45.9 \pm 3.1b	52.2 \pm 3.6b
<i>P. australis</i>	8.7 \pm 0.3c	9.1 \pm 0.4c	4.4 \pm 0.03a	4.8 \pm 0.1a	38.3 \pm 1.6a	43.7 \pm 5.7a
<i>A. calamus</i> L.	7.3 \pm 0.5b	7.9 \pm 0.3b	5.5 \pm 0.1b	6.1 \pm 0.05b	40.2 \pm 3.4a	48.2 \pm 2.2ab

Letters a, b, and c indicate significant difference among the three plant species ($p < 0.05$)

P. australis, *T. augustifolia*, and *A. calamus* L. developed many more roots and leaves. In terms of maximum root length, *T. augustifolia* was 1.5–2 times longer than *A. calamus* L. and *P. australis*, while *T. augustifolia* had significantly higher maximum leaf length than *P. australis* and *A. calamus* L. Comparing the results of the two $\text{NH}_4^+/\text{NO}_3^-$ ratios, plants had 2.5–10.7 % greater fresh mass production under the $\text{NH}_4^+/\text{NO}_3^-$ ratio of 2:1 than that under the ratio of 1:5.

3.2 Removal Performance

NH_4^+ in the solution was almost completely removed by all three plant species during the 30-day experiment under both $\text{NH}_4^+/\text{NO}_3^-$ ratios but at different rates (Fig. 2). *T. augustifolia* and *A. calamus* L. removed nearly 100 % of NH_4^+ during days 15–21 for both $\text{NH}_4^+/\text{NO}_3^-$ ratios, in contrast to *P. australis*, which reached that level after 27 days. These indicated that the influence of plant species was inferior to that of $\text{NH}_4^+/\text{NO}_3^-$ ratio. Low dissolved oxygen was in the experimental system (oxygen filling the experimental device was not mandatory), which was not conducive to aerobic nitrification of ammonium; NH_4^+ removed mainly through plant absorption without considering ammonia volatilization losses; *T. augustifolia* and *A. calamus* L. had faster absorption rate of NH_4^+ compared to *P. australis*. However, degradation of NO_3^- was mainly influenced by $\text{NH}_4^+/\text{NO}_3^-$ ratio. Under the 1:5 ratio, *P. australis* and *A. calamus* L. had similar degradation rates; by day 30, the NO_3^- concentrations were 1.96 and 1.35 mg L^{-1} , respectively, and removal efficiencies were 81–86 %. In contrast, the removal efficiency was only 44–54 % with the 2:1 ratio. At the same time, NO_3^- was completely removed by *T. augustifolia* during the 30-day experiment under both ratios. Above all, the three species responded differently to inorganic nitrogen

composition: *T. augustifolia* had the highest nitrogen removal efficiency under both $\text{NH}_4^+/\text{NO}_3^-$ ratios, and *P. australis* had the lowest NH_4^+ removal efficiency under both $\text{NH}_4^+/\text{NO}_3^-$ ratios. Further, considering different $\text{NH}_4^+/\text{NO}_3^-$ ratios, *A. calamus* L. and *P. australis* had greater NO_3^- removal efficiency with a $\text{NH}_4^+/\text{NO}_3^-$ ratio of 1:5 than under the $\text{NH}_4^+/\text{NO}_3^-$ ratio of 2:1. As the result, for the configuration of plant, *T. augustifolia* should be preferred, and in lower $\text{NH}_4^+/\text{NO}_3^-$ ratio, *A. calamus* L. and *P. australis* had greater NO_3^- removal efficiency.

3.3 Plant Uptake of Nitrogen

Plant nitrogen accumulation was the result of multiplication by the dry biomass production and plant nitrogen mass concentration. Plant nitrogen mass concentration will not change obviously in a short time (Krywult et al. 2013), so the nitrogen accumulation can be determined by the biomass production, the more biomass growth, the more nitrogen accumulation. The mean nitrogen mass concentrations were significantly different between the plant species, with *T. augustifolia* < *A. calamus* L. < *P. australis*. Over 30 days, plant nitrogen accumulation was *P. australis* < *A. calamus* L. < *T. augustifolia*. This order is mainly affected by the plant's dry mass production: *T. augustifolia*, even though its nitrogen mass concentration was the least, had the highest dry mass production. All species had a greater plant nitrogen accumulation under the $\text{NH}_4^+/\text{NO}_3^-$ ratio of 2:1 than that under the $\text{NH}_4^+/\text{NO}_3^-$ ratio of 1:5 (Table 3). Under the 2:1 ratio, plants had much greater dry mass production, indicating that in the higher $\text{NH}_4^+/\text{NO}_3^-$ ratio, these plants grew better, possibly because of preferred N forms: some plants are better adapted to NH_4^+ than to NO_3^- supply (Rewald et al. 2016).

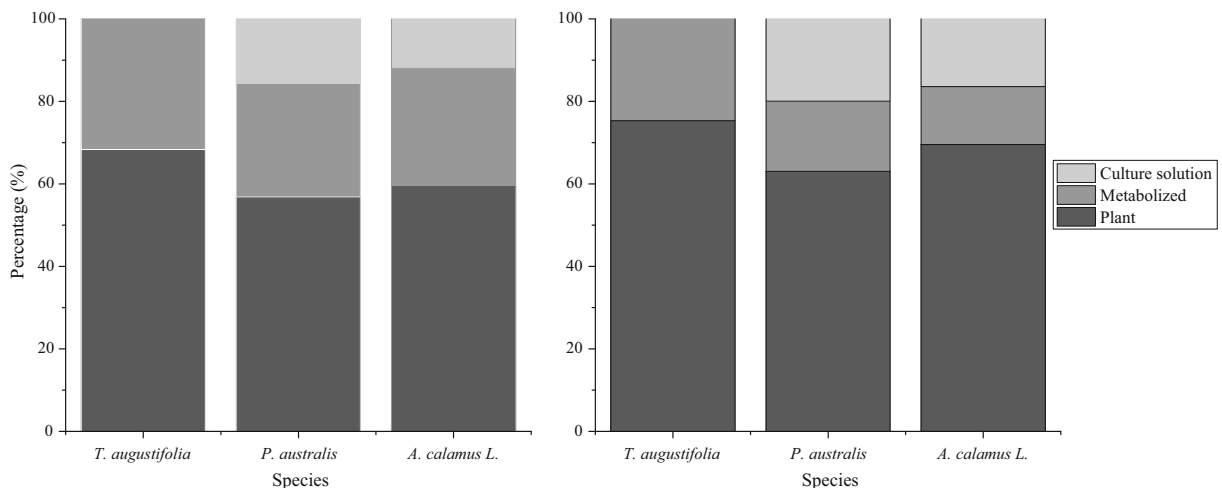


Fig. 3 The percentage contribution of nitrogen removal in the form of plant uptake, microbially metabolized and remaining in the culture solution at the end of the 30-day experiment (the left

figure is the percentage under the NH₄⁺/NO₃⁻ ratio of 1:5; the right figure is the percentage under the NH₄⁺/NO₃⁻ ratio of 2:1)

3.4 Mass Balance of Nitrogen

Although the plants used in the experiments were cleaned carefully prior to setup in the hydroponic cultures, the root systems were not axenic; therefore, microorganisms may have been contributing to metabolism.

In general, large percentages (from 56.9 to 75.3 %) of plant nitrogen uptake were found in six different treatments (Fig. 3). These figures were similar if compared with experiments at similar scale and same plant species reported in the literature, different species made some difference (Table 4). The highest plant uptake (75.3 %) was observed for *T. augustifolia* under the NH₄⁺/NO₃⁻ ratio of 2:1; further, the plant uptake for *T. augustifolia* was also the highest (69.9 %) with the NH₄⁺/NO₃⁻ ratio of 1:5. However, the metabolized portion negatively correlated with the NH₄⁺/NO₃⁻ ratio, and the metabolized percentage was lower under NH₄⁺/NO₃⁻ ratio of 2:1 than that under NH₄⁺/NO₃⁻ ratio of 1:5. The

differences in the plants' contributions were essentially attributed to their different biomass productions. TN removal efficiency and the plant removal contribution percentage were significantly correlated with the biomass production of plants ($r=0.937$ and $r=0.949$, respectively, $p<0.01$). NO₃⁻ removal efficiency was significantly correlated with microbial metabolism ($r=0.873$, $p<0.01$).

3.5 Root Exudates

Biotic interactions are crucial for the understanding of ecosystem functioning. These interactions are often governed through chemical signals, especially between plants and microorganisms at the root level. Given this context, the quality of root exudates is a key element in the establishment and regulation of such interactions.

Figure 4 shows the concentrations of the root secretion TOC, the total organic acids, and the total soluble sugars in the culture solution dealt with six different

Table 4 Examples of nitrogen assimilated in plant tissues in wetland

Plant species	Daily TN loading rates (g m ⁻²)	Macrophyte assimilated percentage (%)	Reference
<i>Typha latifolia</i> L.	0.4	62	Chung et al. (2008)
<i>Phragmites australis</i>	0.5	61.4	Borin and Tocchetto (2007)
<i>Eichhornia crassipes</i>	1.3	63.8	Wang et al. (2013)
<i>Carex elata</i> All.	0.48	43	Borin and Salvato (2012)
<i>Juncus effusus</i> L.	0.48	76	Borin and Salvato (2012)

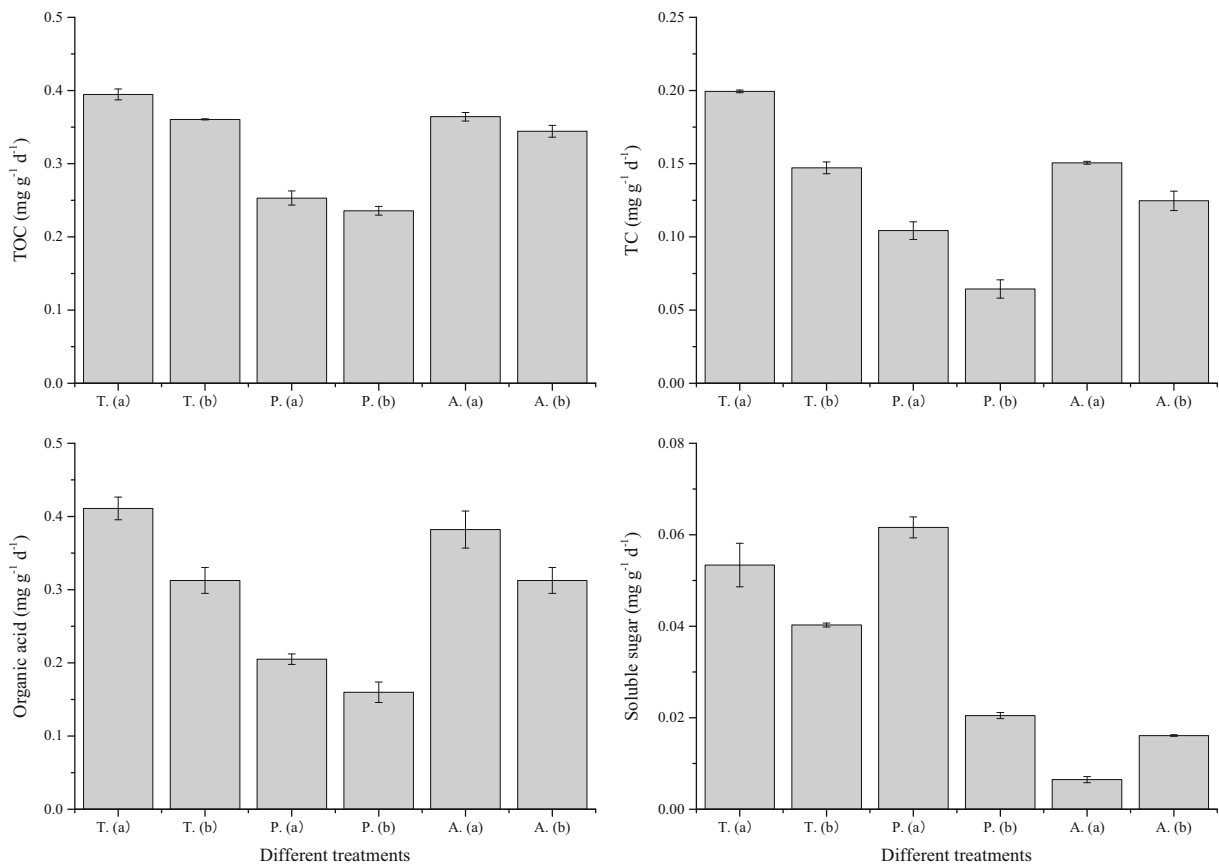


Fig. 4 Concentrations of TOC, total organic acids, and total soluble sugars in the culture solutions dealing with three plant species and two $\text{NH}_4^+/\text{NO}_3^-$ ratios. The six treatments are as follows: T. (a) and T. (b) are *T. augustifolia* cultured under ratios of 1:5 and 2:1, respectively; P. (a) and P. (b) are *P. australis*

cultured under ratios of 1:5 and 2:1, respectively; and A. (a) and A. (b) are *A. calamus* L. cultured under ratios of 1:5 and 2:1, respectively. Note that TC is organic acids and soluble sugars converted to carbon concentration

treatments in 30-day experiment. Organic acids and soluble sugars were converted to reflect carbon concentration in the form of total carbon (TC). Among the three plant species, *T. augustifolia* had the highest exudate concentrations, including TOC, organic acids, and soluble sugars; the reason for this may be that *T. augustifolia* had the highest biomass production of the plant species. TC was about half of TOC, which demonstrated that root exudates were mainly composed of organic acids and soluble sugar.

The exudate composition differed between the six treatment systems (Fig. 5). In every system, five types of organic acid - tartaric acid, malonic acid, malic acid, succinic acid, and citric acid - were detected; succinic acid was always detected in high concentrations. Glucose and arabinose were detected in every system, but sucrose and galactose were only present in some

systems, and arabinose appeared in a higher concentration compared to other soluble sugars. This indicates that the different $\text{NH}_4^+/\text{NO}_3^-$ ratios affected the root's secretions. The concentration of organic acids was ten times higher than that of soluble sugar.

4 Discussion

During the study, all three plant species grew well and conducted nitrogen degradation. However, biomass, root length, and leaf length of the three species turned out differently during the experiment. These differences are possibly associated with adjustments to their physiology (i.e., net assimilation) and morphology (Ma et al. 2010), as many species have various mechanisms for aquatic/drought adaptation, including the enhancement of root

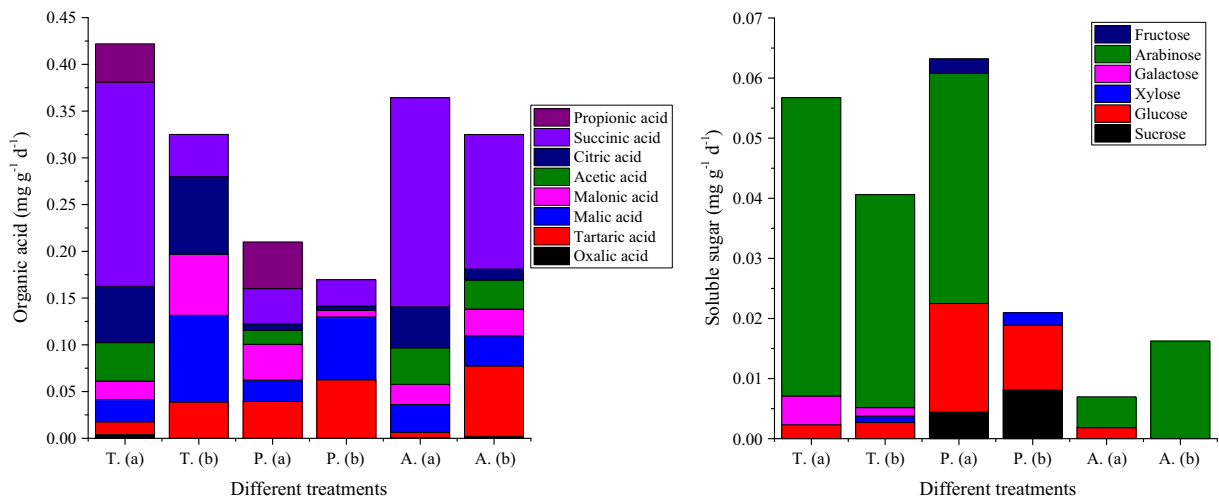


Fig. 5 Concentrations of organic acids and soluble sugars in the culture solutions dealing with three plant species and two $\text{NH}_4^+/\text{NO}_3^-$ ratios. The horizontal ordinate is the same as in Fig. 4

systems, adjustments to growth rate, modifications to plant structure, and more efficient water utilization. According to Li et al. (2014) and Wang et al. (2013), environmental conditions during the test may also have influenced their adaptability and growth. At the end of the study, the mean nitrogen mass concentration of *T. augustifolia* was the lowest compared with *P. australis* and *A. calamus* L., but the former had the highest biomass production, which likely plays a decisive role in its highest rank in the final TN removal. The linear relationship between dry biomass and TN removal efficiency was positively significant ($r=0.946$, $p<0.05$), which meant that the dry biomass best reflects the ability of plant species to remove nitrogen from wastewater; these results are similar to those reported by Zhu et al. (2011). Generally, plants that can store large amounts of nitrogen in their tissue are usually the best choice for nitrogen removal, making total biomass an important factor in nitrogen removal from wastewater (Chang et al. 2010). Thus, plant biomass is not only the important factor for the bioenergy production after plant harvest but also constitute an important index for choosing macrophytes to remediate environmental pollution (Zhen et al. 2016; Kumar et al. 2016).

Different sources of wastewater have distinct $\text{NH}_4^+/\text{NO}_3^-$ ratios. The inorganic nitrogen composition in wastewater can range from 0.4 to 99.6 % for NH_4^+ or NO_3^- . Plant species may differ in their tolerance to and preference for NH_4^+ or NO_3^- (Sundaravadivel and Vigneswaran 2001; Cea-Barcia et al. 2014). Some

species, such as peas (*Pisum sativum* L.) and tomatoes (*Lycopersicon esculentum* Miller), prefer NO_3^- (Lasa et al. 2001; J. Li et al. 2007), while tea (*Camellia sinensis* L.) prefers NH_4^+ as its nitrogen source (Ruan et al. 2007). In this study, *T. augustifolia* removed more nitrogen than the other two species because of its greater biomass. Meanwhile, there was difference in nitrogen mass balance for species between the two $\text{NH}_4^+/\text{NO}_3^-$ ratios. For all three plant species, the higher $\text{NH}_4^+/\text{NO}_3^-$ ratio (2:1) was better for N removal than the lower $\text{NH}_4^+/\text{NO}_3^-$ ratio (1:5). Plants generally prefer NH_4^+ rather than NO_3^- in habitats with NH_4^+ and NO_3^- mixes. In general, the NH_4^+ absorbed from water by macrophytes can be used directly in the synthesis of amino acids, while NO_3^- must be metabolized; therefore, acquisition and assimilation of NH_4^+ is considered less carbon-costly than that of NO_3^- (Rewald et al. 2016; Bloom et al. 1992). Hence, most plants absorb NH_4^+ preferentially. Results from the current study correspond to findings from some previous studies, which showed that different $\text{NH}_4^+/\text{NO}_3^-$ ratios could significantly impact plant biomass, thus greatly affecting their nitrogen removal ability (Rewald et al. 2016).

The nitrogen balance did not account for organic nitrogen or the volatilization of nitrogen; the metabolized percentage in the nitrogen mass balance was microbially metabolized. The NO_3^- removal efficiency significantly correlated with the metabolized percentage ($r=0.873$, $p<0.05$). It was found that the metabolized percentage was higher under the $\text{NH}_4^+/\text{NO}_3^-$ ratio of 1:5

than the $\text{NH}_4^+/\text{NO}_3^-$ ratio of 2:1. These results suggest that denitrification may be the main contribution of microbes, and more NO_3^- may be conducive to microbial metabolism.

Combining the above analyses, we conclude that there was denitrification in our research. Plants secreted some organic matter that contributed to microbe denitrification. It has been found that root exudates, mainly low molecular weight organic matter, are able to act as sources of energy and as hydrogen donors (Zhai et al. 2013; Salvato et al. 2012). In the research, detected organic acids and soluble sugars were converted to TC. This TC was about half of the TOC of exudates; additionally, TC was significantly correlated with TN removal ($r=0.820$, $p<0.05$), at the same time, TOC was not significantly correlated with TN removal ($r=0.741$). These findings demonstrate that organic acids and soluble sugars, as low molecular weight organic matter, are contributors to microbial denitrification, but there is some organic matter in the TOC that are non-beneficial to denitrification microbes. Among different plant species, higher TOCs were accompanied by higher biomass. Additionally, the same plants under a $\text{NH}_4^+/\text{NO}_3^-$ ratio of 1:5 had more TOC than those under a ratio of 2:1, even if plants in the latter solution had more biomass production, which indicates that plant secretion is affected by not only biomass but also solution composition.

In the analysis of organic acids, different organic acids and soluble sugars expressed different concentrations under different treatments. Succinic acid, malic acid, and tartaric acid were the main organic acids, while arabinose and glucose were the main soluble sugars. This phenomenon may be due to differences in the plant species, differences in growth conditions, and sampling. Moreover, we found that soluble sugar detection was trace contrasted to organic acids; more research on organic acids is needed to further understanding of these acids.

5 Conclusions

This study investigated nitrogen transformations in three macrophyte species cultivated in hydroponic systems representing the presence of wastewater with two $\text{NH}_4^+/\text{NO}_3^-$ ratios. Our results suggest that the linear relationship between dry biomass and TN removal efficiency was positively significant ($r=0.946$, $p<0.05$),

indicating that dry biomass could best reflect the ability of plant species to uptake nitrogen out of wastewater, and the choice of larger mass macrophyte species should be priority selection. Different $\text{NH}_4^+/\text{NO}_3^-$ ratios could significantly impact plant biomass, thus greatly affecting their nitrogen removal. NO_3^- removal efficiency was significantly correlated with microbial metabolism ($r=0.873$, $p<0.01$), meaning that denitrification may be the main role of microbes; higher levels of NO_3^- may be conducive to microbial metabolism. Organic acids, as a low molecular weight organic matter, were mainly root secretions that contributed to microbial denitrification. Above all, plant absorption occupies large proportion in the process of wetland nitrogen removal, and choice of plants needs combination with water quality, and microbial denitrification also should be considered.

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