Fate of $^{15}NO_3^-$ and $^{15}NH_4^+$ in the Treatment of Eutrophic Water Using the Floating Macrophyte, *Eichhornia crassipes*

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Use of the floating aquatic macrophyte, *Eichhornia crassipes*, to improve eutrophic water quality is practiced on a large scale in China. Limited information is available on the relative importance of the biological NO_3^- or NH_4^+ removal process during the treatment of eutrophic water using *Eichhornia crassipes*. To investigate the key process responsible for the removal of NO_3^- and NH_4^+, ^{15}N-NO_3^- (9.98 atom % [at.%] ^{15}N) or ^{15}N-NH_4^+ (10.08 at.% ^{15}N) was added to obtain eutrophic water with or without the cultivation of *Eichhornia crassipes*. In the unplanted water, considerable proportions of the added ^{15}N-NO_3^- (27.13 ± 4.87%) or ^{15}N-NH_4^+ (42.08 ± 7.22%) were assimilated by the developed algae. The growth of *Eichhornia crassipes* controlled algae development in the planted water. Furthermore, the cultivation of *Eichhornia crassipes* stimulated gaseous loss of N by microbial denitrification (8.61 ± 1.70% N_2O-N loss from ^{15}N-NO_3^-–labeled water). Apart from N loss by denitrification, considerable proportions of the added ^{15}N-NO_3^- (62.01 ± 6.93%) or ^{15}N-NH_4^+ (76.76 ± 6.21%) were assimilated into the macrophyte N pools. The fine root detritus of *Eichhornia crassipes* contained a proportion of N (4.37 ± 1.39% in $^{15}NO_3^-$–labeled water, 2.03 ± 0.52% in $^{15}NH_4^+$–labeled water) that will be returned to the water after decomposition. In addition to ^{15}N loss via N_2O emission, an unaccounted proportion of ^{15}N could be mainly due to gaseous loss as N_2 by denitrification (25.00% in ^{15}N-NO_3^-–labeled water with *Eichhornia crassipes*).

The eutrophication in lakes and rivers is accelerating in developing and developed countries (Albay et al., 2003; Qin, 2009). However, traditional wastewater treatment processes are unsuitable for reducing eutrophication because lakes and rivers have lower nutrient concentrations and larger volumes than wastewater (Wang et al., 2009). Therefore, processes that can treat larger volumes of nutrient-enriched water at lower costs are desirable.

Macrophytes are receiving greater attention as an alternative treatment of surface water and wastewater due to their efficacy in assimilating nutrients and creating favorable conditions for the microbial decomposition of organic matter (Hu et al., 2008; Wang et al., 2009). In China, large-scale cultivation of the floating macrophyte, water hyacinth (*Eichhornia crassipes*), is being used to reduce eutrophication in Lake Taihu and Lake Dianchi (Zheng et al., 2008; Deng et al., 2009). Confined cultivation of *Eichhornia crassipes* prevents it from becoming an invasive weed while treating polluted water. This process permits simple mechanical harvest after nitrogen (N) and phosphate assimilation by *Eichhornia crassipes* (Zheng et al., 2008).

Limited information is available on the importance of the biological removal process of nutrient elements during the treatment of eutrophic water using *Eichhornia crassipes*. Nitrogen plays a predominant role in the eutrophication of aquatic systems (Saunders and Kalff, 2001). In past studies, much attention was given to N assimilation by *Eichhornia crassipes* during the purification of eutrophic water. Consequently, other biological processes through which N was dissipated, such as nitrification and denitrification, were neglected (Fox et al., 2008; Polomski et al., 2009). Nitrogen is lost when NO_3^- and NH_4^+ are converted to gaseous end products, N_2O and N_2 (Ruser et al., 2006; Fernandes et al., 2010). *Eichhornia crassipes* suspended in the water column has the potential to stimulate nitrification and denitrification in eutrophic water (Snooknah, 2000).

Eichhornia crassipes releases oxygen from roots, which facilitates the creation of aerobic microsites on the roots

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Abbreviations: at.%, atom %; MPN, most probable number; $^{15}NH_4^+$EW, ^{15}N-NH_4^+–labeled eutrophic water without cultivation of water hyacinth; $^{15}NH_4^+$EW+WH, ^{15}N-NH_4^+–labeled eutrophic water with cultivation of water hyacinth; $^{15}NO_3^-$EW, ^{15}N-NO_3^-–labeled eutrophic water without cultivation of water hyacinth; $^{15}NO_3^-$EW+WH, ^{15}N-NO_3^-–labeled eutrophic water with cultivation of water hyacinth; TN, total nitrogen.

(Moorhead and Reddy, 1988). The consumption of organic carbon by the attached bacteria on the roots removes oxygen from the water faster than it can diffuse back, thereby creating anaerobic microsites in which denitrification occurs (Hamersley and Howes, 2002). Studies suggest that the role of macrophytes as nitrifier and denitrifier hosts could be increased by selecting macrophytes with longer roots (10–20 cm) and increasing root densities to 20% of the water column (Austin, 2000; Hamersley et al., 2003). A water hyacinth root can grow from 5 to 100 cm, with the surface area approximately 2.5 to 8.0 m^2 kg^{-1} on a dry weight basis (Kim and Kim, 2000; Yi et al., 2009). Therefore, the water hyacinth root can be a good supporting medium for nitrifying and denitrifying bacteria to propagate and stimulate nitrification and denitrification in eutrophic water (Snooknah, 2000). However, limited information is available concerning the effect of *Eichhornia crassipes* in the conversion of NO_3^- and NH_4^+ through nitrification and denitrification in eutrophic water.

We hypothesized that the cultivation of *Eichhornia crassipes* would stimulate the microbial nitrification and/or denitrification that influences the fate of NO_3^- and NH_4^+ in eutrophic water. If the hypothesis is proven by this study, the outcome of NO_3^- and NH_4^+ in eutrophic water cultivated with *Eichhornia crassipes* will include: (i) gaseous loss as N_2O-N and N_2–N by nitrification and/or denitrification; (ii) N assimilation by *Eichhornia crassipes*; and (iii) the restitution of N assimilated by *Eichhornia crassipes* to water through root detritus decomposition. The current study employs the ^{15}N stable isotopic tracing method to quantitatively trace the fate of NO_3^- and NH_4^+ in eutrophic water with or without the cultivation of *Eichhornia crassipes*.

Materials and Methods

Preparation of Eutrophic Water with $^{15}NO_3^-$ or $^{15}NH_4^+$

Eutrophic water was prepared according to the method of preparing artificial wastewater by Vermaat and Hanif (1998) when they studied the performance of macrophytes on wastewater. The artificial wastewater composed of sucrose, acetate, and propionic acid (10 mg L^{-1} chemical oxygen demand) was added to 60 L of one-fourth modified Hoagland nutrient solution. The amount of chemical oxygen demand (10 mg L^{-1}) was approximately that normally found in Lake Taihu, the largest freshwater lake in China, which has suffered serious eutrophication in recent years (Wang et al., 2007). Hoagland nutrient solution was prepared using tap water. ^{15}N-labeled KNO_3 (9.98% at.% ^{15}N) or $(NH_4)_2SO_4$ (10.08% at.% ^{15}N) was added separately to the prepared wastewater to obtain the final eutrophic water (5.35 ± 0.48 mg L^{-1} NO_3^- and 7.63 ± 0.45 mg L^{-1} total nitrogen [TN]; 5.60 ± 0.55 mg L^{-1} NH_4^+ and 9.06 ± 0.18 mg L^{-1} TN).

Preparation of *Eichhornia crassipes*

Eichhornia crassipes was collected from the No. 2 Pond at Jiangsu Academy of Agricultural Sciences. The pond receives domestic wastewater and rainwater. The concentration of TN in this pond ranges from 2.0 to 5.8 mg L^{-1} during the year (unpublished data, 2011). Full-size individuals of *Eichhornia crassipes* grown under natural light and having a length of approximately 20 cm were collected from the pond in October 2011 for use in the experiment. Each treatment received 0.90 to 0.93 kg of macrophytes (6–7 individuals).

Experiment Design

The experiment consisted of four treatments with three replicates for each:

1. ^{15}N-NO_3^-–labeled water without cultivation of water hyacinth ($^{15}NO_3^-$EW)
2. ^{15}N-NO_3^-–labeled water with cultivation of water hyacinth ($^{15}NO_3^-$EW+WH)
3. ^{15}N-NH_4^+–labeled water without cultivation of water hyacinth ($^{15}NH_4^+$EW)
4. ^{15}N-NH_4^+–labeled water with cultivation of water hyacinth ($^{15}NH_4^+$EW+WH)

The experiment was performed in a closed system (Fig. 1), with a Plexiglas headspace chamber (length by width by height, 45 cm by 30 cm by 45 cm) and a cubic base container made from polyvinyl chloride materials (45 cm long by 30 cm wide by 35 cm high). *Eichhornia crassipes* grew in the cubic base container filled with 60 L of prepared eutrophic water. The shoot of *Eichhornia crassipes* extended to the Plexiglas headspace chamber, where gas samples were taken through a sampling port with rubber septum (Shimadzu) on the chamber. The Plexiglas headspace chamber and the cubic base container were connected by a groove (2 cm in width, 4 cm in depth) into which tap water was filled to ensure it was gastight. To minimize the initial background of gaseous products that can be derived from denitrification in air of the system, 60 L of eutrophic water in the cubic base container was exchanged against 79% He + 21% O_2 before starting the experiment. The Plexiglas headspace chamber was then put into a groove on the cubic base container. The atmosphere of the headspace chamber was replaced by flushing with 79% He + 21% O_2 for 10 min through the inlet and outlet on the top of the headspace chamber. Finally, the inlet and outlet were closed, and the grooves were filled with tap water.

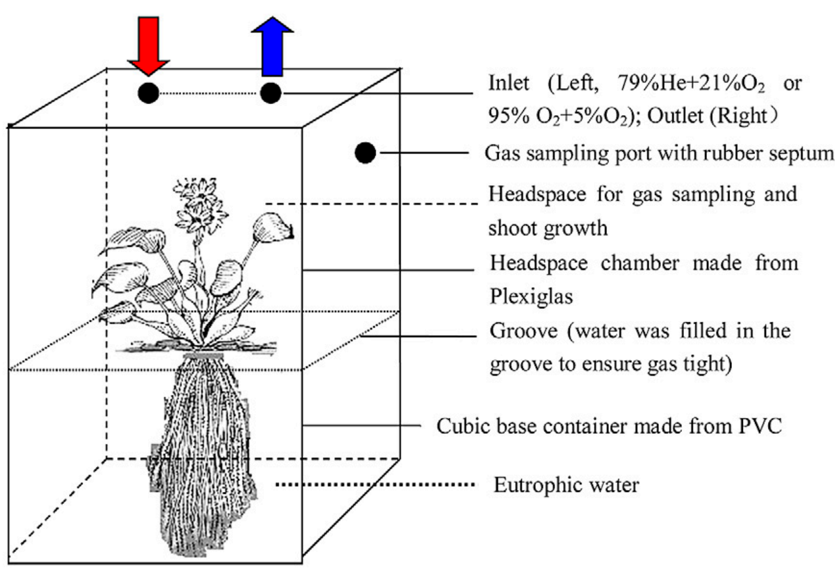

Fig. 1. Illustration of enclosed system for collecting gaseous products derived from nitrification and/or denitrification as well as for plant growth.

In the treatment with the cultivation of *Eichhornia crassipes*, approximately 0.9 kg of *Eichhornia crassipes* was transplanted into the experimental water. During the experiment, 95% O_2 + 5% CO_2 was blown into the closed chamber through the inlet on the top of the headspace chamber every day to maintain the ideal photosynthesis and respiration.

Eichhornia crassipes was harvested after 20 d in $^{15}NO_3^-$EW+WH treatment and after 28 d in $^{15}NH_4^+$EW+WH treatment because of the possible longer reaction time for $^{15}NH_4^+$ to produce gaseous products. Shoots and roots of *Eichhornia crassipes* were separately analyzed for N content and ^{15}N at.% abundance after tissue was oven-dried at 60°C and ground to pass through a 245-μm (60-mesh) sieve. One-liter water samples were collected when *Eichhornia crassipes* was harvested. Water samples were filtered through a 0.45-μm membrane filter, chemically preserved with 1 mL of $HgCl_2$ solution (200 mg L^{-1}), and stored at −4°C until analysis. The concentrations of NO_3^-, NO_2^-, and NH_4^+ as well as their corresponding ^{15}N at.% abundance in filtered water samples were analyzed (Du et al., 2009). Root detritus in water was collected by passing all 60 L of water through a 74-μm (200-mesh) nylon net. Nitrogen content and ^{15}N at.% abundance of root detritus were analyzed (Wang et al., 2011). In the treatment without *Eichhornia crassipes*, algae developed in the water, with most algae attached to the wall of the cubic base flume. The algae attached to the wall were collected by carefully scraping with a stainless steel slice, and the algae in the water were collected by passing all 60 L of water through a (25-μm) 500-mesh nylon net. Nitrogen content and ^{15}N at.% abundance of the collected algae were also analyzed (Wang et al., 2011). Gas samples were taken with 100-mL syringes attached to a three-way stopcock at intervals of 0, 2, 10, and 19 d in the ^{15}N-NO_3^-–labeled treatments and intervals of 0, 11, 22, and 28 d in the $^{15}NH_4^+$–labeled treatments treatment. The collected gas samples were analyzed for N_2O concentration and ^{15}N at.% abundance (Cao et al., 2008).

Chemical Analyses

The concentrations of NO_3^-, NO_2^-, NH_4^+, and TN in filtered water samples were analyzed using a continuous flow analyzer (Seal, AutoAnalyzer 3). The concentration of N_2O was measured using the gas chromatograph (Agilent 7890A) equipped with a 4.5- by 3-mm packed Porapak Q (198/165 μm [80/100 mesh]) and a Ni63 electron capture detector. The column and detector were conditioned at 60°C and 300°C, respectively. A mixture of Ar/CH_4 (95/5 v/v) was used as a carrier gas at a flow rate of 40 mL min^{-1}. The N content of the shoots, roots, root detritus, and algae was analyzed according to the H_2O_2–H_2SO_4 decomposition method (Jiang et al., 2007), and was quantitated by a DigiPREP total Kjeldahl nitrogen system (SCP Science).

Samples were analyzed for ^{15}N content with the help of the Analysis and Test Center of the Institute of Soil Science, Chinese Academy of Sciences. The ^{15}N content analysis of macrophyte roots and shoots, root detritus, and algae was determined using a Flash-EA elemental analyzer coupled to a Delta V isotope ratio mass spectrometer (Thermo Finnigan Corp.) (Wang et al., 2011). NH_4^+–N, NO_3^-–N, and NO_2^-–N in the water sample were transformed to N_2, N_2O, and N_2O, respectively, using chemical methods according to Du et al. (2009). The ^{15}N analysis of N_2O and N_2 was performed by a MAT 253 stable isotope ratio mass spectrometer (Thermo Finnigan Corporation) via a gas injection and preconcentration device (Cao et al., 2008).

Denitrifying Bacteria Enumeration

The collected water samples were filtered using the quantitative filter paper to remove the root detritus before determining bacterial number. *Eichhornia crassipes* root samples (2 g), collected from fresh macrophytes, were immediately ground using a mortar and pestle. The obtained homogenate was suspended in 100 mL of sterilized Milli-Q water to obtain the original inoculum. A microtechnique based on the most-probable-number (MPN) method was adopted for the enumeration of the denitrifying bacteria in the samples (Rowe et al., 1977; Staley and Griffin, 1981).

Statistical Analyses and Calculations

To examine the effect of *Eichhornia crassipes* over time on the ^{15}N at.% excess of N_2O released, repeated-measures multivariate analyses of variance (MANOVA) were conducted. The effects of cultivation of *Eichhornia crassipes* vs. without *Eichhornia crassipes* cultivation on N_2O-N ^{15}N recovery and denitrifying bacteria number in water were examined by paired-samples *t* test. The difference between denitrifying bacteria number in water and that attached to *Eichhornia crassipes* roots was examined by independent-samples *t* test. The differences of ^{15}N at.% excess in *Eichhornia crassipes* or algae between ^{15}N-NH_4^+–labeled treatments and ^{15}N-NO_3^-–labeled treatments were also compared by independent *t* test.

The ^{15}N at.% excess and ^{15}N recovery of the samples were calculated as follows: (i) ^{15}N at.% excess = ^{15}N at.% in samples − ^{15}N at.% of natural abundance (0.3663%); (ii) ^{15}N recovery (%) = (amount ^{15}N in sample/total ^{15}N added) × 100.

Results

$^{15}NO_3^-$, $^{15}NH_4^+$, and $^{15}NO^{2-}$ Pools in Planted and Unplanted Water

Table 1 shows the results of ^{15}N at.% excess and ^{15}N recovery of N-NO_3^-, N-NH_4^+, and N-NO_2^- in planted and unplanted water. Nearly all (99–100%) of the $^{15}NO_3^-$ or $^{15}NH_4^+$ added to water was transformed during the experimental period when *Eichhornia crassipes* was cultivated in the water. The ^{15}N recoveries of ^{15}N-NO_3^-, ^{15}N-NO_2^-, and ^{15}N-NH_4^+ (sum) in planted water were <0.01%. Accumulation of at.% excess ^{15}N-NO_3^- (6.44 ± 0.074) or ^{15}N-NH_4^+ (6.74 ± 0.84) in unplanted water was higher than that in water planted with *Eichhornia crassipes* (Table 1). The ^{15}N recovery of ^{15}N-NO_3^-, ^{15}N-NO_2^-, and ^{15}N-NH_4^+ was 54.49 ± 4.47% in unplanted water to which $^{15}NO_3^-$ was added and 40.49 ± 2.50% in unplanted water to which $^{15}NH_4^+$ was added.

The ^{15}N-NH_4^+ was not detected when $^{15}NO_3^-$ was added, but ^{15}N-NO_3^- was detected in water when $^{15}NH_4^+$ was added to both planted and unplanted water. Extremely low ^{15}N recoveries of NO_2^-–N were detected in planted water, whereas ^{15}N recoveries of ^{15}N-NO_2^- in unplanted water were relatively higher (1.03 ± 0.47% when $^{15}NO_3^-$ was added to water, 0.051 ± 0.011% when $^{15}NH_4^+$ was added to water).

Eichhornia crassipes Assimilation for ^{15}N Derived from $^{15}NO_3^-$ or $^{15}NH_4^+$ in Water

Table 2 shows the results of ^{14}N + ^{15}N content, ^{15}N at.% excess, and ^{15}N recovery in *Eichhornia crassipes* shoots and roots. During the experimental period, *Eichhornia crassipes* assimilated 887.9 ± 16.57 mg (^{14}N + ^{15}N) from ^{15}N-NO_3^-–labeled water ($^{15}NO_3^-$EW+WH treatment) and 914.2 ± 33.91 mg (^{14}N + ^{15}N) from ^{15}N-NH_4^+–labeled water ($^{15}NH_4^+$EW+WH treatment). The ^{15}N recoveries in *Eichhornia crassipes* (shoots + roots) were 58.01 ± 0.01% from $^{15}NO_3^-$EW+WH treatment and 76.76 ± 6.21% from $^{15}NH_4^+$EW+WH treatment, respectively. Independent-samples *t* test determined that ^{15}N at.% excess of *Eichhornia crassipes* grown in ^{15}N-NH_4^+–labeled water (2.90 ± 0.39 in shoots and 1.53 ± 0.22 in roots) was significantly ($p < 0.05$) higher than that grown in ^{15}N-NO_3^-–labeled water (1.95 ± 0.04 in shoots and 1.09 ± 0.18 in roots). The ^{15}N at.% excess and ^{15}N recovery in *Eichhornia crassipes* shoots were significantly ($p < 0.05$) higher than those in *Eichhornia crassipes* roots grown either in ^{15}N-NO_3^-–labeled water or ^{15}N-NH_4^+–labeled water.

^{15}N in Algae and Root Detritus Derived from $^{15}NO_3^-$ or $^{15}NH_4^+$ in Water

During the experimental period, algae developed in the unplanted water, while none developed in water planted with *Eichhornia crassipes*. High ^{15}N at.% excess values were found in the algae that developed. The ^{15}N at.% excess in algae that developed in ^{15}N-NH_4^+–labeled water (8.19 ± 0.11, $^{15}NH_4^+$EW treatment) was significantly higher ($p < 0.05$) than that in ^{15}N-NO_3^-–labeled water (5.27 ± 0.66, $^{15}NO_3^-$EW treatment). The ^{15}N recoveries of algae were 27.13 ± 4.87% from $^{15}NO_3^-$EW treatment and 42.08 ± 7.22% from $^{15}NH_4^+$EW treatment (Table 3). In the planted water, root detritus accumulated in water. The ^{15}N recoveries of root detritus were 4.37 ± 1.39% from $^{15}NO_3^-$EW+WH treatment and 2.03 ± 0.52% from $^{15}NH_4^+$EW+WH treatment (Table 3).

^{15}N in N_2O-N Derived from $^{15}NO_3^-$ or $^{15}NH_4^+$ in Water

During the experimental period, ^{15}N-labeled N_2O was detected in the collected gas samples. The ^{15}N enrichment of N_2O increased with elapsed incubation time (Fig. 2). According to results of the repeated-measures MANOVA, incubation time, cultivation of *Eichhornia crassipes*, and their interactions had a significant effect on N_2O-N ^{15}N at.% excess ($p < 0.001$). The ^{15}N at.% excess of N_2O-N ranged from 0.0057 ± 0.0000 to 2.05 ± 0.23 in samples collected from ^{15}N-NO_3^-–labeled treatment. These were greatly higher than values observed in samples collected from ^{15}N-NH_4^+–labeled treatment (ranged from 0.0059 ± 0.00027 to 0.12 ± 0.014) (Fig. 2). Moreover, ^{15}N at.% excess of N_2O-N released from the planted water was significantly higher than from the unplanted water (Fig. 2). Accordingly, the recovery of ^{15}N as N_2O-N was 8.61 ± 1.70% in the planted water to which $^{15}NO_3^-$-N was added ($^{15}NO_3^-$EW+WH treatment), while the recovery was 0.32 ± 0.036% in the planted water to which $^{15}NH_4^+$–N was added ($^{15}NH_4^+$EW+WH treatment) (Fig. 3).

Table 1. ^{15}N atom % (at.%) excess and ^{15}N recovery of N-NO_3^-, N-NH_4^+, and N-NO_2^- in water with and without cultivation of *Eichhornia crassipes*.

Treatment†	N form	Concentration	^{15}N at.% excess	^{15}N recovery
		mg L^{-1}	——— % ———	
$^{15}NO_3^-$EW	NO_3^-	3.87 ± 0.62	6.44 ± 0.074	53.7 ± 4.12
	NO_2^-	0.097 ± 0.039	5.34 ± 0.50	1.03 ± 0.47
	NH_4^+	0.012 ± 0.012	ND‡	–
$^{15}NO_3^-$EW+WH	NO_3^-	0.190 ± 0.260	0.013 ± 0.003	0.005 ± 0.007
	NO_2^-	0.023 ± 0.014	0.32 ± 0.19	0.006 ± 0.002
	NH_4^+	0.005 ± 0.002	ND	–
$^{15}NH_4^+$EW	NO_3^-	1.66 ± 0.04	0.071 ± 0.032	0.23 ± 0.11
	NO_2^-	0.106 ± 0.026	0.26 ± 0.09	0.051 ± 0.011
	NH_4^+	2.98 ± 0.06	6.74 ± 0.84	40.2 ± 2.41
$^{15}NH_4^+$EW+WH	NO_3^-	0.063 ± 0.015	0.038 ± 0.034	0.005 ± 0.005
	NO_2^-	0.006 ± 0.008	ND	–
	NH_4^+	0.198 ± 0.289	ND	–

† $^{15}NO_3^-$EW, ^{15}N-NO_3^-–labeled water without cultivation of water hyacinth; $^{15}NO_3^-$EW+WH, ^{15}N-NO_3^-–labeled water with cultivation of water hyacinth; $^{15}NH_4^+$EW, ^{15}N-NH_4^+–labeled water without cultivation of water hyacinth; $^{15}NH_4^+$EW+WH, ^{15}N-NH_4^+–labeled water with cultivation of water hyacinth.

‡ Not detected.

Table 2. ^{14}N + ^{15}N content, ^{15}N atom % (at.%) excess, and ^{15}N recovery in *Eichhornia crassipes*.

Treatment†	Item	Shoots	Roots
$^{15}NO_3^-$EW+WH	^{14}N + ^{15}N uptake (mg)	565.45 ± 2.07	322.42 ± 14.51
	^{15}N at.% excess (%)	1.95 ± 0.04	1.09 ± 0.18
	^{15}N recovery (%)	45.32 ± 5.59	19.02 ± 5.38
$^{15}NH_4^+$EW+WH	^{14}N + ^{15}N uptake (mg)	568.67 ± 3.36	340.56 ± 23.25
	^{15}N at.% excess (%)	2.90 ± 0.39	1.53 ± 0.22
	^{15}N recovery (%)	65.12 ± 7.66	20.62 ± 3.59

† $^{15}NO_3^-$EW+WH, ^{15}N-NO_3^-–labeled water with cultivation of water hyacinth; $^{15}NH_4^+$EW+WH, ^{15}N-NH_4^+–labeled water with cultivation of water hyacinth.

Table 3. ^{15}N atom % (at.%) excess and ^{15}N recovery in algae and root detritus.

Target	Treatment†	Item	N content
Algae	$^{15}NO_3^-EW$	N accumulated (mg)	155.96 ± 8.12
		^{15}N at.% excess (%)	5.27 ± 0.66
		^{15}N recovery (%)	27.13 ± 4.87
	$^{15}NH_4^+EW$	N accumulated (mg)	156.48 ± 22.58
		^{15}N at.% excess (%)	8.19 ± 0.11
		^{15}N recovery (%)	42.08 ± 7.22
Root detritus	$^{15}NO_3^-EW+WH$	N accumulated (mg)	67.47 ± 2.38
		^{15}N at.% excess (%)	2.24 ± 0.51
		^{15}N recovery (%)	4.37 ± 1.39
	$^{15}NH_4^+EW+WH$	N accumulated (mg)	30.67 ± 8.20
		^{15}N at.% excess (%)	2.08 ± 0.15
		^{15}N recovery (%)	2.03 ± 0.52

† $^{15}NO_3^-EW$, $^{15}N\text{-}NO_3^-$–labeled water without cultivation of water hyacinth; $^{15}NH_4^+EW$, $^{15}N\text{-}NH_4^+$–labeled water without cultivation of water hyacinth; $^{15}NO_3^-EW+WH$, $^{15}N\text{-}NO_3^-$–labeled water with cultivation of water hyacinth; $^{15}NH_4^+EW+WH$, $^{15}N\text{-}NH_4^+$–labeled water with cultivation of water hyacinth.

Quantity of Denitrifying Bacteria in Water and Attached to *Eichhornia crassipes* Roots

Figure 4 shows the results of the quantity of denitrifying bacteria in water and attached to *Eichhornia crassipes* roots. Denitrifying bacteria was detected in the unplanted eutrophic water (2.23×10^2 to 4.31×10^2 MPN mL^{-1}). The number of the denitrifying bacteria was significantly lower ($p < 0.05$) than that observed in the planted water as well as on *Eichhornia crassipes* roots (Fig. 4). The quantity of denitrifying bacteria observed in the planted water was 1.58×10^3 to 1.95×10^3 MPN mL^{-1} in the $^{15}NO_3^-EW+WH$ treatment and 9.57×10^2 to 1.58×10^3 MPN mL^{-1} in the $^{15}NO_3^-EW+WH$ treatment. The quantity of denitrifying bacteria on *Eichhornia crassipes* roots was 1.97×10^7 to 4.62×10^7 MPN mL^{-1} in the $^{15}NO_3^-EW+WH$ treatment and 1.70×10^7 to 4.62×10^7 MPN mL^{-1} in the $^{15}NH_4^+EW+WH$ treatment.

Discussion

Transformation of $^{15}NO_3^-$ and $^{15}NH_4^+$ in the Unplanted Water

In the unplanted water, the accumulation of excess $^{15}N\text{-}NO_3^-$ or $^{15}N\text{-}NH_4^+$ was higher than in the water planted with *Eichhornia crassipes*. The distinct reduction of ^{15}N abundance of the added $^{15}N\text{-}NO_3^-$ or $^{15}NH_4^+$ in the unplanted water indicated that the biological transformation processes of $^{15}N\text{-}NO_3^-$ or $^{15}NH_4^+$ occurred in the water.

Nitrification or/and denitrification were the dominant fate of added $^{15}N\text{-}NO_3^-$ or $^{15}N\text{-}NH_4^+$ in the water. Nitrate reduction to ammonium was negligible. Therefore, the low recovery of ^{15}N as N_2O-N detected in the water was a result of gaseous loss of N by microbial denitrification in the unplanted water.

In the unplanted water, a considerable proportion of the added $^{15}N\text{-}NO_3^-$ or $^{15}N\text{-}NH_4^+$ was assimilated by the algae that developed. A preferential uptake of NH_4^+ over NO_3^- by the algae that developed was found because ^{15}N at.% excess and ^{15}N recoveries of algae collected from $^{15}NH_4^+$–labeled water were all significantly higher ($p < 0.05$)

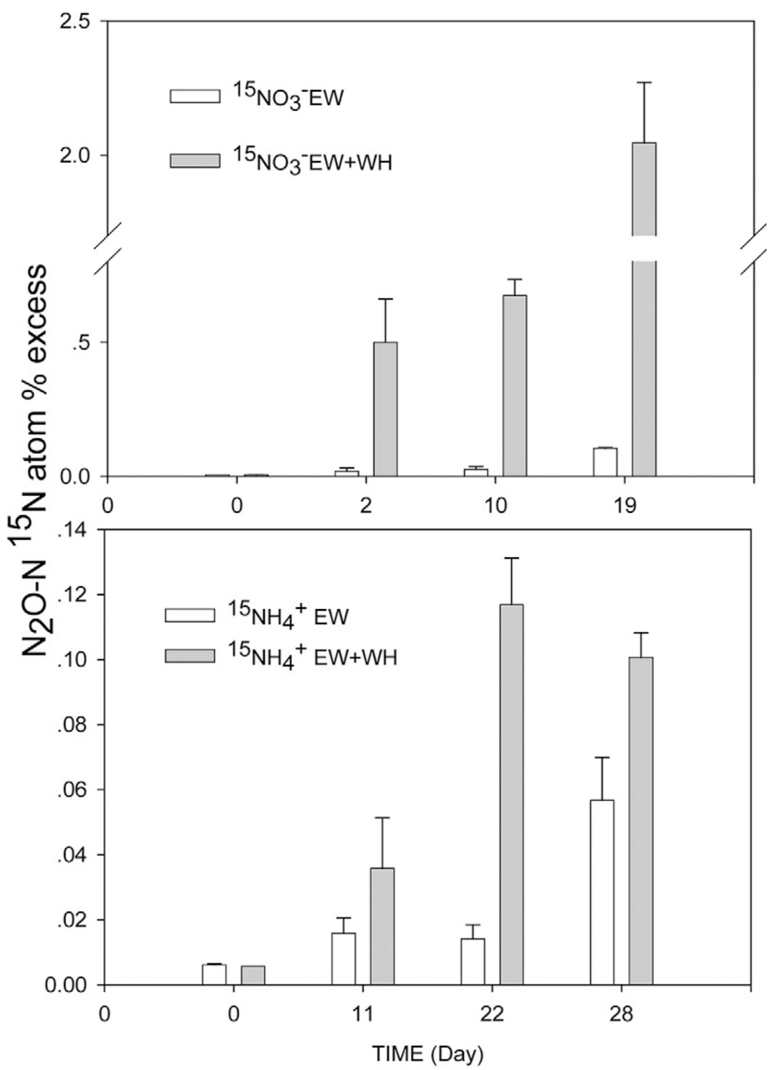

Fig. 2. ^{15}N **atom % excess of** N_2O **released from water with or without cultivation of** ***Eichhornia crassipes*****. Vertical bars represent standard deviations.** $^{15}NO_3^-EW$, $^{15}N\text{-}NO_3^-$**–labeled water without cultivation of water hyacinth;** $^{15}NO_3^-EW+WH$, $^{15}N\text{-}NO_3^-$**–labeled water with cultivation of water hyacinth;** $^{15}NH_4^+EW$, $^{15}N\text{-}NH_4^+$**–labeled water without cultivation of water hyacinth;** $^{15}NH_4^+EW+WH$, $^{15}N\text{-}NH_4^+$**–labeled water with cultivation of water hyacinth.**

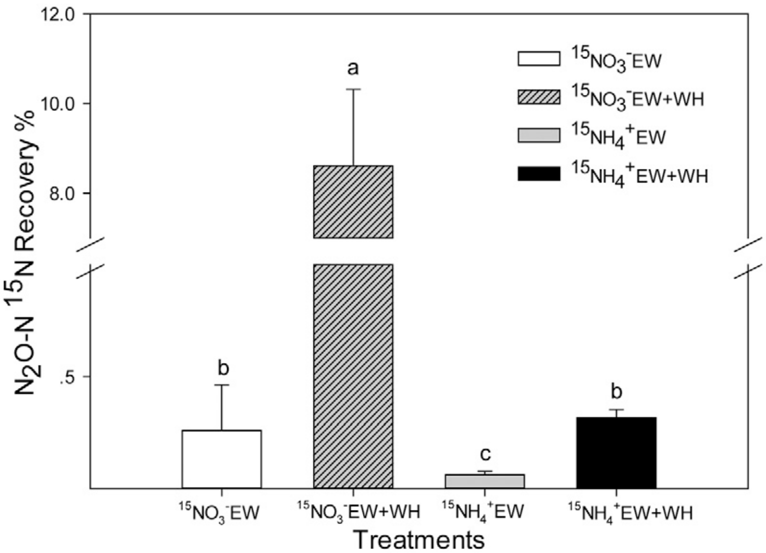

Fig. 3. ^{15}N **recovery of** N_2O**-N released from water with or without cultivation of *Eichhornia crassipes*. Vertical bars represent standard deviations. Bars with the same letters are not significantly different (*p* < 0.05) between different treatments.** $^{15}NO_3^-$**EW,** ^{15}N**-**NO_3^-**–labeled water without cultivation of water hyacinth;** $^{15}NO_3^-$**EW+WH,** ^{15}N**-**NO_3^-**–labeled water with cultivation of water hyacinth;** $^{15}NH_4^+$**EW,** ^{15}N**-**NH_4^+**–labeled water without cultivation of water hyacinth;** $^{15}NH_4^+$**EW+WH,** ^{15}N**-**NH_4^+**–labeled water with cultivation of water hyacinth.**

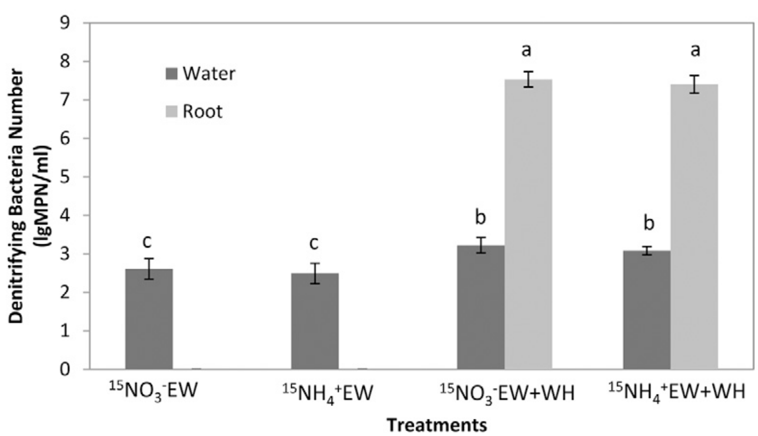

Fig. 4. Quantity (log most probable number [MPN] mL^{-1}**) of denitrifying bacteria in water and attached to *Eichhornia crassipes* roots. Vertical bars represent standard deviations. Bars with the same letters are not significantly different (*p* < 0.05) between the bacteria quantity attached to the root and bacteria quantity in water from different treatments.** $^{15}NO_3^-$**EW,** ^{15}N**-**NO_3^-**–labeled water without cultivation of water hyacinth;** $^{15}NO_3^-$**EW+WH,** ^{15}N**-**NO_3^-**–labeled water with cultivation of water hyacinth;** $^{15}NH_4^+$**EW,** ^{15}N**-**NH_4^+**–labeled water without cultivation of water hyacinth;** $^{15}NH_4^+$**EW+WH,** ^{15}N**-**NH_4^+**–labeled water with cultivation of water hyacinth.**

than those collected from the $^{15}NO_3^-$–labeled water (Table 3). This is consistent with most previous studies that demonstrated that algae have a preference for assimilating NH_4^+ over NO_3^- (Page et al., 1999; Padhi et al., 2010).

Effect of Macrophyte Cultivation on Biological Transformation of $^{15}NO_3^-$ and $^{15}NH_4^+$ in Water

The floating macrophyte, *Eichhornia crassipes*, strongly influenced the fate of the added $^{15}NO_3^-$ or $^{15}NH_4^+$ in water. Nearly all (99–100%) of the ^{15}N-NO_3^- or ^{15}N-NH_4^+ added to the water was transformed during the experimental period when *Eichhornia crassipes* was cultivated in the water. The ^{15}N recoveries of ^{15}N-NO_3^-, ^{15}N-NO_2^-, and ^{15}N-NH_4^+ (sum) in the planted water were $<0.01\%$. In the planted water, the key processes responsible for NO_3^--N or N-NH_4^+ removal include macrophyte assimilation and denitrification. In addition, no algae developed in the planted water. Previous studies found that *Eichhornia crassipes* was the most beneficial macrophyte for preventing algae development in water (Kim and Kim, 2000; Kim et al., 2003).

A considerable proportion of the added ^{15}N-NO_3^- (55.01–70.01%) or ^{15}N-NH_4^+ (72.37–81.16%) was assimilated into the macrophyte N pools. This result was consistent with other studies that reported that the uptake of N by *Eichhornia crassipes* or other floating aquatic macrophytes (e.g., pennywort [*Hydrocotyle umbellata* L.], water lettuce [*Pistia stratiotes* L.], and water spinach [*Ipomoea aquatica* Forssk.]) is one of the most important pathways to remove N from water (Sooknah and Wilkie, 2004; Li et al., 2007; Fox et al., 2008). Our results that *Eichhornia crassipes* has a preference for assimilating NH_4^+–N over NO_3^-–N are consistent with previous studies (Reddy and Tucker, 1983; Snooknah, 2000).

During the growth of a macrophyte, the production of fine root detritus leads to N loading in its habitat through the decomposition of the detritus (Chen et al., 2002; Fornara et al., 2009). In the current study, 1.56 ± 0.12 g dry wt. root detritus was produced from *Eichhornia crassipes* roots grown in ^{15}N-NO_3^-–labeled water (duration of 19 d), and 0.86 ± 0.28 g dry wt. was produced from *Eichhornia crassipes* roots grown in ^{15}N-NH_4^+–labeled water (duration of 28 d). Correspondingly, ^{15}N recoveries of root detritus were 4.37 ± 1.39% collected from $^{15}NO_3^-$–labeled water and 2.03 ± 0.52% collected from $^{15}NH_4^+$–labeled water. Therefore, a proportion of N accumulated by *Eichhornia crassipes* from eutrophic waters will be released back to the water after the detritus decomposes (Reddy and DeBusk, 1991). This may cause overestimation of the N removal rates due to macrophyte assimilation when only plant N content is analyzed. According to a previous study by Moorhead et al. (1988), annual net N recovered in *Eichhornia crassipes* detritus represented 21 and 28% of the total N removed by plants in the fertilized and control reservoirs, respectively. Net N loading to the reservoirs from detritus was 92 to 148 kg N ha^{-1} yr^{-1}. In another study by Reddy and DeBusk (1991), annual averages for C, N, and P deposited through detritus at the sediment–water interface in eutrophic Lake Apopka were 2870, 176, and 19 kg ha^{-1} yr^{-1}, respectively. This further supports the above implication that simply analyzing N content in macrophytes would overestimate N removal rates due to macrophyte assimilation. It is clear that N in the deposited detritus will be finally subjected to microbial transformation.

Effect of Macrophyte Cultivation on Nitrous Oxide Emission through Biological Denitrification

Nitrous oxide (N_2O) is an obligatory intermediary product of denitrification (Tilsner et al., 2003), and is a by-product of nitrification and coupled nitrification–denitrification

(Bateman and Baggs, 2005; Mathieu et al., 2006). Under different conditions, emissions of N_2O can represent 0 to 100% of denitrification products (Aulakh et al., 1992; Mathieu et al., 2006).

The lower recovery of ^{15}N as N_2O-N when $^{15}NH_4^+$-N was added may be due to the competition for nitrogen between macrophytes and microorganisms that are responsible for the biological denitrification reaction (Kaye and Hart, 1997; Hodge et al., 2000). The high affinity of *Eichhornia crassipes* for assimilating $^{15}NH_4^+$ may lead to a reduced nitrification and/or coupled nitrification–denitrification potential of $^{15}NH_4^+$ in the eutrophic water because macrophytes compete with microorganisms for NH_4^+ (Verhagen et al., 1995; Xu et al., 2011).

When $^{15}NO_3^-$ was added to water that was cultivated with *Eichhornia crassipes*, obvious N_2O emission was observed. Moreover, ^{15}N at.% excesses of N_2O released from the planted water were higher than observed values released from the unplanted water (Fig. 2). This indicates that the cultivation of *Eichhornia crassipes* stimulated the gaseous loss of N by microbial denitrification in eutrophic water. A well-developed macrophyte rhizosphere enhances microbial density and activity by providing the root surface for microbial growth, a source of carbon compound through root exudates and a favorable alternation of aerobic and anaerobic environment via root oxygen release (Gagnon et al., 2007; Vymazal, 2011). In this study, the quantity of denitrifying bacteria on *Eichhornia crassipes* roots was higher than that observed in the planted water and the quantity of the denitrifying bacteria in the planted water was significantly higher ($p < 0.05$) than that observed in unplanted water. This condition provided support to the stimulated microbial denitrification process in the planted eutrophic water.

The amount of gaseous loss of N is related to the N concentration in the soil, water, or sediment according to Ambus (2005) and Fernandes et al. (2010). In a previous study, the proportion of gaseous loss of N through nitrification and/or denitrification to the total N loss in water cultivated with *Eichhornia crassipes* was estimated using the mass balance method. According to the results, 22.32, 37.73, and 55.34% of N were lost through denitrification in water with different initial TN concentrations of 6.22, 15.06, and 20.08 mg L^{-1}, respectively (Zhang, 2009). This result indicated that the extent to which N was lost through microbial nitrification and/or denitrification in the planted water may be higher in water with higher TN concentrations. This implies that plant-mediated microbial nitrification and/or denitrification could be the dominant factor affecting N reduction in a water body with high concentration of N. It is consistent with other studies that the role of macrophytes in aquatic ecosystems should not be underrated, as aquatic vegetation also exerts considerable indirect effects (e.g., mediating denitrification) that may have a greater impact than the direct uptake of N into the macrophyte biomass (Knops et al., 2002; Desmet et al., 2011).

Overall Fate of $^{15}NO_3^-$ and $^{15}NH_4^+$ in Water with or without the Cultivation of *Eichhornia crassipes*

The total recovery of ^{15}N as $^{15}NO_3^-$ or $^{15}NH_4^+$ that was added to water did not reach 100% in either planted or unplanted water. Many reasons were considered for the incomplete recovery of ^{15}N, including sampling uncertainty, measurement error, and unaccounted for biological transformation process (e.g., gaseous loss as N_2 by denitrification). The unaccounted fraction of recovery of the added ^{15}N could mainly represent gaseous loss as N_2 by denitrification (approximately 25% in the planted water to which $^{15}NO_3^-$ was added, and 20.85% in the planted water to which $^{15}NH_4^+$ was added). This is in addition to the N loss via N_2O emission mentioned above. In aquatic systems, N_2 was the main gaseous product by denitrification (McCutchan et al., 2003; McCutchan and Lewis, 2008) and denitrification removed a large fraction of the fixed N that reaches a body of water. Our recent studies, through direct measurement of N_2 produced by denitrification, also reveal that N_2 was the major product by denitrification whether in *Eichhornia crassipes*–planted water or unplanted water (unpublished data, 2011), and the proportion of N loss via N_2 emission could be as high as approximately 60% in the planted water with high concentration of nitrogen (NH_4^+–N 6.0–7.2 mg L^{-1}, NO_3^-–N 0.81–5.14 mg L^{-1}, TN 8.9–12.07 mg L^{-1}).

Conclusions

Eichhornia crassipes strongly influenced the fate of N in water. Considerable proportions of N in the water will be assimilated by algae. *Eichhornia crassipes* can control the development of algae in water by direct uptake of N; however, fine root detritus of *Eichhornia crassipes* will be subject to microbial transformation, which can return N to water when the detritus decomposes. *Eichhornia crassipes* can also facilitate considerable denitrification. The results indicated that both indirect (plant-mediated nitrification and/or denitrification) and direct effects of *Eichhornia crassipes* cause N to be removed.

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