Aquatic Botany 120 (2014) 193–200

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Nitrogen-removal ability and niche of *Coix lacryma-jobi* and *Reineckia carnea* in response to NO_3^-/NH_4^+ ratio

Biqing Chen[a,b], Dong Liu[a,c], Wenjuan Han[a], Xing Fan[a], Haiqin Cao[a], Qinsu Jiang[a], Yu Liu[a], Jie Chang[a], Ying Ge[a,*]

[a] College of Life Sciences, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, PR China
[b] Peking-Tsinghua Center for Life Sciences, Peking University School of Life Sciences, Beijing 100871, PR China
[c] Nanjing Institute of Environmental Sciences, Ministry of Environmental Protection, Nanjing 210042, PR China

ARTICLE INFO

Article history:
Received 13 September 2013
Received in revised form 16 May 2014
Accepted 22 May 2014
Available online 2 June 2014

Keywords:
Biomass
Constructed wetland
Nitrogen accumulation
Nitrogen concentration

ABSTRACT

Wastewater comes from various sources with remarkably different nitrate (NO_3^-)/ammonium (NH_4^+) ratios. In order to choose coexisting plant species for treating wastewater with various compositions in constructed wetlands, it is important to know the performances of plant species under different NO_3^-/NH_4^+ ratios. In this study, we investigated the growth traits and nitrogen accumulation of *Coix lacryma-jobi* and *Reineckia carnea* under five NO_3^-/NH_4^+ ratios (100/0, 75/25, 50/50, 25/75 and 0/100) in a hydroponic system. Results showed that, the total biomass, aboveground biomass and aboveground nitrogen accumulation were the greatest under a NO_3^-/NH_4^+ ratio of 50/50 for *C. lacryma-jobi*, but those were the highest under a ratio of 75/25 for *R. carnea*. Under the NH_4^+-only (0/100) treatment, *C. lacryma-jobi* exhibited ammonium toxicity with leaf nitrogen concentration (43 mg g^{-1}) exceeding the maximum limit for plants. In contrast, *R. carnea* showed less sensitivity to NO_3^-/NH_4^+ ratios as the coefficient of variance for biomass of *R. carnea* (17%) was less than half of that of *C. lacryma-jobi* (40%). The two plant species differed in nitrogen niche (niche overlap = 0.42).

1. Introduction

Excessive wastewater discharge into surface water without treatment usually causes eutrophication with environmental impacts such as hypoxia, serious water pollution and reduction in biodiversity. Constructed wetlands (CWs) have emerged as a viable wastewater treatment technology throughout the world, with an increasing number being established in recent years in developing countries, especially in China (Liu et al., 2009). In CWs, nitrogen (N) uptake by plants and denitrification by rhizosphere microorganisms are two main mechanisms responsible for N removal (Stottmeister et al., 2003; Ge et al., 2007; Vymazal, 2007). Plants contribute directly and indirectly to N removal in CWs. The direct contribution is the N uptake by plants that accounts for 5–23% of the total N removal in CWs (Tanner, 2001; Ge et al., 2007, 2011), and the indirect contribution is the release of organic compounds and oxygen from plant roots, providing a viable environment for rhizosphere microorganisms (Sundaravadivel and Vigneswaran, 2001; Menon et al., 2013), which enhances N transformation and removal from CWs. The dominance of nitrification or denitrification is associated with N chemical forms (nitrate and ammonium in this paper). Wastewater from different sources shows great differences in the nitrate/ammonium (NO_3^-/NH_4^+) ratio, and the percentage of N in the form of NH_4^+ and/or NO_3^- typically covers a wide range from 0.4% to 99.6% in total inorganic N in wastewater (Liu et al., 2009; Cao et al., 2011).

Plants react differently in response to different NO_3^-/NH_4^+ ratios in the N supply, reflected in several plant biometric characteristics. Most species grow better and accumulate more N when supplied with mixed NO_3^- and NH_4^+ (Guo et al., 2002; Jampeetong et al., 2013). Many plant species prefer N in the form of NO_3^-, such as *Pisum sativum* L. and *Lycopersicon esculentum* Miller (Lasa et al., 2001; Li et al., 2007). However, a few species, such as *Camellia sinensis* L. and *Oryza sativa* L., exhibit a preference for NH_4^+ as the N source (Sasakawa and Yamamoto, 1978; Ruan et al., 2007). Plant species that prefer NO_3^- will develop symptoms of toxicity when NH_4^+ is the sole N source, especially showing severely impaired root growth (Britto and Kronzucker, 2002); in contrast, plant root growth may not be inhibited when NO_3^- is the sole N source (Britto and Kronzucker, 2002; Ruan et al., 2007). It is reported that only a few plant species used in CWs for wastewater treatment favor

* Corresponding author. Tel.: +86 571 8820 6465; fax: +86 571 8820 6465.
E-mail address: geying@zju.edu.cn (Y. Ge).

http://dx.doi.org/10.1016/j.aquabot.2014.05.016
0304-3770/

NH_4^+, including *Phragmites australis* (Cav.), Trin. ex Steud., *Glyceria maxima* (Hartm.) Holmb. (Tylova et al., 2005), *Canna indica* L. (Konnerup and Brix, 2010), *Lolium perenne* L. (Cao et al., 2011), *Acorus calamus* L. and *Iris pseudacorus* L. (Chang et al., 2010). Different N forms in wastewater affect plant growth, thus species that favor a particular form of N can be used in CWs treating wastewater dominated by that form (Sundaravadivel and Vigneswaran, 2001; Cao et al., 2011).

The demand for N is similar among plant species as N is an essential element for plants, and therefore their N niches overlap heavily (Tilman and Wedin, 1991). Niche partitioning is one of the major mechanisms of coexistence (Mikkelson, 2005; Silvertown, 2004; Sterck et al., 2011). Plant species coexisting in a community may segregate in N chemical form preference (Araya et al., 2010), which influences the growth and resource allocation of plants (Bruck and Guo, 2006) as well as interspecific competition does (Zhang et al., 2007b). The N niche in this paper is defined as the occupation of a plant species for N source in an ecosystem due to its N form preference (NO_3^-/NH_4^+ ratios). The N niche overlap can uncover whether different plant species can coexist in a community, and whether there is a complementary effect between species (Loreau and Hector, 2001). It suggests that the mixed cultivation of plant species with different N niche may enhance N uptake, and thus, increase the rate of N removal from CWs.

Coix lacryma-jobi and *Reineckia carnea* are plant species used in CWs (Yang et al., 2005; Ge et al., 2011). They are both perennial grasses with strong root systems. It has been reported that *C. lacryma-jobi* prefers NO_3^- nutrition under various pH (Jampeetong et al., 2013), while the preference of this species under different NO_3^-/NH_4^+ ratios is unknown. Besides, it is unknown about which N form is preferred by *R. carnea*. Therefore we investigated the effects of N form (NH_4^+ versus NO_3^-) on the growth and N accumulation of *C. lacryma-jobi* and *R. carnea* in a microcosm experiment under easily controlled conditions. The study aimed to: (1) analyze the N uptake and potential N removal ability of the two species in response to NO_3^-/NH_4^+ ratio in wastewater; (2) study the preference of the two species for NH_4^+ versus NO_3^- and thus determine their theoretical N niche; and (3) provide a basis for choosing species in CWs aiming at niche complementarity.

2. Materials and methods

2.1. Plants and growth conditions

Coix lacryma-jobi, belonging to the family Poaceae, was a tall (1.0–1.5 m high) grain-bearing tropical perennial plant native to South-East Asia (Jampeetong et al., 2013). It grew exclusively in summer and autumn, and could grow in acidic and moist soils without shades (Ge et al., 2011). *R. carnea*, belonging to the family Liliaceae, was perennial evergreen herbs with a height of 0.3–0.5 m, and it preferred acidic and neutral moist soils for growth (Flora of China, online version).

The experiment was conducted in the greenhouse at Zhejiang University (120°05′ E, 30°18′ N), Hangzhou, Southeast China. The seedlings of both species were collected from Tianjing Botanical Garden for Aquatic Plants in Hangzhou, washed clean and cultivated in distilled water. After 10 days in distilled water, uniform seedlings of each species were selected and transplanted to 4 L plastic pots filled with 2 L nutrient solution with different inorganic N compositions (NO_3^-/NH_4^+ ratios in percentage were 100/0, 75/25, 50/50, 25/75 and 0/100, respectively; hereafter described as 'treatments') at a constant N concentration of 8 mmol L^{-1}, simulating the N loading rate for the CWs. There were 60 pots, 30 for each plant species. All treatments were replicated six times with four plants of the same species grown in each pot, simulating the natural density of the plant species. The treatments were arranged in a completely randomized block design. The nutrition supply was modified from Hoagland's nutrient solution (Hoagland and Arnon, 1950), which contained the following macronutrients (in mmol L^{-1}): K^+ 7, Mg^{2+} 2, PO_4^{3-} 1, Ca^{2+} 5 (Table 1); and the following micronutrients (in mg L^{-1}): H_3BO_3 2.86, $CuSO_4·5H_2O$ 0.08, $ZnSO_4·7H_2O$ 0.22, $MnCl_2·4H_2O$ 1.81, $H_2MoO_4·4H_2O$ 0.09, and Fe-EDTA 7.645 (Zhang et al., 2005). The solution pH was adjusted to 6.0 ± 0.2 with diluted NaOH or HCl. Plants grew under conditions of a 14 h photoperiod, day/night temperatures of 25/15 °C, relative humidity of 70–80%, and artificial light with an irradiance of ca. 280 μmol photon m^{-2} s^{-1}. The volume of solution in each pot was maintained by adding distilled water every 2 days. Nutrient solution was replaced every 10 days in order to ensure that no nutrients were depleted and to avoid transformations between N forms by microorganisms.

Table 1
Compositions (mmol L^{-1}) of nutrient solutions with different NO_3^-/NH_4^+ ratios but the same N concentration (8 mmol L^{-1}).

Nutrient source	NO_3^-/NH_4^+ ratio				
	100/0	75/25	50/50	25/75	0/100
KNO_3	4.8	2.8	0.8	2.0	0.0
$Ca(NO_3)_2·4H_2O$	1.6	1.6	1.6	0.0	0.0
$CaCl_2·2H_2O$	3.4	3.4	3.4	5.0	5.0
$(NH4)_2SO_4$	0.0	1.0	2.0	3.0	4.0
KH_2PO_4	1.0	1.0	1.0	1.0	1.0
$MgSO_4·7H_2O$	2.0	2.0	2.0	2.0	2.0
KCl	1.2	3.2	5.2	4.0	6.0

2.2. Experimental measurements and calculations

Before plants were transplanted into pots, the initial fresh weight and length of shoot (stems and leaves) were recorded for both *C. lacryma-jobi* and *R. carnea*. Plants were harvested after 40 days of growth in summer, and then separated into leaf, stem and root. The lengths of the shoot and of the root of each plant were measured. All leaves from each plant were collected and scanned (ScanMaker 4900, Microtek International Inc., Ellicott, USA) immediately after harvest. The WinFLORA Pro 2002a software (Regent Instruments, Quebec, Canada) was used to determine leaf area. The leaf, stem and root samples were dried in a forced-air oven at 65 °C for 72 h to determine dry weight (DW), referred to as leaf DW, stem DW, root DW, total DW (the sum of leaf, stem and root DW), and aboveground DW (the sum of leaf and stem DW). The dried plant material was grounded, and NH_4^+ and NO_3^- concentrations were determined as described below. Material from each sample (0.2 g) was weighed and digested in $H_2SO_4 + H_2O_2$ following the Kjeldahl method. NH_4^+ and NO_3^- concentrations in the digested solution were determined colorimetrically at wavelengths of 420 and 210 nm, respectively (Lü et al., 2004).

Root/shoot ratio (R/S) was calculated as root DW divided by shoot DW (Hunt, 1978; Tylova et al., 2005). N accumulation was calculated as N concentration in plant tissues multiplied by DW of that tissue part.

Coefficient of variance (CV) of total DW for each species was calculated as,

$$CV = \frac{\sigma}{\mu} \quad (1)$$

where, μ is the average value of total DW under the five treatments and σ is the standard deviation.