Ecological Engineering 99 (2017) 114–118

Contents lists available at ScienceDirect

Ecological Engineering

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

Tracking uptake of submerged macrophytes (*Ceratophyllum demersum*)—Derived nitrogen by cattail (*Typha angustifolia*) using nitrogen stable isotope enrichments

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ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form 10 October 2016
Accepted 13 November 2016
Available online 18 November 2016

Keywords:
Ceratophyllum demersum
Nitrogen assimilation
Stable isotope labeling
Typha angustifolia
Wetland

ABSTRACT

External inputs and internal cycling are two pathways of nutrients to the aquatic macrophytes in the wetland ecosystem. Internal sources of nutrients include plant die-off and the decomposition of organic matter. In this study, we used a stable nitrogen isotope (^{15}N) enrichment experiment to track the timing and magnitude of cattail (*Typha angustifolia*) use of coontail (*Ceratophyllum demersum*) – derived nitrogen (CDN) in a freshwater wetland. Dry coontail powder enriched in ^{15}N ($\delta^{15}N = 1259 \pm 15‰$) was buried in the soil close to the roots of cattail plants in field mesocosms at the Yeyahu National Preserve, Beijing, China, from July 22 to September 30, 2014. Different parts (roots, rhizomes, stems, leaves and leaf apexes) of the cattail plants were collected at day 0, 14, 28, 42, 49, 56, 63 and 70 for stable isotope analysis. Results reveal uptake of the nitrogenous nutrient from coontail detritus by cattail four weeks after being exposed to CDN. The maximum uptake rate for excess ^{15}N in roots, leaves, stems, rhizomes and leaf apexes were 1.55 ± 0.84, 0.69 ± 0.11, 0.33 ± 0.08, 0.14 ± 0.11, 0.06 ± 0.02 mg ^{15}N m^{-2}. The root system is the largest pool of nitrogen in cattails, which serves as the source of nitrogen for other plant parts. The uptake rate per unit biomass was highest in the leaf apexes.

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1. Introduction

In wetlands, aquatic macrophytes can assimilate nutrients from the overlying water and sediments. Upon their death, a substantial amount of plant biomass is buried in the sediments, thereby contributing to nutrient immobilization within aquatic systems (Davis, 1991; Pietro et al., 2006). A significant portion of the organic matter is released back into the water column through microbial decomposition, which may affect the nutrient removal capacity of macrophytes in constructed wetlands (Chimney and Pietro, 2006). As a result, assimilation and decomposition of aquatic plants are a key process in nutrient cycling of aquatic ecosystems (Gallagher, 1978; Brinson et al., 1981; Nicholas and Polunin, 1984; Benfield, 1996). Emergent macrophytes are believed to play several important roles in aquatic ecosystems. They stabilize sediments, reduce flow velocity and provide habitats for aquatic animals (Li et al., 2010). Emergent macrophytes are also capable of retaining suspended particles (Horppila and Nurminen, 2001; Wang et al., 2006) and assimilate nutrients from sediment (Carignan and Kalff, 1980; Brix, 1994; Horppila and Nurminen, 2001). Cattail (*Typha angustifolia*) is a perennial emergent aquatic plant with a worldwide distribution. Cattail grows in warm environments with adequate light. The plant is considered an effective treatment species for nutrient removal and water purification in constructed wetlands (Liu et al., 2012; Bachand and Horne, 1999) and lakes (Horppila and Nurminen, 2001). Cattail has a higher removal rate for nitrogen than reed (*Phragmites australis*), *Sparganium* and scirpi (*Scirpus triqueter*) (Liu et al., 2012; Bachand and Horne, 1999). Horppila and Nurminen (2001) indicated that cattail can reduce water turbidity and sediment resuspension.

Submerged macrophytes are also an important component of the aquatic primary production (Brothers et al., 2013) and biogeochemical cycles (Carpenter and Lodge, 1986). Submerged macrophytes can reduce water turbidity caused by phytoplankton and suspended solids (René et al., 2014), and have better nitrogen removal efficiency than emergent macrophytes. Coontail (*C. demersum*) is a perennial and widely distributed submerged macro-

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http://dx.doi.org/10.1016/j.ecoleng.2016.11.027
0925-8574/Published by Elsevier B.V.

phyte. The stems, leaves and epidermis of submerged macrophytes can assimilate nutrients (Paterniti and Mantai, 1986). Hence, coontail are capable of removing nutrients from the water column effectively. Nevertheless, coontail decomposes easily after death. Chimney and Pietro (2006) indicated that the decomposition rate (0.056 8 d^{-1}) of coontail was higher than other macrophytes. That implies that the organic matter in coontail will affect nutrient removal efficiency as plants decompose. However, there is lack of research that specifically addresses the fates of nutrients such as nitrogen in submerged macrophytes detritus.

In recent years, stable isotopes have been successfully used to study nutrient cycling in aquatic ecosystems (Fry, 2006). The ^{15}N signature of the labeled materials is typically more enriched than that of the unlabeled materials from natural ecosystems and can be detected by analyzing major inorganic and organic pools from the study system. For example, Barrón et al. (2006) used ^{15}N to assess the fate of sedimented planktonic material in a seagrass (*Posidonia oceanica*) meadow. They found that the amount of ^{15}N recovered during the experiment in the *P. oceanica* meadow was fourfold greater than that recovered in unvegetated sediment, indicating that the *P. oceanica* meadow was more efficient in retaining the nitrogen deposited as algal material than unvegetated sediments. They concluded that enhanced trapping of sestonic particles by seagrass canopies can be an efficient nutrient acquisition strategy in the oligotrophic environments that seagrasses inhabit. Gribsholt et al. (2009) used ^{15}N to follow ammonium cycling pathway in unvegetated sediment and sediment vegetated by common reed. Within weeks, all added $^{15}NH_4^+$ was transformed and/or assimilated by the biota. Between 42% and 48% of the added N was recovered in plants and sediment after 3–6 months, whereas 24% remained after 1 year. The majority of ^{15}N was retained in the organic matter pool within the sediment, primarily through efficient recycling of the ^{15}N within the microbial community. This study shows the strong potential of reed roots and especially bacteria to retain nitrogen in tidal freshwater sediment over longer periods of time. Li et al. (2010) tracked the fate of decayed *Microcystis* sp. in unvegetated sediment and sediment unvegetated by common reed. They found that *Microcystis*-derived nitrogen was quickly assimilated by reed plants and used to support new growth in a short time period.

In this study, nitrogen stable isotope tracer (^{15}N) was used to track the fate of coontail-derived nitrogen (CDN) in a natural wetland dominated by cattail. The objective of this study was to investigate if nitrogenous nutrients from coontail detritus in wetland sediments can be used by cattail. In this experiment, ^{15}N was used to label coontail, which was deposited into sediment with cattail stands. The isotopic signals were followed by analyzing the organic nitrogen pools of various parts of the cattail plants periodically during a 70-day experiment conducted in a natural wetland.

2. Methods

2.1. Study site

This study was conducted at the Yeyahu National Wetland Preserve located in northwestern Beijing, China (115°46′16′–115°59′48′N and 40°22′04′–40°30′31′E). The preserve has a surface water area of 9.8 km^2, and a marsh area of 12 km^2 (Hong et al., 2012). The annual average air temperature is 13.1 °C.

2.2. Labeling experiments

Live coontail were collected from Yuyuantan Park (Beijing, China) in June of 2014. Filamentous algae and large debris were carefully removed from coontail reared in tap water for 2 days and then incubated with 98% ^{15}N as NH_4Cl (Sigma–Aldrich), at 0.3 mg L^{-1} for 7 days under natural regimes of light and temperature. After incubation, the coontail were washed repeatedly with distilled water to remove unassimilated $^{15}NH_4^+$ and were dried at 70 °C for 72 h in an oven. The dried coontail were ground into fine powder that passed the 100-mesh metal sieve. The sample powder was stored in centrifuge vials prior to stable isotope analysis. The resulting $\delta^{15}N$ value of the labeled coontail was 1259 ± 14.7‰ (n = 3).

An outdoor experiment was conducted at the Yeyahu National Wetland Preserve from July 22 to September 30, 2014. At the experimental site, 26 polyvinyl chloride (PVC) cylinders with a height of 1 m and an inner diameter of 0.16 m (total area of 0.02 m^2) were inserted into sediment to a depth of 50 cm. The cylinders were 30 cm higher than the surface of the water column. Each cylinder enclosed an individual cattail plant. The area within each cylinder was regarded as an experimental plot. One day after the deployment of the plots, the water inside the cylinder was drained and 2 g of dry ^{15}N-labeled coontail detritus was mixed into 40 mL of water, which was injected into the sediment of each experimental plot at a depth of 1 − 2 cm using a syringe. Four injections of 10 mL of the dissolved ^{15}N-labeled coontail detritus were made to optimize the distribution of the labeled material in the experimental plot. After the injection, the roots of cattail were covered with sediment (about 3 cm) for every experimental plot.

2.3. Sample collection and analysis

Samples of plant tissues were taken on days 0, 14, 28, 42, 49, 56, 63 and 70 after injection. For the purpose of analysis, the samples taken on day 0 were labeled as the controls. During each sampling event, three randomly selected plots (replicates) were collected by harvesting the entire cattail biomass within each of the three PVC cylinders and separated in the laboratory into roots, rhizomes, stems, leaves and leaf apexes, and were cleaned with deionized water. Samples of plant material were dried at 70 °C for 72 h in an oven and weighed. The dried plants were ground into a fine powder that passed the −100-mesh metal sieve and was stored securely in centrifuge vials. Stable isotope and total nitrogen (N%) analyses were performed at the Chinese Academy of Forestry Sciences in Beijing using an Isotope Ratio Mass Spectrometer (DELTA V Advantage) coupled with an Elemental Analyzer (Flash EA1112 HT).

2.4. Excess ^{15}N and the mass of excess ^{15}N calculations

$\delta^{15}N$ (‰) is the excess ^{15}N relative to the atmospheric nitrogen (R_{air}); R_{sample} is ^{15}N: ^{14}N ratio of the sample:

$$\delta^{15}N(‰) = \left(\frac{R_{sample}}{R_{air}} - 1\right) \times 1000 \quad (1)$$

Excess ^{15}N is the atom% (at%) of ^{15}N per gram of dry sample, calculated according to Veuger et al. (2007):

$$\text{excess}^{15}N(\%) = \frac{\text{at\%}^{15}N_{sample} - \text{at\%}^{15}N_{control}}{100} \times N\% \quad (2)$$

$$\text{at\%}^{15}N_{sample} = \frac{100 \times R_{air} \times \left(\frac{\delta^{15}N_{sample}}{1000} + 1\right)}{1 + R_{air} + R_{air} \times \frac{\delta^{15}N_{sample}}{1000}} \quad (3)$$

where $\text{at\%}^{15}N_{sample}$ represents the atomic abundance of ^{15}N (%) in a sample taken at a given sampling event; at% ^{15}N $N_{control}$ is the percent of ^{15}N in a plant sample on day 0. N% is the nitrogen content (%) in the plant tissue.