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Using the $\delta^{15}N$ of submerged biomass for assessing changes in the nitrogen cycling in a river receiving wastewater treated effluent

Eduardo Cejudo[1,*], Jennifer L. Hood, Sherry L. Schiff, Ramon O. Aravena

Department of Earth Sciences, University of Waterloo, 200 University Ave. West, Waterloo, ON N2L 3G1, Canada

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ABSTRACT

We measured the changes in concentration and isotopic composition of the two major dissolved inorganic nitrogen species (ammonium and nitrate) and the isotopic composition of submerged biomass, in order describe the changes in the nitrogen cycling at a river receiving treated effluent before and after upgrades at a wastewater treatment plant in southwestern Ontario (Canada). We observed that changes in the $\delta^{15}N$ of macrophytes and periphyton are correlated to changes in the quality of treated effluent. We explored two scenarios, differential assimilation of dissolved nitrogen species without isotopic fractionation using a two end-member mixing model, and isotopic fractionation due to assimilation ($\alpha_{assimilation}$) in macrophytes. The two end-member mixing model suggested different contribution per N specie, and $\alpha_{assimilation}$ was estimated to be around 1.005 and 1.012 before and after upgrades, respectively. Thus, we provided evidence that the $\delta^{15}N$ of submerged biomass can be used as a proxy for in-stream nitrogen cycling and complementary to water quality monitoring for evaluating changes in the nitrogen cycling in urban catchments.

1. Introduction

Nitrogen's role in regulating primary productivity is crucial. Its movement and transformations through ecosystems influences biomass accumulation and energy flux, and it is central for the global productivity (Bernhard, 2010). Although most freshwater systems are primarily phosphorus limited (Howarth, 1988; Schindler and Hecky, 2009), there is evidence that plants and algae may become nitrogen limited in aquatic environments with high biomass (Mischler et al., 2014). Nitrogen and phosphorus from nonpoint sources are largely responsible for eutrophication (Carpenter et al., 1998; Kaushal et al., 2011; Baker et al., 2014); however, point sources (such as wastewater treatment facilities) might be equal or greater contributors of nutrients into aquatic ecosystems (Pieterse et al., 2003; Kiedrzyńska et al., 2014). Nitrogen assimilation by submerged attached autotrophic and heterotrophic organisms is likely influenced by the nutrient dynamics of the water column, as they incorporate available nutrients into their biomass (Allen and Spence, 1981; Reddy, 1982; Ogura et al., 2009). When the nitrogen cycling in an aquatic ecosystem is altered, the changes among organisms and at different scales can be measured with stable isotopes analyses. Stable isotopes analyses in aquatic ecosystems have been used to describe trophic levels, trophic networks and effects of land use, among others (Loomer, 2008; Peipoch et al., 2012; Pastor et al., 2013; Dalu et al., 2016; Mao et al., 2016).

Stable isotopes analyses consider that some biological processes can discriminate against the heavy isotope ^{15}N (Needoba et al., 2003; Jones et al., 2004), and isotopic fractionation is the result of factors such as substrate saturation, enzymatic expression, water temperature and physiological stress, among the most important (Mariotti et al., 1981; Handley and Raven, 1992, Cernusak et al., 2009). Nitrogen isotopic composition ($\delta^{15}N$) has been used to determine the sources of nutrients in surface water and groundwater as a regular practice, especially when the nitrogen species come from single or well identified source, such as commercial fertilizers, sewage systems or treatment plants (Komor and Anderson, 1993; Boon and Bunn, 1994; McClelland et al., 1997; Aravena and Roberston, 1998; Lake et al., 2001; Miyajima et al., 2009). Changes in the $\delta^{15}N$ of primary producers and consumers in aquatic ecosystems have been used to estimate changes in nutrient sources and the metabolic status of rivers and streams. For example, Kohl et al. (1971) suggested that the nitrogen assimilated by submerged macrophytes was mainly from agricultural provenance given that the isotopic composition ($\delta^{15}N$) of the plant tissue and the dissolved inorganic nitrogen derived from fertilizers are similar. Fry (1991) found that $\delta^{15}N$ close to 0‰ (particularly in seston) suggested nitrogen fixation close to

* Corresponding author.
E-mail address: eduardo.cejudo@cicy.mx (E. Cejudo).
[1] Present address: Centro de Investigación Científica de Yucatán (CICY A.C.) Unidad de Ciencias del Agua. Calle 8, No. 39, Mz. 29, S.M. 64. Cancun, Quintana Roo, Mexico.77500.

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the water channel, whereas algae was constrained to $\delta^{15}N$ values between −2 and +3‰, a common isotopic composition of soil organic matter and synthetic fertilizers. Anderson and Cabana (2006) found that streams impacted by point-source discharges had ^{15}N enrichment at three different trophic levels. Finlay and Kendall 2007 provided examples of changes in the $\delta^{15}N$ values in human-impacted rivers as a result of external input of nutrients. Pastor et al. (2013) found that nutrient availability had an effect upon the $\delta^{15}N$ of periphyton, algae and macrophytes. However, when multiple sources are involved, the interpretation of the stable isotopes analyses may not be straightforward due to mixing of substrates, products or changes in the chemical species involved (Mayer et al., 2002; Hood et al., 2014).

In 2013, the Region of Waterloo (south western Ontario, Canada) completed major upgrades to the Kitchener Wastewater Treatment Plant (henceforth referred as KWTP), which is a conventional activated sludge process plant with chemical phosphorus removal and sodium hypochlorite disinfection and treatment capacity of 123 million litters per day (Region of Waterloo, 2013). Before upgrades, the effluent was discharged with high ammonium concentration; after upgrades, better aeration led to almost complete nitrification (Region of Waterloo, 2014). Preliminary analyses showed that the isotopic composition of the epilithon growing in the river had large variability before the completion of the upgrades at the wastewater treatment plant (Cejudo et al., 2014); thus, the before-and-after approach hereby presented, help to properly interpret the effects of upgrading a wastewater treatment plant upon the nitrogen cycling or the receiving ecosystem. Although there is much literature discussing the need to control the nutrient content of human effluent into aquatic environments (Lewis et al., 011; Minaudo et al., 2015; Dodds and Smith, 2016), there are few studies quantifying the effects of changes in the nitrogen cycling in aquatic ecosystem due to upgrades at wastewater treatment facilities. We measured the changes in biomass $\delta^{15}N$ in a river downstream of treated effluent discharge as the result of changes in the concentration and $\delta^{15}N$ of the two major DIN species; longitudinal data of concentrations and isotopic composition of DIN and biota allowed us to infer in-stream nitrogen cycling and to explore the relative proportion of ammonium and nitrate incorporated into biomass. The objective of this paper is to provide evidence that changes in the isotopic composition of submerged biomass can be used as a proxy of changes at the nitrogen cycling in stream and as a complementary tool for water quality monitoring.

2. Materials and methods

2.1 S. tudy area

The Grand River (south western Ontario, 43.3 N, −80.4 W, Fig. 1) is the largest river in southwestern Ontario, it contributes with about ten percent of the drainage to Lake Erie and drains a watershed of approximately 6800 km^2 (Grand River Convervation Authority 2013). The bedrock is mainly composed by limestones and dolostones, the watershed is physiographically divided into three sections: Northern till plains, Central moraines and Southern Lake plains. The Central Moraines (the most densely populated area) comprises uneven formations, rolling to hilly land crossed by ridges or recessional and terminal moraines (Karrow, 1987; Nelson et al., 2003). There is no obvious rainy season in the region, though a large amount of the precipitation fall in form of snow (winter). During summer months (mid June to mid September), river base-flow is maintained by flow control reservoirs (Nelson et al., 2003). Temperature range from −7.1 to 20.5 °C. The average precipitation is around 750 mm (506–952 mm, Environment Canada stations 6149387 and 6141095). The land use is mainly agricultural in the north and urban and agricultural in the central area. Approximately 93% of the watershed land area is considered rural, yet the 7% of the urban area homes 81% of the total population (800,000 in 2013). The sampling locations are located in the Central Grand River at variable distance from the effluent discharge (Fig. 1). The location representing upstream of the KWTP effluent is considered as the baseline conditions for the Central Grand River above the KWTP, and accounts for agricultural sub-catchments and one other wastewater treatment plant (city of Waterloo). The downstream locations collect the effects of the KWTP effluent at different distances from the discharge.

2.2. Sampling protocols

River water samples were collected during the summer (July 2011 and August 2013) at one location upstream (14 km above the KWTP effluent, assumed to be background conditions) and five locations downstream of the KWTP effluent discharge. All samples downstream of the KWTP effluent were collected inside the plume of nutrients created by the effluent (identified by highest electrical conductivity in the water column). Water samples were filtered to 0.45 µm (Whatman® membrane filter) and stored cold or frozen before analysis in HDPE containers. Macrophytes, epilithon, epiphyton and seston samples for Total Nitrogen isotopic composition ($\delta^{15}NTN$) were collected at the same time and at the same locations. The concentrations at the location 0 m downstream of the effluent were estimated as the instantaneous volumetric mixing of effluent to the river discharge (effluent data provided by the Region of Waterloo).

The biotic samples intended to represent the biomass of the current-year growing season (*i.e,* the year where they were collected, 2011 and 2013) due to the fact that most macrophytes and epiphyton die and are washed out in the fall and are not present in winter. However, a certain quantity of the epilithon may have overwinter or did not detach completely after decaying; thus, a mixture of live and detritus biomass could have existed in the samples collected, and some material may be from the previous year or from upstream washout. However, this residual material is considered only a small fraction of the total amount collected. Macrophytes, epiphyton, epilithon and seston samples were collected as follows:

2.2.1. Macrophytes

Two whole organism samples were collected at each location, including above and below ground biomass. The species collected were *Myriophyllum spicatum, Elodea* sp. *Fontinalis* sp., *Potamogeton* spp. and *Stuckenia* spp. For simplicity in this paper we will refer to species of *Stuckenia* and *Potamogeton* genera as *Potamogeton* spp. Mats of the benthic algae *Cladophora* spp. were also sampled and because they were intertwined within dense macrophyte beds, they were considered within the macrophytes samples. The material was rinsed *in situ* to eliminate excess sediments and superficial debris; stored in plastic bags (Ziploc ®) with sufficient water to preserve them wet and then stored on ice. In the laboratory, each macrophyte (stem, roots, leaves and reproductive structures if present) was placed in a mason jar (1 L) with approximately 50 ml of nanopure water and vigorously agitated. The slurry, comprising all detached material was considered epiphyton (*see below*). Each macrophyte was then submitted to extensive washing (distilled water), then blotted and oven dried in paper bags (60 °C, 48–72 h). The dry material of each macrophyte was pulverized (Retsch Mixer Mill MM200), acid-washed (5% v/v HCl) to remove carbonates, oven dried and stored in acid-washed glass vials until elemental analysis.

2.2.2. Epiphyton

The slurry obtained from each macrophyte (n = 2) was decanted into centrifuge tubes, frozen and freeze-dried. Sub-samples were acidified (5% v/v HCl), oven dried and stored in acid-washed glass vials until elemental analysis.

2.2.3. Epilithon

Two cobbles (≈10 cm diameter) at each location were collected directly from the river and stored in plastic bags (Ziploc ®) with