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Dissolved organic nitrogen in urban streams: Biodegradability and molecular composition studies

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ABSTRACT

A portion of the dissolved organic nitrogen (DON) is biodegradable in water bodies, yet our knowledge of the molecular composition and controls on biological reactivity of DON is limited. Our objective was to investigate the biodegradability and molecular composition of DON in streams that drain a gradient of 19–83% urban land use. Weekly sampling over 21 weeks suggested no significant relationship between urban land use and DON concentration. We then selected two streams that drain 28% and 83% urban land use to determine the biodegradability and molecular composition of the DON by coupling 5-day bioassay experiments with high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Both urban streams contained a wide range of N-bearing biomolecular formulas and had >80% DON in lignin-like compounds, with only 5–7% labile DON. The labile DON consisted mostly of lipid-and protein-like structures with high H/C and low O/C values. Comparison of reactive formulas and formed counterparts during the bioassay experiments indicated a shift toward more oxygenated and less saturated N-bearing DON formulas due to the microbial degradation. Although there was a little net removal (5–7%) of organic-bound N over the 5-day bioassay, there was some change to the carbon skeleton of DON compounds. These results suggest that DON in urban streams contains a complex mixture of compounds such as lipids, proteins, and lignins of variable chemical structures and biodegradability.

1. Introduction

Dissolved organic nitrogen (DON) is commonly the dominant dissolved N form in surface waters in both forested and human dominated watersheds (Pellerin et al. 2004, 2006; Stanley and Maxted, 2008). Thus, DON is a potential source of reactive N in aquatic ecosystems (Berman and Bronk, 2003; Bradley et al., 2010; Jørgensen et al., 2014; Killberg-Thoreson et al., 2013; Seitzinger et al., 2002; Wiegner et al., 2009). Researchers have hypothesized that DON export in streams is affected by land use patterns; however, reports in the literature are mixed. For example, Pellerin et al. (2006) reported that DON concentrations were 2.5–4 times higher in the urban than forested watersheds. Kroeger et al. (2006) reported that DON export increased in coastal watersheds with increase in human population, and they argued that anthropogenic N added to watersheds can be exported as DON. Other researchers found no significant effect of urbanization on DON export (Aitkenhead-Peterson et al., 2009; Stanley and Maxted, 2008). When they observed no significant effect of urbanization, Stanley and Maxted (2008) suggested that increases in anthropogenic DON associated with urbanization (sewage wastes, fertilizers) were being offset by decreased influence of pre-development wetland DON, such that there was no net change in the stream DON concentrations after post development.

A growing number of studies have addressed the bioavailability and ecological significance of DON in the freshwater (Fellman et al., 2009; Petrone et al., 2011; Ylla et al., 2010) and marine ecosystems (Berman and Bronk, 2003; McCarthy et al., 1998; Mozdzer et al., 2010; Petrone et al., 2009). In these studies, connecting the extent of DON bioavailability to specific DON characteristics (e.g., size fraction) is seldom straightforward because DON is not comprised of a single molecule but is a structurally complex mixture of compounds that vary in chemical structure and bioavailability. These include simple compounds (e.g., sugars, proteins, and amino acids) that are readily used by plants and microbes, as well as more complex compounds like polyphenols and

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tannins that are not readily used (Neff et al., 2003).

Recently, high resolution mass spectrometry using high magnetic fields has been used to characterize DON in the marine and stream samples. The very high resolving power of high magnetic field mass spectrometry allows to make compound level assessments of DON and identify individual DON components based on the molecular formulas (Kellerman et al., 2015; Osborne et al., 2013; Lusk and Toor, 2016). This technique has shown promise when some kind of comparison is desirable to evaluate DON trends in time or space, or to elucidate what makes DON biodegradable or refractory. For example, Osborne et al. (2013) and Lusk and Toor (2016) used ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) to assess changes at the compound level in DON in the water samples before and after bioassay experiments.

The objective of this study was to investigate the effect of urban land use gradient on the biodegradability and molecular composition of DON in the streams of the Alafia River watershed, which drains into Tampa Bay estuary, Florida, United States. Our preliminary data showed a weak or no significant correlation between land use and streamwater DON, similar to the findings of other researchers (Aitkenhead-Peterson et al., 2009; Stanley and Maxted, 2008). Recent authors have reported that urbanization increased the lability and changed the chemical composition of bulk dissolved organic matter (DOM) in urban streams in comparison to non-urban streams (Parr et al., 2015; Williams et al., 2015). Thus, we hypothesized that urbanized streams would contain unique and more labile DON compounds. To test this hypothesis, we used 5-day bioassay experiments and positive ion mode atmospheric pressure photoionization (+APPI) coupled with high resolution FT-ICR-MS to determine the biodegradability and molecular composition of DON in two urban streams that drain 28% and 83% of urban land use.

2. Materials and methods

2.1. Preliminary studies to assess nitrogen concentrations and forms in urban streams

The study sites included streams draining various sub-basins of the Alafia river, which is a blackwater stream, with a characteristic low gradient and surrounded by sandy soils that leach DOM into the streams. The waters of the Alafia have a characteristic golden-tan color due to the high levels of DOM, and there are low levels of sediment, which is another common trait of blackwater streams. The headwaters of the Alafia river originate from swamp and prairie lands of Polk County, Florida and extend 38.6 km westward into lower Hillsborough Bay, ultimately discharging into Tampa Bay estuary. The soils in the watershed are sandy, with moderate to slow infiltration and are dominated by spodosols, inceptisols, and alfisols in the Myakka, Winder, Zolfo, Lake, and Chandler soil groups (Doolittle et al., 1989).

First, we selected seven stream sites located in sub-basins of the Alafia river watershed and three sites located on the mainstem of the Alafia river (Fig. 1). We used 2010 land use data from the Southwest Florida Water Management District and ArcGIS 10 to determine the area under urban, forest, agricultural, and wetland land uses, as well as drainage density and the number of septic systems (Supplementary Fig. S1; Table 1).

Grab water samples were collected from these 10 stream sites on a weekly basis from April 15, 2013 to September 30, 2013 ($n = 21$). This captured the 2013 summer rainy season (June to September) as well as six weeks immediately preceding the rainy season. Samples were collected in 250 mL polyethylene bottles and transported to the lab on ice and filtered through 0.45 μm filter paper within 24 h of collection. The filtered and unfiltered samples were stored in 25-mL polyethylene scintillation vials at approximately 4 °C until analysis for N forms and concentrations, as discussed below.

2.2. Sampling sites for DON bioavailability and molecular characterization

The sampling sites for the biodegradability and molecular characterization of DON were selected from the subset of 10 sites used in the preliminary study. These included a highly urbanized stream (Buckhorn Creek) and a less urbanized stream (Turkey Creek). Buckhorn Creek is predominantly urban with 83% urban land use and 3% agriculture and 9% wetland land uses. Turkey Creek is characterized by mixed land uses, with 28% urban land use and 28% agriculture and 12% wetland land uses. Grab samples were collected from the most downstream points in these two streams in June 2014 in sterile 5 L polycarbonate carboys and transported to the lab on ice. The samples were immediately filtered through 0.45 μm filter paper, stored in borosilicate glass jars at approximately 4 °C for <24 h, and used in the bioassay and FT-ICR-MS experiments. For the biodegradability experiment, samples were inoculated with bacteria from streamwater of the Alafia river mainstem, after methods described by Osborne et al. (2013) (Fig. 1; sampling point MS1). The inoculum water was collected and processed on the same day as the stream samples. The bioassay experiments were initiated by mixing 400 mL of filtered stream water sample and 125 mL of inoculum in 1 L glass Erlenmeyer flasks. The initial samples (designated as T_0) were analyzed for N forms and prepared for FT-ICR-MS analysis as described below. A second set of flasks (designated T_5) were incubated for 5 d on a 12:12 light:dark cycle. After 5 d, the incubated samples were filtered through 0.45 μm to remove microbial cells and analyzed for N forms and FT-ICR-MS analysis as described for the T_0 samples.

2.3. Nitrogen forms and concentrations analysis

For NH_3–N and NO_x–N analysis, filtered samples were analyzed according to EPA method 353.2 using a discrete analyzer (AQ2+, Seal Analytical Inc., Mequon, WI). For total dissolved N (TDN) analysis, a subsample of the filtered samples was oxidized with alkaline persulfate as described by Hosomi and Sudo (1986) and then analyzed for NO_x–N with a discrete analyzer as above. For total N (TN) analysis, unfiltered samples were oxidized and analyzed as for TDN analysis. Particulate organic N (PON) was calculated as the difference between TN and TDN and DON was calculated as the difference between TDN and (NOx–N + NH_3–N).

2.4. Molecular characterization of DON by FT-ICR-MS

The DON in the 0.45 μm-filtered samples was concentrated and extracted for FT-ICR-MS analysis using 1 g, 6 mL Varian Bond Elut PPL solid phase extraction (SPE) cartridges that were first rinsed with two cartridge volumes (12 mL) of HPLC grade methanol. Flow through the SPE sorbent bed was set not to exceed 20 mL/min. After sample loading, the sorbent bed was rinsed with 12 mL of deionized water adjusted to pH ~2 with HCl and then dried by applying a light vacuum for approximately 5 min. We used 6 mL of methanol to elute the sorbent bed and collect into 20-mL borosilicate scintillation vials.

We used dopant-assisted photoionization by diluting samples (in methanol) obtained from SPE at 9:1 with toluene (JT Baker, Phillipsburg, NJ) to increase ionization efficiency. The + APPI was performed on a modified ThermoFisher APPI source (ThermoFisher Corp., San Jose, CA) (Purcell et al., 2006, 2007). Samples were analyzed with a custom-built 9.4 T FT-ICR-MS (Kaiser et al., 2011)