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Composition of dissolved organic nitrogen in rivers associated with wetlands

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HIGHLIGHTS

- DON in wetland-associated rivers from three different climates is characterized.
- Proportion of DON as humic substance N increases with decreasing total N content.
- Proportion of DON as amino acids and their composition are similar among the rivers.
- Ratio of primary amine N to peptide/amide N differs among the rivers.
- Aromatic N content and other parameters vary seasonally in the cool temperate rivers.

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ABSTRACT

As basic information for assessing reactivity and functionality of wetland-associated dissolved organic matter (DOM) based on their composition and structural properties, chemical characteristics of N in ultrafiltered DOM (UDON; >1 kD) isolated from wetland-associated rivers in three climates (cool-temperate, Hokkaido, Japan; sub-tropical, Florida, USA; tropical, Sarawak, Malaysia) were investigated. The UDON was isolated during dry and wet seasons, or during spring, summer, and autumn. The proportion of UDON present as humic substances, which was estimated as the DAX-8 adsorbed fraction, ranged from 47 to 91%, with larger values in the Sarawak than at the other sites. The yield of hydrolyzable amino acid N ranged 1.24 to 7.01mg g^{-1}, which correlated positively to the total N content of UDOM and tended to be larger in the order of Florida > Hokkaido > Sarawak samples. X-ray photoelectron N1s spectra of UDON showed a strong negative correlation between the relative abundances of amide/peptide N and primary amine N. The relative abundances of amide/peptide N and primary amine N in the Sarawak samples were smaller (70–76%) and larger (20–23%) respectively compared to those (80–88% and 4–9%) in the Florida and Hokkaido samples. Assuming terminal amino groups and amide N of peptides as major constituents of primary amine N and amide/peptide N, respectively, the average molecular weight of peptides was smaller in the Sarawak samples than that in the Florida and Hokkaido samples. Seasonal variations in UDON composition were scarce in the Sarawak and Florida samples, whereas the distribution of humic substance-N and nonhumic substance-N and compositions of amino acids and N functional groups showed a clear seasonality in the Hokkaido samples. While aromatic N increased from spring to autumn, contributions from fresh proteinaceous materials were also enhanced during autumn, resulting in the highest N content of UDOM for this season.

1. Introduction

Wetlands contain a significant quantity of terrestrial dissolved organic matter (DOM), and dissolved organic carbon (DOC) concentration in river water is generally high when the watershed includes wetlands (Mattsson et al., 2005; Baum et al., 2007; Williams et al., 2010). In

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particular, DOC concentrations can reach 40–60 mg L^{-1} when peatlands exist in the watershed (Laudon et al., 2004; Billett et al., 2006; Moore et al., 2011). DOM plays a central role in biogeochemistry of wetlands and related aquatic environments, as it limits light penetration, fuels the microbial loop through providing energy and carbon (C) to microorganisms, and influences the solubility and bioavailability of trace elements, among others (Findlay and Sinsabaugh, 2003). Furthermore, as a portion of riverine DOM is transported to the coastal ocean, the quality of DOM is also critical to the biological productivity in the coastal zone.

Wetlands are distributed widely from arctic to tropical regions with a wide range of climatic conditions, and consequently, primary production and heterotrophic activities. Since the quality (i.e. composition and reactivity) of DOM reflects its sources and generation processes (Jaffé et al., 2008), the composition of wetland-derived DOM may have a global commonality on the one hand, but specific environmental conditions may leave a characteristic molecular fingerprint on them on the other hand (Cawley et al., 2014). Thus, comparative studies on the quantity and quality of DOM between different climatic regions are required to attain comprehensive understandings of material cycles and biogeochemical processes in wetlands and associated aquatic environments (Watanabe et al., 2012).

Supplying nitrogen (N) to aquatic organisms is among the most basic functions of DOM. Dissolved organic N (DON) accounts for 20% to >90% of total dissolved N in rivers and estuaries (Berman and Bronk, 2003; Wiegner et al., 2006), and generally 10–70% is considered bioavailable (Stepanauskas et al., 2002; Seitzinger et al., 2002; Wiegner et al., 2006). Variations in the bioavailability of DON are attributed, at least in part, to its composition and structure (Amon and Benner, 1996; Amon et al., 2001; Labanowski and Feuillade, 2009). Amino acids are considered relatively labile among DON components (Duan and Bianchi, 2007; Benner and Kaiser, 2011). Although free amino acids and amino acids in low molecular DOM are more labile than those in high molecular or ultrafiltered DOM (UDOM) (Stepanauskas et al., 1999; Cory and Kaplan, 2012), biodegradation and photodegradation of UDOM accompanied by the release of ammonia or free amino acids are also important for aquatic ecosystem (Mopper and Kieber, 2002). A portion of amino acids incorporated into humic substances (HS) are still biodegradable, although the ratio of biodegradable amino acids to total amino acids has been reported to be larger for non-humic substances (NHS) than that for HS (Volk et al., 1997). Heteroaromatic N derived from charred materials has been suggested to be less biodegradable (Jaffé et al., 2012) and has been shown to be ubiquitous in aquatic systems (Ding et al., 2014).

Still, the existing information on the molecular characteristics and reactivity of DON in wetlands is limited (Maie et al., 2006), and thus, the objective of the present study is to determine key structural properties that characterize the functionality and reactivity of UDOM in wetland-associated rivers in various climatic regions. Here we focus on N in UDOM (UDON) in samples collected from rivers flowing through wetlands in three climatic regions, characterized by cool temperate (Hokkaido, Japan), subtropical (Florida, USA), and tropical (Sarawak, Malaysia) climates. The molecular properties of the UDON were investigated in terms of HS-N and NHS-N contents, elemental composition, hydrolyzable amino acid composition, and X-ray photoelectron spectroscopy (XPS) N 1s spectra. An attempt was made to assess seasonal variations in UDON characteristics, but the authors are aware that the limited number of samples may only reflect the actual differences within this specific dataset.

2. Materials and methods

2.1. Location of sampling sites and collection of water samples

Hokkaido samples ($n = 10$) were collected from three rivers: Chiraikaribetsu River (abbreviated 'Chirai') in the Bekanbeushi wetland (8300 ha), Dei River in the Kiritappu wetland (3168 ha), and Kimonto River in the Kimonto wetland (46 ha), three or four times in autumn (2007), summer (2008), and spring (2009). Water samples were not collected in winter due to inaccessibility. Details are shown in Table 1. Average meteorological data through the three Hokkaido wetlands from 2001 to 2010 were as follows: annual precipitation, 1076 ± 105 mm; daily mean temperature, 5.61 ± 0.1 °C; maximum temperature, 30.4 ± 1.6 °C; and minimum temperature, −21.4 ± 3.4 °C (Japan Meteorological Agency, 2011). These wetlands belong to lowmoor or transient moor peatlands. Major vegetations were *Alnus japonica* Steud, *Phragmites communis* Trin, *Eriophorum vaginatam* Linn, *Moliniopsis japonica* (Hack.) Hayata, *Myrica gale* Linn., and *Calamagrostis purpurea* subsp. *langsdorfii*, and *Sphagnum* community was also observed.

Florida samples ($n = 4$; Table 1) were collected from the Taylor Slough and Shark River Slough in the Everglades in October 2007 (wet season) and March 2009 (dry season). Average annual precipitation in the Everglades from 1971 to 2000 was 1323 mm, and mean temperature, mean maximum temperature, and mean minimum temperature during the same period were 23.4 °C, 28.5 °C, and 18.2 °C, respectively (Southeast Regional Climate Center, 2007). The Taylor Slough site is a freshwater marsh dominated by emergent wetland plants such as *Cladium* and *Eleocharis* with high abundance of calcareous periphyton mats. The soil is a wetland marly peat (about 1 m thick) on limestone bedrock. The Shark River Slough site is located at the upper estuary of the Shark River and dominated by fringe mangroves. The soil is a mangrove swamp peat (>1 m).

Sarawak samples ($n = 4$; Table 1) were collected from two rivers, namely the Bakong River and Leban River in December 2007 (wet season) and August 2008 (dry season). Average annual precipitation, mean temperature, mean maximum temperature, and mean minimum temperature from 2007 to 2010 were 3854 mm, 26.4 °C, 34.5 °C, and 22.0 °C, respectively. Along the rivers, mixed swamp forests are developed on peat soil. The dominate vegetation included Ramin (*Gonystylus bancanus*), Jongkong (*Dactylocladus stenostachys*), Kapur (*Drybalanops rappa*), and Alan (*Shorea albida*). During 2006, an area along the Bakong River near the sampling site was deforested and converted to an oil palm plantation.

2.2. Preparation of UDOM samples

Each of the 50–60 L river water samples was collected and filtered with combusted glass fiber filters with nominal pore size of 0.40 μm (GB140, ADVANTEC, Tokyo, Japan), followed by with hydrophilic polyvinylidene difluoride membranes (nominal pore size, 0.22 μm) equipped in a Pellicon-2 Mini tangential flow ultrafiltration system (Millipore, Billerica, MA, USA). DOM in the filtrates was concentrated using the Pellicon-2 Mini equipped with regenerated cellulose membranes having the smallest molecular weight cut-off (1 kD) among those available to the system (Maie et al., 2006). The concentrated UDOM solution was further desalted by repeating diafiltration with an addition of 10-fold volume of ultrapure water three times on the membranes. Both types of the membranes were washed with 0.5 M HCl and 0.1 M NaOH solutions beforehand, and the water samples were cooled on ice water during the entire filtration procedures. The concentrated samples were filtrated with a PTFE membrane (nominal pore size, 0.45 μm; ADVANTEC), freeze-dried, and mixed thoroughly with an agate mortar.

2.3. Measurement of HS-N/NHS-N ratio

To determine the HS-N and NHS-N, 10 mg of sample was dissolved in 50 mL ultrapure water regulated at pH 1.5 with 6 M H_2SO_4. An aliquot was measured for DOC and total N concentrations using TOC-V_{CPH} with T-N units (Shimadzu, Kyoto, Japan) after sparging with N_2. Based on the molecular weight cut-off of the membrane used in the DOM preparation, the total N concentrations were regarded as the DON