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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.

ARTICLE INFO

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 7.01 mg 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.

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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

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 low-moor or transient moor peatlands. Major vegetations were *Alnus japonica* Steud, *Phragmites communis* Trin, *Eriophorum vaginatum* 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$_2$SO$_4$. 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

Table 1
Water samples used.

River	Latitude	Longitude	Sampling date (season)	
Bakong	N2°46.200	E112°09.139	27 Dec. 2007 (rainy)	5 Aug. 2008 (dry)
Leban	N2°51.111	E112°09.983	27 Dec. 2007 (rainy)	5 Aug. 2008 (dry)
Taylor	N25°24.214	W80°36.414	16 Nov. 2007 (rainy)	22 Mar. 2009 (dry)
Shark	N25°24.586	W80°57.859	13 Nov. 2007 (rainy)	23 Mar. 2009 (dry)
Dei	N43°04.845	E145°05.388	24 Nov. 2007 (autumn)	30 Jun. 2008 (summer)
			23 Apr. 2009 (spring)	
Kimonto	N43°37.173	E143°28.698	4 Nov. 2007 (autumn)	5 Sep. 2008 (summer)
			24 Apr. 2009 (spring)	
Chirai	N43°05.927	E144°55.653	24 Nov. 2007 (autumn)	30 Jun. 2008 (summer)
			10 Sep. 2008 (summer)	23 Apr. 2009 (spring)

concentrations. Another 20 mL of this solution was passed through a column (φ10 mm) packed with 3 mL of prewashed Supelite DAX-8 (Supelco, Bellefonte, PA, USA). Then the resin was washed with 3-column volumes of water (at pH 1.5), and the eluent and washing were combined (NHS fraction). The DOC and DON concentrations in the NHS fractions were then measured. The same procedures were also conducted using ultrapure water (DOC < 0.05 mg L^{-1}) to obtain a blank value. The HS-C and HS-N concentrations were estimated from the difference between total DOC (or DON) and NHS-C (or NHS-N) concentrations taking the blank values into consideration.

2.4. Measurement of elemental composition

Contents of C, H, and N were measured using an elemental analyzer (MicroCorder JM10, J-Science Group, Tokyo) after 1-week drying under vacuum on P_4O_{10}. Sulfur in the sample was converted into sulfate by the oxygen flask method (Busman et al., 1983) and determined using an ion chromatograph (Dionex ICS-1600, ThermoFisher, Sunnyvale, CA, USA). Ash content was measured by the combustion method. The O content was obtained as the difference between total weight and the sum of C, H, N, S, and ash contents.

2.5. Measurement of amino acid composition

Hydrolyzable amino acid compositions were measured as follows: 1 mg sample was weighed into a glass ampoule, and 1 mL of 6 M HCl containing 10 mg mL^{-1} of phenol was added. The ampoule was sealed under high vacuum and heated at 110 °C for 24 h. After the insoluble residue was filtered, 100 μL of the hydrolyzate was dried up and dissolved in 500 μL of buffer solution consisting of 0.0167 M sodium citrate and 0.167 M citric acids with 0.15 M sodium chloride, 5 mL L^{-1} ethanol, and 100 μL L^{-1} caprylic acid. Then the solution (100 μL) was injected to an amino acid analyzer (JLC/500V, JEOL, Tokyo). Amino acids were detected by ninhydrin color reaction and quantified using the external standard method.

The amino acid-based degradation index (DI) proposed by Dauwe et al. (1999) was calculated using the following equation:

$$DI = \sum_i \left[\frac{var_i - AVE\,var_i}{SD\,var_i} \right] \times fac.coef_i$$

where var_i is the mol% of amino acid i in a sample to be examined, and $AVEvar_i$, $SDvar_i$, and $fac.coef_i$ are average, standard deviation, and factor coefficient of amino acid i of the dataset obtained from particulate organic matter in various diagenetic states (Dauwe et al., 1999), respectively. Those values are presented in Table SI 1. The DI corresponds to the score plot of the first principal component (PC1) in the principal component analysis of amino acid composition in natural organic matter samples with different diagenetic histories. A higher and lower DI values suggest the lower and higher degrees of diagenetic reworking of the proteinaceous components, respectively.

2.6. Measurement of XPS N1s spectra

N1s spectra were recorded on an X-ray photoelectron spectrometer (AXIS 165, Shimadzu, Japan) using a monochromatic Al Kα X-ray source (1486.6 eV) at an analyzer pass energy of 40 eV, an electric current of 30 mA, and a voltage of 10 kV. A finely powdered sample (ca. 1 mg) was fixed on the surface of a metallic sample block by means of Scotch double-sided non-conducting tape. Vacuum pressure was maintained at 10^{-6} to 10^{-7} Pa during measurement. Spectra were recorded for each visible line at 0.1 or 0.05 eV per step. The time for one scan was 298 ms, and 32 or 64 scanned data were accumulated. Correction of binding energy was made relative to the C–C/C–H signal at 285.0 eV in the C1s spectra measured simultaneously. Data manipulation including curve fitting, integration, and background subtraction was accomplished on a workstation installed with data treatment software (VISION-2, Shimadzu). The spectra were deconvoluted into three Gaussian curves with peak centers at 399.0 ± 0.1 eV (aromatic N including imine, heterocyclic C=N, and aromatic amine), 400.4 ± 0.1 eV (amide/peptide N including pyrrole, secondary and tertiary amines, and imide); and 402.3 ± 0.1 eV (primary amine N including other protonated N), and the proportions of the three N groups in total N were estimated from the relative areas surrounded by Gaussian curves and base line with respect to the spectral area (Abe and Watanabe, 2004).

3. Results

3.1. Ratio of HS and NHS

Fig. 1 shows the proportions of DOC and DON in the forms of HS and NHS in the UDOM samples. The proportion of DON present as HS (%HS-N) ranged from 47 to 91%, which was smaller than that of DOC present as HS (%HS-C), 62–98%. Both %HS-N and %HS-C were larger ($P < 0.005$) in the Sarawak rivers (87 ± 4% and 97 ± 1%, respectively) than in the Florida rivers (62 ± 7% and 75 ± 10%) and Hokkaido rivers (67 ± 9% and 80 ± 5%) irrespective of season. Seasonal variations in %HS-N were small in the Sarawak and Florida rivers (<7%), while %HS-N in the Hokkaido samples particularly that in the Dei River, tended to be larger in the order of spring > summer > autumn.

3.2. Elemental composition

The elemental composition of the UDOM samples is presented in Table 2, and Fig. 2 shows the relationships between the weight% of C and O, C and N, and H and N in the UDOM samples, respectively. The Sarawak samples were low in O (45.5–46.2%), N (0.94–1.10%), H (3.91–4.14%), and S (0.19–0.21%) contents and high in C content (48.5–49.3%), while the Florida samples were high in O content (48.0–51.8%) and low in C content (40.4–42.5%). The N (1.84–3.19%), H (4.37–5.61%), and S (0.68–1.61%) contents of the Florida samples overlapped with those of the Hokkaido samples, 1.51–2.30%,
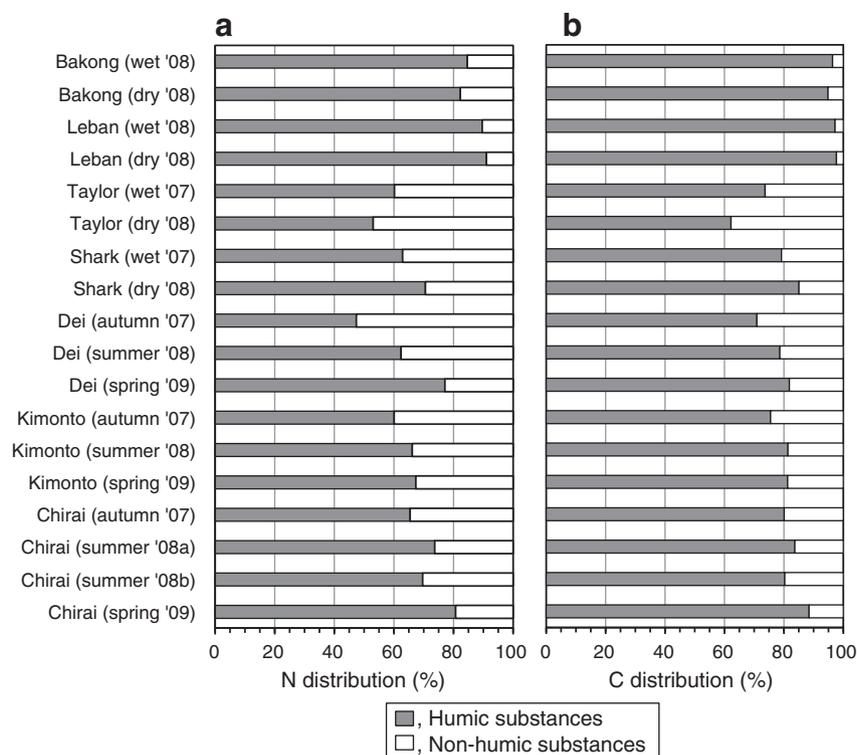
Fig. 1. Distributions of N (a) and C (b) in humic substances (HS) and non-humic substances (NHS) in wetland river UDOM from different climatic regions. Chirai (summer '08a) and Chirai (summer '08b) were collected in Jun. and Sep. 2008, respectively.

4.71–5.14%, and 0.41–0.82%, respectively, and higher than those of the Sarawak samples. Although elemental composition of the UDOM samples collected at the same site in different seasons did not differ notably, the N content in the Hokkaido samples was higher in autumn, 2.01–2.30%, than those in spring and summer, 1.51–1.89%.

A strong negative correlation was observed between the C and O contents throughout the three sampling regions ($r = -0.929$***; Fig. 2a). The relationship between C and N contents ($r = 0.732$***; Fig. 2b) and that between H and N contents ($r = 0.921$***; Fig. 2c) were also significant. The fact that linear correlations were observed for all cross-plots suggests that regardless of climate/region, the

elemental composition of UDOM in these wetland dominated rivers can be evaluated uniformly.

3.3. Amino acid composition

Table 2 also shows the amount of hydrolyzable amino acid N, which ranged from 1.24 mg g^{-1} in the Leban (Sarawak) during the dry season to 7.01 mg g^{-1} in the Taylor (Florida) during the dry season. The amount of hydrolyzable amino acid N correlated positively to the N content in UDOM ($r = 0.861$***; Fig. 3) and thus tended to be larger in the Florida samples and smaller in the Sarawak samples. Yields of amino acids in the Hokkaido samples, which were intermediate between the Florida and Sarawak samples, showed seasonal variation, i.e., smaller in summer than in autumn.

The proportions of DON detected as amino acid N were 10–22%, which did not show distinct variation among the three regions. Variations in amino acid composition (Fig. 4) were also small among all the UDOM samples. Dominant amino acid was always glycine (Gly; 17–23%). The second largest value was recorded by aspartic acid (Asp; 12–14%) or alanine (Ala; 9–12%), followed by threonine (Thr) and glutamic acid (Glu; 8–10%). As such, neutral hydrophilic amino acids and acidic amino acids were commonly dominant, regardless of climatic regions and seasons. Amino acid contents in UDOM samples are presented in Table SI 1.

The amino acid-based DI (Dauwe et al., 1999) is also shown in Fig. 4. This index has been used as a proxy for evaluating the reactivity of DON. Larger values are presumably indicative of fresh, reactive DON, while smaller values are obtained when DON is more degraded. DI of the present samples was always negative, suggesting the presence of more degraded proteinaceous materials. DI was significantly larger ($P < 0.05$) in the Sarawak (-0.04 to -0.53) than in the Florida (-0.71 to -1.43) and Hokkaido (-0.37 to -1.18) samples. In the Sarawak rivers, DI was larger in dry season than that in the wet season, while the order was opposite in the Florida rivers. In the Hokkaido rivers, DI tended to be smaller in summer than in spring and autumn with a significant difference ($P < 0.05$) between summer and autumn.

Table 2
Elemental composition and acid-hydrolyzable amino acid N contents in UDOM samples from wetland associated rivers in different climatic regions.

| Site (season)	Weight %[a]					Amino acid N[a]	
	C	H	N	S	O	(mg g^{-1})	(%total N)
Bakong (rainy '08)	49.34	3.91	0.94	0.19	45.63	1.40	15.0
Bakong (dry '08)	49.14	4.14	1.06	0.20	45.46	1.51	14.3
Leban (rainy '08)	48.85	4.02	1.10	0.21	45.82	1.62	14.7
Leban (dry '08)	48.53	4.06	1.04	0.20	46.17	1.24	11.9
Taylor (rainy '07)	42.20	5.29	2.54	0.68	49.29	3.70	14.6
Taylor (dry '08)	42.48	5.61	3.19	0.76	47.96	7.01	22.0
Shark (rainy '07)	40.70	5.02	2.38	0.98	50.92	3.34	14.1
Shark (dry '08)	40.38	4.37	1.84	1.61	51.79	2.23	12.2
Dei (autumn '07)	46.37	5.09	2.01	0.82	45.71	3.63	18.1
Dei (summer '08)	47.08	4.85	1.62	0.78	45.68	2.23	13.8
Dei (spring '09)	48.72	4.91	1.51	0.51	44.35	2.62	17.3
Kimonto (autumn '07)	45.47	5.00	2.30	0.82	46.42	2.93	12.8
Kimonto (summer '08)	45.77	4.71	1.76	0.66	47.09	2.27	12.9
Kimonto (spring '09)	48.91	4.83	1.89	0.73	43.63	1.84	9.7
Chirai (autumn '07)	45.13	5.14	2.30	0.70	46.73	3.77	16.4
Chirai (summer '08a)	45.58	4.83	1.84	0.75	47.01	1.95	10.6
Chirai (summer '08b)	45.74	4.78	1.81	0.52	47.15	2.11	11.6
Chirai (spring '09)	49.61	4.76	1.68	0.41	43.55	3.56	21.2

[a] On ash free basis.
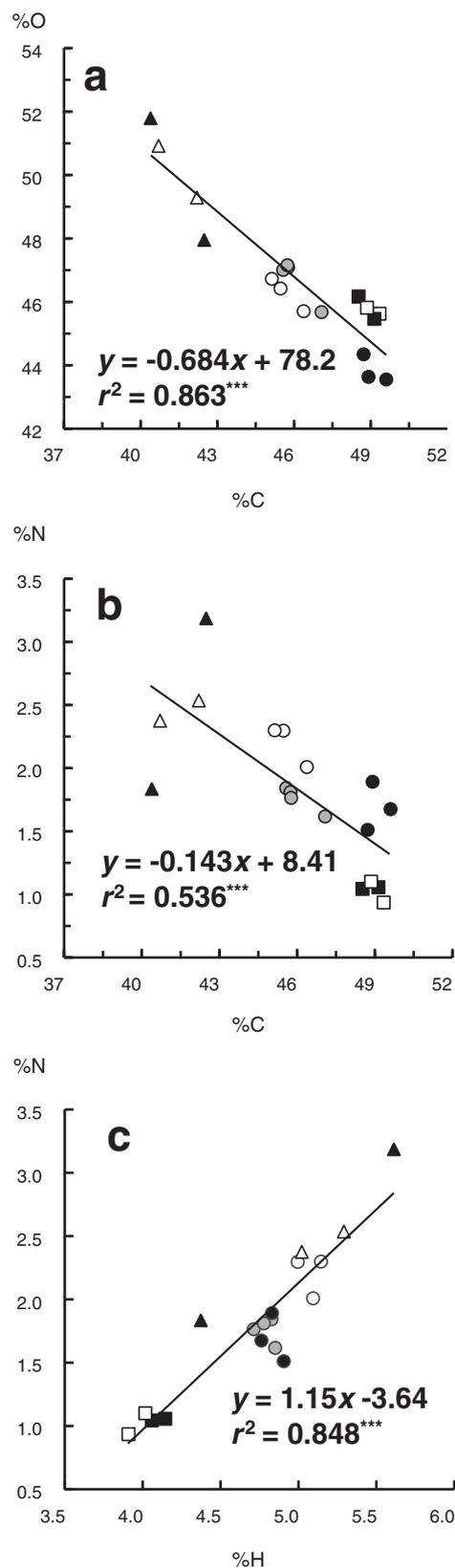
Fig. 2. Relationships between %C and %O (a), between %C and %N (b), and between %H and %N (c) in wetland river UDOM from different climatic regions. Squares, circles, and triangles refer to Sarawak, Hokkaido and Florida samples, respectively. Open symbols indicate wet season or autumn. Closed symbols indicate dry season or spring. Gray symbols indicate summer.
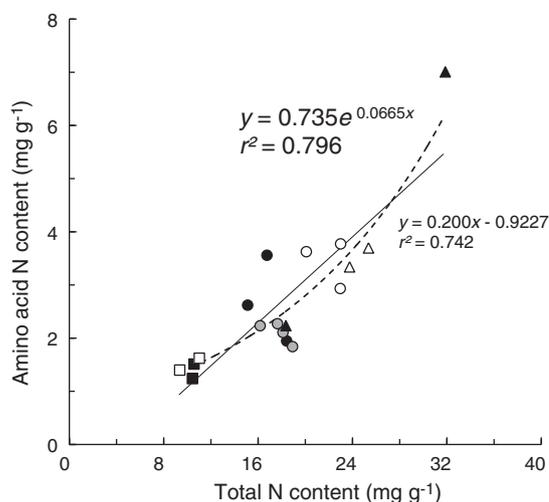

Fig. 3. Relationship between N content and amino acid N content in wetland river UDOM from different climatic regions. Squares, circles, and triangles refer to Sarawak, Hokkaido and Florida samples, respectively. Open symbols indicate wet season or autumn. Closed symbols indicate dry season or spring. Gray symbols indicate summer.

3.4. XPS N 1s spectra

The representative XPS N 1s spectra of the UDOM samples from the three regions are shown in Fig. 5. Composition of N functional groups estimated from the XPS N 1s spectra (Table 3) ranged as follows: %amide/peptide N, 70–88%; %aromatic N, 4–14%, and %primary amine N, 4–23%. As shown by the presence of a shoulder peak in the spectra, the Sarawak samples were characterized by the largest %primary amine N, 20–23%, and smallest %amide/peptide N, 70–76%, among the three sampling regions. The variations in the N composition between the Florida and Hokkaido samples were small, but the %amide/peptide N (84–88%) and %primary amine N (4–7%) in the Florida samples tended to be larger and smaller than those in the Hokkaido samples, 81–88% and 6–9%, respectively. Throughout the three sampling regions, a strong negative correlation was observed between the %amide/peptide N and %primary amine N ($r = -0.892^{***}$).

Seasonal variation in the N composition in the Sarawak and Florida samples was difficult to assess due to the limited number of available samples. However, the N composition of the Hokkaido samples varied between summer and spring or autumn, where %aromatic N was larger ($P < 0.05$) in the order: autumn (12–14%), summer (9–11%), and spring (4–7%).

4. Discussion

The presence of wetlands, particularly peatlands, in river catchments enhances DON flux to surface waters (Worrall et al., 2012). However, the bioavailability or residence time of DON exported in rivers may vary depending on its composition and structure. In the present study, UDON in river water associated with wetlands under very different environmental conditions (climate, vegetation, hydrology, etc.) was characterized through a climatic gradient from cool temperate to the tropics. The DAX-8 fractionation indicated the dominance of HS-N and HS-C in UDOM, which were larger than 60% (16 of 18 samples; Fig. 1) and above 75% (15 of 18 samples) respectively. Linear correlations between HS-C and DOC had previously been reported for these sites, suggesting larger soil-derived DOC contributions with increasing total DOC values (Watanabe et al., 2012) with the Sarawak featuring the highest HS-C content. This location also had the highest HS-N values. Nevertheless, %HS-N correlated negatively to the N content ($r = -0.827^{***}$), amino acid N content ($r = -0.668^{***}$), and %amide/
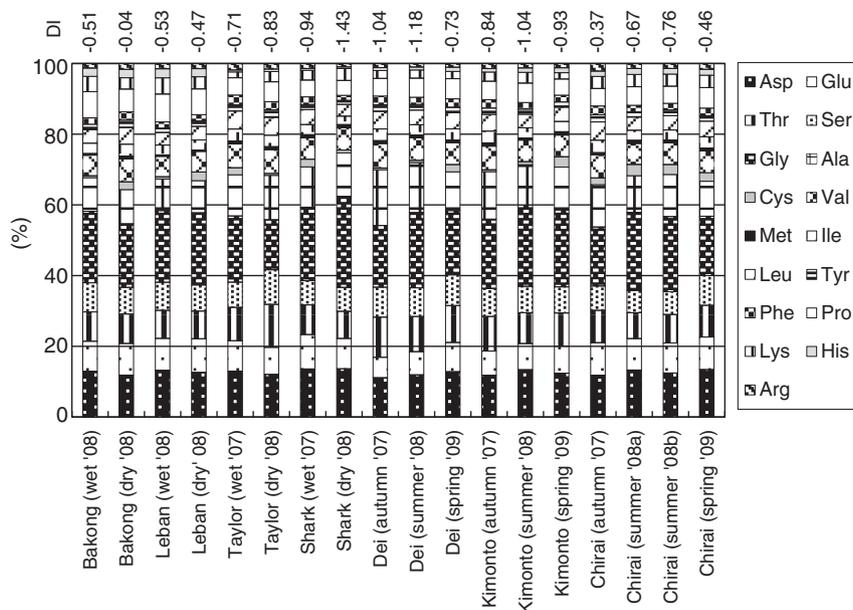
Fig. 4. Composition of amino acids released upon acid hydrolysis and degradation index (DI) values for wetland river UDOM from different climatic regions.

peptide N ($r = -0.599$**), suggesting the greater contribution of NHS for the difference in the N content of UDOM. In fact, the slope in the regression analysis between the N and NHS-N contents in UDOM (mg g^{-1}

on ash free basis) was much larger than that between the N and HS-N contents in UDOM (Fig. 6):

$$HS\text{-}N = 0.386 \times N + 5.207 \quad r^2 = 0.790^{***} \quad (1)$$

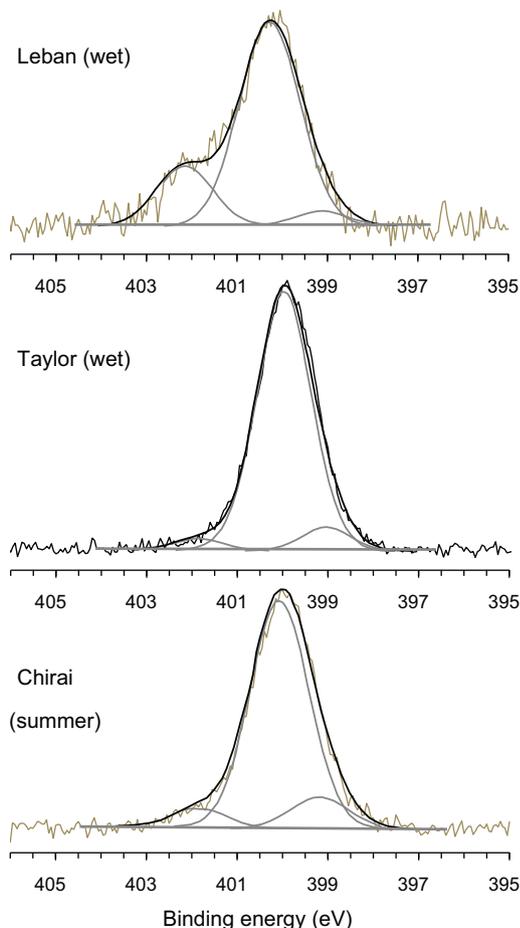$$NHS\text{-}N = 0.614 \times N - 5.207 \quad r^2 = 0.905^{***}. \quad (2)$$

The N content and hydrolyzable amino acid content in UDOM tended to be larger in the order: Florida, Hokkaido, and Sarawak samples (Table 2; Fig. 2). While the elevated N content for the Taylor UDOM samples (Florida) may be due to the presence of N-fixing cyanobacteria in the abundant periphyton mats, the lower N content in the Sarawak UDOM samples may be due to the enhanced contribution of peat soil-derived DOM at this site, which seems more depleted in DON.

Amino acid composition of the present DOM samples was typical for that of DOM in rivers (Duan and Bianchi, 2007; Ylla et al., 2011) and soils (Fischer et al., 2007). Minor differences among the three regions were observed in the proportions of histidine (His) and proline (Pro), which were larger in the Sarawak samples than those in the Florida

Fig. 5. Representative XPS N1s spectra of wetland river UDOM from different climatic regions.

Table 3
Composition of N functional groups in UDOM samples from wetland associated rivers in different climatic regions estimated from XPS N1s spectra.

| Site (season)	%primary amine N	%amide/peptide N	%aromatic N
Bakong (wet '08)	21	72	7
Bakong (dry '08)	23	70	7
Leban (wet '08)	20	76	4
Leban (dry '08)	21	73	6
Taylor (wet '07)	7	84	9
Taylor (dry '09)	5	87	8
Shark (wet '07)	4	88	8
Shark (dry '09)	5	88	7
Dei (autumn '07)	6	82	12
Dei (summer '08)	7	84	9
Dei (spring '09)	8	88	4
Kimonto (autumn '07)	6	81	13
Kimonto (summer '08)	7	83	10
Kimonto (spring '09)	8	88	4
Chirai (autumn'07)	6	80	14
Chirai (summer '08a)	7	82	11
Chirai (summer '08b)	8	84	8
Chirai (spring '09)	9	84	7
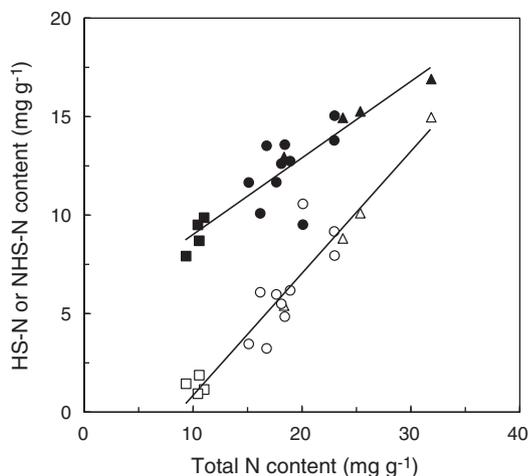
Fig. 6. Relationship between N content and HS-N or NHS-N content in wetland river UDOM from different climatic regions. Regression equations are shown in text. Squares, circles, and triangles refer to Sarawak, Hokkaido and Florida samples, respectively.

and Hokkaido samples, 6.4–7.9% vs 4.2–5.8% and 2.3–2.5% vs 0.4–1.7%, respectively. These observations, in agreement with the above discussed HS-N/NHS-N ratios, may be indicative of a greater contribution of microbially derived amino acids (Cowie et al., 1992; Benner and Kaiser, 2011) to the Sarawak UDON. In the Hokkaido samples, the proportions of tyrosine (Tyr) and phenylalanine (Phe) tended to be larger in autumn (1.8–1.9% and 2.4–2.5%) than those in summer (1.5–1.6% and 1.8–2.1%), suggesting the generation of new (fresh) UDON after the summer season. In agreement, a larger content of Gly during the summer (21–23%) compared to autumn (17–20%) may reflect a higher resistance to photo- and bio-degradation (Benner and Kaiser, 2011; Wu et al., 2003) of the UDON during this period.

The DI values for the studied UDON samples (Fig. 4) were generally in the lower range of those previously reported for rivers and wetlands (Duan and Bianchi, 2007; Maie et al., 2006). DI values for the Florida samples were higher for the wet than for the dry season samples, suggesting higher inputs of reactive DON during the wet season. This may be resulted from periphyton primary productivity, which is commonly higher during the wet season (Pisani et al., 2013 and references therein). While the Shark sample (Florida) collected during the dry season featured the lowest DI value (−1.43), the Bakong sample (Sarawak) collected during the dry season showed the highest DI value (−0.04). As the DI values of the Leban UDON (Sarawak) did not show significant seasonal variations, the increase in DI of the Bakong UDON might reflect the influence of deforestation and the implementation of oil palm plantations in the region. Yamashita and Tanoue (2003) and Ylla et al. (2011) observed that the DI values of DOM were larger when the hydrolyzable amino acid content or the proportion of N present as amino acids was larger. However, the larger average DI values from the Sarawak samples compared to those of the other rivers (except for the Chirai samples, Hokkaido) do not agree with this reported trend. The combination of higher but negative DI values and low total amino acid contents suggests that much of the Sarawak UDON is to some extend degraded. A possible reason for the higher DI in Sarawak is the lower accessibility to low molecular peptides bound to HS which are abundant at this site (Fig. 1). Similarly, amide-N in deep ocean waters has been suggested to be biologically recalcitrant (Aluwihare et al., 2005) possibly through its incorporation into humic-like substances. However, other factors such as partitioning of amino acids between particulate organic matter and DOM (Aufdenkampe et al., 2007) and adsorption of amino acids to sediments (Kawahata and Ishizuka, 1993), may also help explain these results. Further research is needed to better determine the biogeochemical mechanism responsible for the present observations.

The aromatic N accounted for 4 to 14% of total UDON. Similar content of aromatic N in some US rivers has been suggested to be related to the presence of dissolved black carbon (DBC; Jaffé et al., 2012) generated through the dissolution of charcoal as a byproduct of fossil fuel burning (Ding et al., 2013; Dittmar et al., 2012), and has been termed dissolved black nitrogen (DBN; Ding et al., 2014). While the %aromatic N was highest for the Hokkaido and lowest for the Sarawak samples, the environmental reasons for these differences are unlikely the result of differences in recent fire frequency, as this has been suggested to be unrelated to the abundance of DBC in DOM (Ding et al., 2013). The variation in the relative abundance of %aromatic N also did not correlate with the proportion of DON present as HS-N (Fig. 1). These combined results suggest that dissolved HS are heterogeneous in terms of the amount and stability of aromatic N.

The yield of amino acid N released upon acid hydrolysis accounted only for one-fourth of total UDON, which may in part be attributable to a smaller recovery of amino acids in HS after acid hydrolysis (Abe et al., 2005). While the %amide/peptide N estimated by XPS showed a positive correlation to the N content of UDOM ($r = 0.680^{***}$), the %primary amine N also showed a significant but negative correlation ($r^2 = 0.674^{***}$) to the N content of UDOM. Potential contributors of N functional groups to primary amine N signal in XPS N1s spectra include amino group of terminal amino acid in peptides, non-acetylated amino sugars, and amino groups in the side chains of amino acids (Abe and Watanabe, 2004). Other than peptide-bond N, indole, purine C–N, and various amides such as lactam (Thorn and Cox, 2012) and hydantoin (Fang et al., 2011) can give signals in the amide/peptide N region. Among them, proteinaceous components may be the most important. Based on this hypothesis, the average number of peptide bonds in proteinaceous materials was inferred from a ratio of %primary amine N to %amide/peptide N. The ratio was larger for the Florida DOM, suggesting the presence of proteinaceous materials with a larger molecular weight, while more fragmented in the Sarawak DOM. The presence of amino acids bound covalently to UDOM could also contribute to the larger %primary amine N to %amide/peptide N ratio. This tendency seems in good agreement with the larger relative abundance of NHS N in the Florida DOM than that in the Sarawak DOM. The Hokkaido samples also showed the positive and negative correlations between the ratio of %primary amine N to %amide/peptide N and the proportions of DON present as HS and NHS, respectively ($P < 0.05$). Therefore, it is suggested that the major difference in the N composition of riverine UDOM associated with wetlands was not the content of proteinaceous materials but their molecular weight distribution.

Based on the data presented in this study, the significance of the potential seasonal (wet versus dry) variations in DON composition for both Sarawak and Florida rivers is limited due to the low number of samples under study, and suggests that UDON composition is reasonably constant at these sites. For the Hokkaido samples however, the distribution of HS-N/NHS-N, DI, and composition of N functional groups showed apparent seasonal variations that were significantly different. The DI values tended to be smaller in summer, suggesting the progression of the degradation of amino acids. The larger proportion of NHS in total UDON and the larger DI in autumn compared to summer suggested the contribution of fresh UDON to the larger N content of UDOM, possibly through leaf litter leaching inputs. The %aromatic N also increased between spring and autumn, although the HS-N content (N content of UDOM × proportion of UDON present as HS-N) in the UDOM samples did not vary significantly among seasons: 9.5–11.7 (Dei), 11.7–13.8 (Kimonto), and 12.6–15.0 (Chirai) mg g^{-1}, respectively. These observations can be explained by assuming that the heterogeneity of the amount, quality, and reactivity of aromatic N in HS may also be involved in controlling seasonal variations in the Hokkaido UDON.

Although UDON samples were collected from three sites under different climatic conditions, no clear transitions in chemical characteristics of UDON were observed along this cool temperate to tropical gradient. As discussed in the above paragraphs, wetland characteristics,

including types of peatland and vegetation cover, may have significant influences on the molecular composition of UDON. Nevertheless, the effect of climate change or global warming on DON concentration in peat porewater (Kane et al., 2014), soil N cycling and related DON dynamics (Ueda et al., 2013), as well as DON transformation process in river (Xia et al., 2013), suggests the importance of climate control of DON dynamics. Further investigation on the relationship between chemical characteristics and functionality of DON from various wetland associated rivers is of value to address how climate change will affect the biogeochemistry of DON.

5. Conclusions

The composition of DON in wetland-associated riverine UDOM from three different aquatic systems was characterized. Throughout the three sampling regions, positive correlations were observed between the N content and H, S, and amino acid N contents as well as the proportion of amide/peptide N in total UDON, suggesting a generally common UDON makeup for vastly different riverine systems. However, specific differences and trends were also observed throughout the dataset. Sarawak tropical peat-derived UDOM was poorer in N, and in particular NHS-N, compared to Florida and Hokkaido UDOM samples. However, the proportions of hydrolyzable amino acid N in total UDON at Sarawak were similar to those in the other regions, due probably to the association of amino acids and low molecular peptides with HS. While seasonal variations in UDON composition were generally scarce or undetectable in the subtropical and tropical sampling sites, the distribution of HS-N/NHS-N, DI, and %aromatic N in the cool temperate Hokkaido region showed variations during three seasons.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.05.095.

Conflict of interest statement

We have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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