Occurrence of unsaturated C₂₅ highly branched isoprenoids (HBIs) in a freshwater wetland

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ABSTRACT

Eight C₂₅ highly branched isoprenoid (HBI) alkenes were detected in the freshwater wetland of the Florida Everglades and tentatively assigned as a C₂₅ diene, three C₂₅ trienes, two C₂₅ tetraenes and two C₂₅ pentaenes based on their mass spectra, retention index and literature reports. One diene and one triene were observed more frequently than the others. The HBIs were present in varying amount of up to 1000, 19000, 780 and 150 ng/g dry weight in the periphyton, floc, surface soils and deeper soils (ca. 1900 AD), respectively. Compound specific carbon isotope analysis of the two most dominant HBIs (one diene and one triene) showed them to be highly depleted in ¹³C (δ¹³C -40.0 to -38.5‰) in freshwater floc, suggesting recycled CO₂ produced from the decomposition of organic matter as an important C source in the biosynthesis of these compounds. HBIs were present across this freshwater wetland in greater diversity and abundance at locations with higher surface water N concentration and longer hydroperiod (inundation). Historical variation in the occurrence of the HBIs in soil cores from multiple slough and ridge environments was assessed. Their increased abundance after ca. 1960 AD in almost

all cores suggests proliferation of their precursor biota (freshwater diatoms) over the past five decades.

Key words: Highly branched isoprenoids (HBIs), diatoms, stable carbon isotopes, freshwater wetland, nutrients, hydroperiod

1. Introduction

Since the discovery of C₂₅ highly branched isoprenoids (HBIs) in sediments from the Gulf of Mexico (Gearing et al., 1976), these hydrocarbons have been widely reported in various locales, especially coastal and marine environments (e.g. Dunlop and Jefferies, 1985; Rowland and Robson, 1990; Hird and Rowland, 1995; Jaffé et al., 2001; Massé et al., 2004; Schouten et al., 2000; Xu et al., 2006a; McKirdy et al., 2010a), but also in saline lacustrine settings (Wang and Williams, 2001; McKirdy et al., 2010b, 2013; Zhang et al., 2011a). Over the past 20 yrs, they have been used extensively as biomarkers for diatom-derived organic matter (OM), based on the first identification (Volkman et al., 1994) of polyunsaturated homologues in two marine diatom species *Haslea ostrearia* (family Naviculaceae) and *Rhizosolenia setigera* (family Rhizosoleniaceae). Numerous C₂₅ HBIs have been reported in five genera of diatoms, namely *Haslea*, *Navicula*, *Pleurosigma*, *Rhizosolenia* and recently, *Berkeleya*, covering both benthic and planktonic species (e.g. Wraige et al., 1999; Belt et al., 2001a,b; Allard et al., 2001; Grossi et al., 2004; Sinninghe Damsté et al., 1999a,b, 2004; Brown et al., 2014).

C₂₅ HBIs have traditionally been considered biomarkers of saline environments, due to their extensive presence in marine diatom species. However, a few studies have detected them in freshwater diatoms and other environments suitable for diatom growth. For instance, a sole C₂₅ HBI triene (IV; see Appendix for structures) was identified in a culture of the freshwater *N. sclesvicensis* (Belt et al., 2001c). A diene (II) was found in freshwater sediments from Rostherne Mere (Rowland, 1983) and a monoene (structure not reported) in freshwater sediments from Lake Koucha, eastern Tibetan Plateau, China (Aichner et al., 2010). Two monoenes were detected in low abundance in recent sediments from freshwater Lake El Junco, Galápagos Islands (Zhang et al., 2011b). Other than these reports, C₂₅ HBIs have not been reported in any other freshwater

environmental settings.

The Florida coastal Everglades is the largest naturally oligotrophic wetland in the USA. It is characterized by a variety of environmental, hydrologic, soil-type and vegetation gradients (Davis et al., 1994). Anthropogenic alteration of the landscape and water and nutrient flow patterns, as well as the predominant seasonality, have led to unique vegetation gradients along the Everglades landscape from sloughs (deeper water) with submerged and emergent aquatic plants, to ridges (shallow water) with sawgrass dominated communities, to Tree Island hammocks (Davis et al., 1994). Within the diverse distribution of plant species, periphyton mats are widely distributed throughout this ecosystem (Gaiser et al., 2011). With a negligible presence of free floating phytoplankton, most of the suspended particulate OM is present in the form of flocculent material (floc), consisting of a non-consolidated layer of inorganic and organic particles (detritus and disaggregated periphyton remains) and microorganisms (generally bacteria). HBIs are abundant in this ecosystem, where C₂₀ HBIs are present in freshwater marshes (Jaffé et al., 2001; Pisani et al., 2013; Saunders et al., 2014), while C₂₅ HBIs mainly occur in estuarine and marine settings (Jaffé et al., 2001; Xu et al., 2006a,b; He et al., 2014). Here, we examine the presence, abundance and spatial distribution of up to eight unsaturated C₂₅ HBIs in various periphyton and floc samples, surface soils and soil cores across the freshwater wetland of the Everglades. Compound specific carbon isotope data for the most abundant C₂₅ HBIs (II and IV) are used to further constrain their potential sources. Other environmental factors potentially influencing the distribution and abundance of these compounds, including surface water nutrient level and hydroperiod, are also explored.

2. Methodology

2.1. Sample locations and description

2.1.1. Locations

The freshwater sites (Fig. 1) encompass nutrient and hydroperiod gradients, as described by Gao et al., 2007, and most are established sampling stations for the Florida Coastal Everglades Long Term Ecological Research program (FCE-LTER;

<http://fcelter.fiu.edu/>). Site WCA3 is to the north of Everglades National Park (ENP) in Water Conservation Area 3. It has a longer hydroperiod and higher nutrient (P and N) level than all the other freshwater sites here (see supplementary data in Table S2, Supplementary Material). Sites SRS1 to SRS3 are in the Shark River Slough (SRS) of ENP, featuring peat soil, and intermediate hydroperiod and nutrient levels. Sites in the Taylor Slough Panhandle (TSPH2-4) are within the freshwater section of Taylor Slough, characterized by marl soils, shorter hydroperiod and lower nutrient level.

2.1.2. Sample type

Periphyton and floc samples were collected from various locations (Table 1). *Chara* sp. (macroalga) and the leaves and roots of dominant Everglades plants such as *Cladium* sp., *Eleocharis* sp., *Nymphaeaceae* sp., *Utricularia* sp., *Typha domingensis* P. and *Typha latifolia* L. were collected throughout ENP at randomly selected locations (He et al., 2015a). Surface soil samples (0–2 cm) were obtained from WCA3, SRS1-3 and TSPH2. Several soil cores (up to 30 cm in depth) were collected from SRS1-SRS3 and TSPH2. They had previously been dated by Saunders et al. (2014).

2.1.3. Sampling methods

Periphyton, floc, surface soil and soil cores were collected following the procedures as described previously (e.g. Neto et al., 2006; Pisani et al., 2013; Saunders et al., 2014). The plant samples were wrapped in Al foil immediately after collection, and kept cool on ice during return to the laboratory. They were then transferred to pre-combusted glass jars with Teflon lined caps and stored in a freezer (–20 °C) until analysis.

2.2. Sample preparation and analytical methods

The detailed analytical procedure has been described elsewhere (e.g. He et al., 2014, 2015b). Briefly, samples were freeze-dried at –40 °C and extracted with ultrasonication using pure CH₂Cl₂ (Optima, Fisher, USA). Activated Cu was added in order to remove elemental S from the floc, periphyton and soil extracts. The extracts were separated using silica gel chromatography, whereby the aliphatic and aromatic hydrocarbon fractions (fractions 1 and 2, respectively) containing the HBIs were eluted

with pure *n*-hexane and a mixture of *n*-hexane and toluene (v:v, 15:5; Optima, Fisher, USA). A known amount of squalane was added to each fraction as an internal standard for semi-quantitative analysis.

A Hewlett-Packard 5973 gas chromatography-mass spectrometry (GC-MS) system was used for analysis. The column was coated with DB-1 MS (30m, 0.25mm i.d., 0.25µm film thickness; Agilent) or DB-5 MS (30m, 0.25mm i.d., 0.25µm film thickness; Agilent). The GC oven was programmed from 60 (held 1 min) to 300 °C (held 20 min) at 6 °C/min. Compounds were assigned by comparison of mass spectra and retention index (RI) values with published data, and interpretation of mass spectra fragmentation patterns. The stable carbon isotopic composition of a C_{25:2} HBI (II) and a C_{25:3} HBI (IV) were determined with a Finnigan Delta V Plus mass spectrometer coupled to a Hewlett-Packard 6890 gas chromatograph (GC-IRMS). The same DB-1 column was used in the GC-IRMS instrument, and the detailed chromatographic conditions have been described by He et al. (2015b).

2.3. Data analysis

Environmental data across multiple locations were obtained from the FCE-LTER database and used for comparison with the abundance of the C₂₅ HBIs. The data included hydroperiod and surface water nutrients, periphyton primary productivity, periphyton composition and soil nutrient concentration. Statistical analysis was performed using SPSS version 13.0 for Windows. Outliers were tested using the two-sided Grubbs test ($P < 0.05$). Significant correlation ($P < 0.05$) between environmental parameters and the abundance of C₂₅ HBIs were determined using Pearson correlation.

3. Results and discussion

3.1. GC and MS characteristics of C₂₅ HBIs

GC-MS analysis of the aliphatic and aromatic hydrocarbon fractions from various periphyton and floc sample extracts revealed the presence of up to eight compounds with chromatographic and MS properties consistent with C₂₅HBI alkenes (Fig. 2). In aliphatic fractions, they eluted between C₂₀ and C₂₃ *n*-alkanes on both DB-1 and DB-5 columns

(Fig. 2 and Table 1). Hydrogenation of the total hydrocarbons with PtO₂ catalyst produced a single C₂₅HBI alkane with a mass spectrum identical to that identified by Rowland and Robson (1990) as 2,6,10,14-pentamethyl-7-(3'-methylpentyl)pentadecane, indicating that all the C₂₅HBI alkenes had a similar carbon skeleton. Examination of their spectra revealed that the group of HBIs consisted of one diene (M⁺, *m/z* 348; C₂₅H₄₈, II in Fig. 3), three trienes (*m/z* 346; C₂₅H₄₆, I, III and IV in Fig. 3), two tetraenes (*m/z* 344; C₂₅H₄₆, V and VI in Fig. 3) and two pentaenes (*m/z* 342; C₂₅H₄₄, VII and VIII in Fig. 3). They elute in the same order from I to VIII on both DB-1 and DB-5 columns. Their retention index (RI) values and major characteristic fragment ions are listed in Table S1.

Examination of the fragmentation pattern and ion distribution of diene II (Fig. 3), and comparison of its RI with previous reports suggests the double bond positions to be at C-6/C-17 and C-23/C-24, respectively (Johns et al., 1999). For the trienes (I, III and IV), III was tentatively assigned as the structure in Fig. 3 on the basis of its MS fragmentation pattern and comparison of its RI with literature values (Belt et al., 1996, 2000; Wraige et al., 1999). Compounds I and IV have very similar fragmentation patterns, suggestive of geometric or configurational isomers rather than positional isomers (Belt et al., 2000). However, their RI values are very different (2065 and 2106, respectively, on DB-1). By comparing the RI with that from Belt et al. (2000), IV was tentatively assigned a structure as shown in Fig. 3. This C₂₅ HBI is one of the two major biomarkers in *H. ostrearia* (Volkman et al., 1994; Wraige et al., 1997). Compound I has a nearly identical mass spectrum to a novel C_{25:3} HBI in a culture of the diatom *Pleurosigma intermedium* (compound I in Fig. 1b of Brown and Belt, 2016). For the tetraenes and pentaenes, only V was tentatively assigned (see structure in Fig. 3) based on the RI reported by Wraige et al. (1997), while the structures of I, VI, VII and VIII were not determined here and require further investigation (Table S1). Compounds VI (RI_{DB-1} 2157) and VII (RI_{DB-1} 2176) have almost the same MS features and RI values as two unidentified HBIs (RI_{HP-1} 2159 and 2175, respectively) found in axenic *H. ostrearia* cultures by Wraige et al. (1999). Compound VI (RI_{DB-1} 2157) also shows similar MS features to an unknown C_{25:4} HBI in *Pleurosigma strigosum* (Grossi et al., 2004). Compound VIII (RI_{DB-1} 2217) has not been reported previously.

3.2. Source assessment of C₂₅ HBIs in the freshwater wetland of the Everglades

While C₂₅ HBIs have been reported in various marine diatom species (Rowland and Robson, 1990; Volkman et al., 1994; Belt et al., 1996; Schouten et al., 1998), those found here are all from freshwater wetland locations and thus should not be derived from marine diatoms. They were not detected in either leaves or roots of dominant wetland plant species across the Everglades ecosystem (e.g. *Cladium*, *Eleocharis*, *Nymphaeaceae*, *Utricularia*, *Typhadomingensis* and *Typhalatifolia*), but were abundant in periphyton. They are therefore most likely derived from freshwater diatoms, one of the most abundant organisms in Everglades periphyton (Vymazal and Richardson, 1995; Gaiser et al., 2006, 2011; Hagerthey et al., 2011). More than 50 freshwater diatom species, including *Navicula* species (*N. radiosa* Kutz. var. 01, *N. subtilissima* Cleve and *N. cryptotenella* L.-Bert), have been identified in periphyton across the freshwater wetland of the Everglades (Gaiser et al., 2006). It is therefore likely that some of these species or other genera of freshwater diatoms (in addition to *N. sclesvicensis*) could be the source of the C₂₅ HBIs here. Interestingly, II was also reported in the top ca. 20 cm sediment (ca. 1984 AD) from a Florida Bay core (compound V in Xu et al., 2006a). Fossils of several freshwater species including *Mastogloia smithii*, *Encyonema evergladianum*, *Nitzschia semirobusta*, *Fragilaria synegrotasca* were identified in that core, although they were dominant only in the basal section (ca. 140-160 cm). This C₂₅ HBI diene significantly correlated with the abundances of planktonic marine diatom species such as *Cyclotella* cf. *distinguenda* and *Cyclotella litoralis* in that study. As such, the occurrence of II in both freshwater wetland and Florida Bay samples suggests its production by both freshwater and marine diatoms. Apart from II, all the other C₂₅ HBIs here differ in number and position of double bonds from those detected in Florida Bay (Xu et al., 2006a), even though they all share the same parent skeleton. Specifically, the C₂₅ HBIs in the Florida Bay sediments were different isomers from those here, featuring only one or two double bonds and with the monoenes dominant (Xu et al., 2006a). The C₂₅ HBIs in the Everglades samples have 2–5 double bonds, with dienes and trienes the most dominant. In addition, their RI values on DB-1 and MS fragmentation patterns are also different (Table S1; Table 1 in Xu et al., 2006a).

The $\delta^{13}\text{C}$ values of the most abundant C₂₅ HBIs (II and IV) range from –40.0‰ to

214 -38.5‰ (Table 2) reflecting greater depletion in ^{13}C than reported previously for *n*-
 215 alkanes in the same samples (-36.0‰ to -25.1‰ ; He et al., 2015b). These isotopically
 216 lighter values suggest that the organisms synthesizing II and IV at least partially utilize
 217 recycled (^{13}C depleted) CO_2 produced from the decomposition of OM as their C source.
 218 Likewise, significantly lighter $\delta^{13}\text{C}$ values for co-occurring monomethyl alkanes (-42.0‰
 219 to -36.1‰ ; He et al., 2015b) and botryococcenes (-37.4‰ to -36.2‰ ; Gao et al., 2007)
 220 reflect a similar CO_2 source for the organisms producing these compound classes. The
 221 $\delta^{13}\text{C}$ values for the Everglades freshwater C_{25}HBIs contrast with those reported for
 222 lacustrine and marine C_{25}HBIs ($\delta^{13}\text{C}$ usually $> -25\text{‰}$). For instance, values of C_{25}HBIs
 223 (with one degree of unsaturation) were found to range from -24.6‰ to -19.4‰ in top
 224 sediments (ca. 4.7 cal ka BP) from a sediment core from Lake Koucha, eastern Tibetan
 225 Plateau, where freshwater phytoplankton were the major source of OM from during that
 226 period (Aichner et al., 2010). The values for C_{25}HBIs (with one or two degrees of
 227 unsaturation) in Florida Bay ranged from -21.9‰ to -17.6‰ (Xu et al., 2006a), as
 228 expected for compounds derived from marine diatoms (Volkman et al., 1994; Sinninghe
 229 Damsté et al., 2004). Similarly, for the C_{20}HBIs detected throughout the Everglades (data
 230 not shown), $\delta^{13}\text{C}$ values ranged from -41.8‰ to -36.4‰ for freshwater and estuarine
 231 samples, while values from -30.1‰ to -18.3‰ were determined in the hypersaline,
 232 seagrass-dominated, Florida Bay. Although C_{20}HBIs have been suggested to be derived
 233 from cyanobacteria (Kenig et al., 1995; Jaffé et al., 2001) or the green alga *Enteromorpha*
 234 *prolifera* (Rowland et al. 1985), recent studies have also attributed their origin to
 235 epiphytic diatoms (Volkman et al., 1998; Atahan et al., 2007; McKirdy et al., 2010a,b,
 236 2014). A similar isotope fractionation or source variation could also apply to the C_{25}
 237 HBIs , and be likely associated with the periphyton mats, which are known to have a high
 238 abundance of epiphytic diatoms (Gaiser et al., 2006, 2011; Pisani et al., 2013).

240 3.3. Spatial distribution and environmental controls on C_{25}HBIs in the freshwater 241 wetland

242 A wider diversity and higher abundance of C_{25}HBIs were present in floc/periphyton
 243 samples than in soils (Table 1), which could be caused by the more complex microbial
 244 composition of periphyton and floc than of soils (Gaiser et al., 2011; Hagerthey et al.,

2011), a variable degree of incorporation of periphyton and floc into soils (Neto et al., 2006) and/or early diagenetic reworking of the more labile, polyunsaturated compounds (Leahy and Colwell, 1990; Rontani et al., 2011). Thus, while the presence of polyunsaturated C₂₅ HBIs (with 4–5 double bonds) suggests a fresh input from periphyton and floc containing freshwater diatoms, their lack in soils is attributed to be from degradation and transformation during early diagenesis. Our result is consistent with reports of the rare occurrence of these diatom biomarkers in soils (Rowland and Robson, 1990; Rontani et al., 2011).

The C₂₅ HBIs in floc seem to correlate with hydroperiod across the Everglades freshwater wetland (R^2 0.61, P 0.11, n =5; Fig. 4; Table S2). However, while the trend is not statistically significant, likely due to the limited number of samples, it is in agreement with findings that (i) freshwater diatoms are more abundant at long hydroperiod sites across the wetland (Gottlieb et al., 2005) and (ii) the longer hydroperiod induces sub-oxic or anoxic conditions in the floc layer and thus enhances OM preservation. Generally, a higher concentration of C₂₅ HBIs was present in slough surface soil (deeper water; long hydroperiod) than in ridge surface soils (shallower water; short hydroperiod), and only IV was detected in slough surface soils (SRS3 and TSPh2; Fig. 5).

The concentration of C₂₅ HBIs in periphyton, floc and surface soil all correlated (P < 0.05) with total N in surface water among the different freshwater sites (Fig. 4). The concentration in floc also correlated with total P concentration in surface water (R^2 0.60, P < 0.05). However, no significant correlation was observed between dissolved P and C₂₅ HBIs in periphyton or surface soils, most likely due to the limited sampling size and the fact that P is the limiting nutrient in this oligotrophic environment. Therefore, we suggest that higher abundance and wider diversity of C₂₅ HBIs in periphyton, floc and surface soils at the WCA3 site vs. all the other locations (Table 2), could in part be attributed to higher nutrient levels in surface water (especially N) and a longer hydroperiod in Water Conservation Area 3 (Fig. 1).

3.4. Depth profiles of C₂₅ HBIs in the freshwater wetland of the Florida Everglades

Only IV (a triene) was detected across all soil cores. The tetraenes and pentaenes were not detected in the soils, indicating a faster degradation rate than the trienes and

dienes (Volkman et al., 1983). Higher concentrations of C₂₅ HBIs were observed at the top of slough cores than in the ridge cores, suggesting better preservation due to the longer hydroperiod. The drying out associated with a shorter hydroperiod enhances aerobic respiration in floc and surface soils in the ridge cores. Although C₂₅ HBIs were present at depths of 4 to 16 cm below ground level (Fig. 5), IV rapidly disappeared in sediments older than 1900 AD, possibly due to diagenetic reworking (cf. Requejo and Quinn, 1983; Kohnen et al., 1990; Cooke, 1995; Wakeham et al., 2002). However, similar C₂₅HBI alkenes can occur in much older Holocene sediments from the Coorong coastal lakes in South Australia (McKirdy et al., 2010a, 2013), suggesting that the rapid rise in abundance of C₂₅ HBIs in the Everglades soil cores after 1960 AD is more likely attributable to an increasing contribution of diatom-derived OM input. The coincidence between recent higher nutrient conditions in the Everglades (Craft and Richardson, 1998) and the increased abundance of C₂₅ HBIs in the surface soils supports the idea that C₂₅ HBIs could serve as indicators of an anthropogenically enhanced nutrient level in the Everglades ecosystem. Similar suggestions have been made on basis of the depth profiles of a C₂₀ HBI in Everglades soil cores (Saunders et al., 2014).

4. Conclusions

The series of C₂₅ HBIs detected in periphyton, floc, surface and deep soils across the Everglades freshwater wetlands is likely produced by freshwater diatoms. The HBI diene and triene (II and IV) were present in higher abundance and greater frequency than the tetraenes and pentaenes, suggesting that they were preferentially produced or better preserved across this freshwater wetland. Their tight correlation and similar $\delta^{13}\text{C}$ values suggest a similar source. Their abundance correlated with dissolved N in the surface water ($P < 0.05$), indicating that nutrients are one of the controlling factors for the distributions of these compounds within this ecosystem. C₂₅ HBIs were better preserved at long hydroperiod sites, such as slough soils, rather than at short hydroperiod sites such as ridge soils where frequent drying out leads to higher rates of OM oxidation. Therefore, both nutrients (N and, to a lesser extent, P) and hydroperiod may play a key role as an environmental driver for the abundance and preservation of C₂₅ HBIs within the Everglades. In all the soil cores, C₂₅ HBIs were below detection limit prior to ca. 1960

AD and rapidly increased in abundance afterwards, implying increased abundance of their precursor biota (i.e. freshwater diatoms) recently and concurrent with increasing nutrient level in the Everglades wetlands. Further investigation is needed to better constrain the sources and environmental factors controlling the distributions and abundance of C₂₅ HBIs in freshwater wetlands.

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Figure captions

Fig. 1. Location map of sampling sites in Everglades National Park. SRS and TSPH denote Shark River Slough and Taylor Slough respectively.

Fig. 2. Partial GC-MS TIC (total ion current) trace of hydrocarbon fractions for typical flocc and surface soil samples: (a) WCA3 ridge flocc (hydrocarbon fraction 1), (b) WCA3 surface soil (hydrocarbon fraction 1), (c) WCA3 ridge flocc (hydrocarbon fraction 2), (d) WCA3 slough flocc (hydrocarbon fraction 1), (e) WCA3 slough surface soil (hydrocarbon fraction 1) and (f) WCA3 slough flocc (hydrocarbon fraction 2). Note: (a-c) are runs on DB-1 column, while (d-f) are runs on DB-5 column; C₂₅ HBIs and *n*-alkanes are indicated by gray triangles and black dots, respectively.

Fig. 3. Mass spectra of eight C₂₅ HBIs detected in the freshwater wetland of the Florida Everglades ecosystem.

Fig. 4. Correlations between concentrations of C₂₅ HBIs in different environmental matrices and the corresponding locations' nutrient levels and hydroperiods across the Everglades freshwater wetlands. Note: the C₂₅ HBI concentration is based on all eight compounds identified in Figure 3.

Fig. 5. Depth profiles of C_{25:3} HBI (IV, ng/g dw) in the (a) SRS1 slough core, (b) SRS1 ridge core, (c) SRS2 slough core, (d) SRS2 ridge core, (e) SRS3 slough core, and (f) TSPH2 slough core. Note: all core dates are from Saunders et al. (2014).

Appendix. Structures of compounds II – V.

Fig. 1

Fig. 2

Fig. 3

Fig. 5

1
2
3

Appendix

Table captions

Table 1

Average concentration (ng/g dw) of C₂₅ HBIs in typical periphyton, floc and surface soils across the freshwater wetlands of the Florida Everglades. SD, standard deviation; -, not determined; see Fig. 1 for locations and abbreviations of sample names.

Table 2

δ¹³C values, ‰ vs. PDB of selected C₂₅ HBIs (II and IV; SD, standard deviation in parentheses).

Sample list	I C _{25:3} (SD)	II C _{25:2} (SD)	III C _{25:3} (SD)	IV C _{25:3} (SD)	V C _{25:4} (SD)	VI C _{25:4} (SD)	VII C _{25:5} (SD)	VIII C _{25:5} (SD)
Periphyton								
WCA3 (n=4)	-	363 (182)	-	107 (97)	52 (48)	23 (17)	-	-
SRS1 (n=1)	-	153	-	30	-	-	-	-
SRS2 (n=2)	-	82 (62)	-	24 (11)	-	-	-	-
SRS3 (n=1)	-	-	-	76	-	-	-	-
TSPH2 (n=1)	-	126	-	25	-	-	-	-
TSPH3 (n=1)	-	-	-	104	-	-	-	-
TSPH4 (n=1)	-	-	-	64	-	-	-	-
Floc								
WCA3 (n=86)	11 (47) ^a	386 (481)	49 (136) ^b	1770 (1573)	91 (279) ^c	24 (68) ^c	47 (137) ^c	12 (35) ^c
SRS1 (n=1)	-	201		982	-	-	-	-
SRS2 (n=4)	-	321 (295)		1017 (862)	-	-	-	-
SRS3 (n=1)	-	-		240	-	-	-	-
TSPH2 (n=4)	-	47 (34)		130 (119)	-	-	-	-
TSPH3 (n=1)	-	-		66	-	-	-	-
TSPH4 (n=1)	-	-		-	-	-	-	-
Surface soil								
WCA-3 (n=9)	-	50 (39)	79 (68)	386 (179)	33 (38)	-	-	-
SRS1 (n=9)	-	-	-	236 (249)	-	-	-	-
SRS2 (n=9)	-	-	-	340 (297)	-	-	-	-
SRS3 (n=9)	-	-	-	26 (31)	-	-	-	-
TSPH2 (n=9)	-	-	-	30 (33)	-	-	-	-

a, b, c - Detected in 2, 10 and 4 of 86 floc samples in WCA-3, respectively.

Table 1

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Sample list	C _{25:2} HBI (II)	C _{25:3} HBI (IV)
WCA3Periphyton (n = 2)	-39.7 (0.7)	-40.0 (1.3)
SRS2 floc (n = 3)	-38.9 (1.3)	-39.3 (1.2)
WCA3 ridge floc (n = 8)	-39.3 (0.9)	-39.0 (0.8)
WCA3 slough floc (n = 7)	-39.7 (0.8)	-39.5 (1.0)
WCA3 surface soil (n = 3)	-38.5(0.6)	-39.1 (0.7)

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16 **Table 2**

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