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Seasonal and diel relationships between the isotopic compositions of dissolved and particulate organic matter in freshwater ecosystems

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Abstract. A study of the isotopic composition of organic matter was conducted in a freshwater marsh over seasonal and diel time scales to determine the sources of dissolved organic matter (DOM) and the processes leading to its formation. Bulk C and N isotopic compositions of the bacterial fraction (0.2–0.7 μm) and particulate organic matter (POM; 0.7–10 μm) were compared on a seasonal basis with the change in $\delta^{13}C$ of DOM. The bulk isotopic data support the idea that DOM was, in part, derived from the breakdown of larger organic matter fractions. The bacterial fraction and POM were compositionally similar throughout the year, based on a comparison of the $\delta^{13}C$ of individual amino acids in each fraction. Annual variation in the $\delta^{13}C$ of amino acids in DOM was greater relative to the variation in larger fractions indicating that microbial reworking was an important factor determining the proteinaceous component of DOM. The ^{13}C enrichment of serine and leucine in each organic matter fraction suggested microbial reworking was an important factor determining organic matter composition during the most productive times of year. Changes in the bulk $\delta^{13}C$ of DOM were more significant over daily, relative to seasonal, time scales where values ranged by 6‰ and followed changes in chlorophyll *a* concentrations. Although bulk $\delta^{13}C$ values for POM ranged only from −29 to −28‰ during the same diel period, the $\delta^{13}C$ of alanine in POM ranged from −30 to −22‰. Alanine is directly synthesized from pyruvate and is therefore a good metabolic indicator. The $\delta^{13}C$ of individual amino acids in DOM revealed the diel change in the importance of autotrophic versus heterotrophic activity in influencing DOM composition. Diel changes in the $\delta^{13}C$ of phenylalanine, synthesized by common pathways in phytoplankton and bacteria, were similar in both DOM and POM. The diel change in $\delta^{13}C$ of isoleucine and valine, synthesized through different pathways in phytoplankton and bacteria, were distinctly different in DOM versus POM. This disparity indicated a decoupling of the POM and DOM pools, which suggests a greater source of bacterial-derived organic matter at night. The results of this study demonstrate the use of the isotopic composition of individual amino acids in determining the importance of microbial reworking and autotrophic versus heterotrophic contributions to DOM over both diel and seasonal time scales.

Introduction

Dissolved organic matter (DOM) often represents the largest source of carbon and reduced nitrogen fueling microbial activity in the aquatic environment (Azam et al. 1983; Hobbie and Wetzel 1992; Kaplan and Bott 1983). The biogeochemical cycling of carbon and nitrogen is often driven by DOM; therefore an understanding of the actual sources and fate of DOM are important goals in aquatic ecosystems science and global biogeochemistry (Meyer et al. 1988). Investigations of both the biological reactivity and chemical composition of various size classes of organic matter suggest low molecular weight DOM, the largest pool of DOM, is primarily derived from larger fractions of organic matter (Amon and Benner 1996; Benner et al. 1992, 1997; Skoog and Benner 1997). The composition and reactivity of different size fractions of organic matter support the evidence that photosynthetic microalgae and bacteria are important sources of DOM in the ocean (Hedges 1992). The nitrogenous component of marine DOM, however, is primarily in the amide form and thought to be derived primarily from bacteria rather than photosynthetic eukaryotes (McCarthy et al. 1997, 1998). In freshwater ecosystems, there are often multiple organic matter sources including material derived from both autochthonous and allochthonous sources (Kaplan and Bott 1982; McDowell and Likens 1988), and much of the nitrogenous DOM is asssociated with humic substances (Lytle and Perdue 1981; Volk et al. 1997).

Biological reactivity of marine and freshwater DOM reveal the existence of two differentially labile pools (Amon and Benner 1996; Kaplan and Bott 1983). In addition to rapidly cycling components, such as amino acids and carbohydrates, a large and more refractory pool of DOM may also be derived from microorganisms and their reworking of organic matter (McCarthy et al. 1998; Tranvik 1993). In contast to most other environments (Cowie and Hedges 1994), the refractory pool of DOM appears to contain a large proteinaceous component both in marine (McCarthy et al. 1997) and freshwaters where it appears to be associated with humic substances (Lytle and Perdue 1981; Volk et al. 1997). The surprisingly large proteinaceous component of DOM suggests the importance of mechanisms by which biological molecules are preserved, and evidence for the biological origins of DOM in the aquatic environment may actually be held within these proteinaceous compounds.

The stable isotopic analysis of individual compounds makes the investigation of the fate of individual molecules with multiple sources possible (Prahl et al. 1992; Hayes 1993; Keil and Fogel 2001). Stable isotopes from bulk stable carbon (C) and nitrogen (N) analyses have been instructive in identifying sources of organic matter and trophic dynamics in many ecosystems (Cabana and Rasmussen 1996; Peterson 1999; Zieman et al. 1984). Bulk isotopic signatures provide an integrated signal of photosynthesis, other carbon dioxide fixation pathways, and energy transfer. For example, stable carbon isotopic signatures have been used to identify the floodplain origins of much of the organic matter supporting a river ecosystem (Quay et al. 1992). Due to the complex nature of and myriad sources for DOM, it is often

very difficult to clearly identify the sources and dynamics of this complex pool from its integrated bulk stable isotopic signatures.

More recently, the stable isotopic composition of individual compounds have been used for identifying sources and the biological processes responsible for the composition of organic matter in sediments (Boschker et al. 1999; Prahl et al. 1992; Keil and Fogel 2001) and DOM in wetlands (Kracht and Gleixner 2000). The C and N isotopic compositions of amino acids have been used to study bacterial reworking of organic matter (Fogel and Tuross 1999; Keil and Fogel 2001), and may serve to further our understanding of the sources and processes responsible for producing DOM in aquatic ecosystems. The range in carbon isotopic composition of amino acids within a given organism are a result of the different biosynthetic pathways of each amino acid (Abelson and Hoering 1961; Macko et al. 1987; Fogel and Cifuentes 1993). Alanine, for example, is directly derived from pyruvate therefore changes in its isotopic composition will directly reflect changes in the source of carbon fixed or metabolized by the organism (Gottschalk 1988). Valine and Leu, however, are derived from pyruvate after multiple steps including decarboxylation, and assimilation of acetyl-CoA which creates a greater difference in their carbon isotopic composition relative to pyruvate. There are amino acid biosynthetic pathways unique to broad taxonomic or functional classes of organisms. Different pathways employing unique enzymes in plants and other eukaryotes (acetolactetate mutase) versus bacteria (acetohydroxy acid synthase), for example, are used to catalyze the first step in the biosynthesis of both Val and isoleucine (Ile) (Gottschalk 1988; Voet and Voet 1990). The isotopic composition of Val and Ile may potentially be used to distinguish between bacterial and plant or algal sources of organic matter.

The incorporation of newly synthesized microbial biomass, or microbial reworking of plant or algal-derived organic matter has been elucidated in sedimentary environments by investigating the $\delta^{13}C$ composition of glycine (Gly), Val, and Ile (Fogel and Tuross 1999; Keil and Fogel 2001). Valine and Ile, as describe above, are synthesized via different enzymes in plants or algae versus the bacteria, and the bacterial enzyme leads to greater fractionation and ultimately the depletion of ^{13}C in the amino acids generated. Depleted values of Val and Ile in organic matter fractions relative to sources or parent material were found and attributed to bacterial reworking of original organic matter sources in the sedimentary environment (Fogel and Tuross 1999; Keil and Fogel 2001). Additionally, enrichment in ^{13}C content of Gly has also been attributed to microbial reworking. The enrichment in ^{13}C of Gly likely occurs when serine (Ser), the precursor to Gly, is utilized during the biosynthesis of other amino acids, such as cysteine. This enrichment in ^{13}C of Ser and Gly is likely to increase during microbial activity and the recycling of organic matter during the degradation of original material.

The objective of the present study was to assess the variation and factors controlling both the bulk and individual amino acid isotope composition in organic matter fractions from the water column of a freshwater wetland. Wetlands are often important sources of DOM and usually provide the primary source of humic substances to adjacent freshwater ecosystems (Hemond 1990; Kortelainen 1993; Mulholland and Kuenzler 1979; Thurman 1985). Although humic substances are often

the major component of DOM in wetlands, a large proportion of wetland DOM is bioavailable, supporting elevated microbial activity (Bano et al. 1997; Mann and Wetzel 1995, 2000). DOC concentrations in surface waters of wetlands often vary seasonally, exhibiting greatest fluctuations during periods of intensive macrophyte growth and microbial activity (Briggs et al. 1993), suggesting the potential importance of macrophyte and microbial sources. Stable isotopic composition of pyrolysis products from a peat bog and adjacent lake have, for example, provided evidence suggesting DOM is formed primarily through microbial production (Kracht and Gleixner 2000).

Stable isotopic composition of organic matter fractions, collected on both seasonal and diel time scales, were determined to assess the impact of biological processing on the isotopic composition of organic matter within the waters of a highly-productive wetland. Results of this study revealed seasonal and diel variations in the isotopic composition of bulk and the proteinaceous component of DOM and the larger organic matter fractions studied. We will demonstrate that differences in the isotopic composition of both the bulk organic matter and individual amino acids reveal that the pathways by which phytoplankton- and bacterial-derived material enters the DOM pool change seasonally and on daily time scales. The bulk stable isotopic composition of DOM indicate that it was derived in part from larger organic matter fractions, as suggested by past studies. Our investigation of the $\delta^{13}C$ of individual amino acids, however, demonstrates the importance of microbial reworking of organic matter and the seasonality of its contribution to the DOM pool not revealed by bulk isotope compositions. Additionally, part of the seasonal variability in the isotopic composition of DOM was attributed to amino acids from central metabolic pathways indicating the seasonal importance of microheterotrophs and terrestrial sources.

Methods

Study site and sampling

This study was conducted in a freshwater tidal wetland within the Jug Bay Wetland Sanctuary (JBWS), an Anne Arundel County park located along the Patuxent River, Maryland. Samples were collected from a site within a marsh channel on the downstream side of an abandoned railroad levee constructed in the late 1890s. Water depth varied from 0.1 at low tide to 1.5 m at high tide. Salinity was generally less than < 0.5 ppt.

Thirty liters of water were collected from the study site at approximately 2 month intervals between December 1998 to November 1999 (6 sampling times), to investigate seasonal changes in organic matter isotopic composition. Each sample was collected within 1 h of slack tide, using an acid washed, distilled water rinsed bucket. Water temperature, dissolved oxygen, and pH were measured using a YSI 95 Dissolved Oxygen probe (Yellowsprings, Ohio) and Orion 230 A meter. Sub-

samples of the whole water were taken for bacterial enumeration, chlorophyll *a*, DOC, total organic carbon (TOC), and ammonium analyses. Samples collected for ammonium analyses and DOC concentration were immediately filtered through a precombusted GF/F filter. The remainder of the sample was passed through a 35 μm mesh then filtered under pressure through a series of three 142 mm diameter filters: 10 μm polycarbonate filter (Nuclepore), a glass fiber filter (GF/F) with nominal pore size of 0.7 μm, and a 0.2 μm polycarbonate filter (Nuclepore). Filtration was conducted under pressures less than 150 mm Hg (~ 3 psi) to avoid cell lysis (Lee 1993). Subsamples of the final 0.2 μm filtrate were taken for bacterial abundance and was always found to contain < 4% of the whole water bacterial abundance. Filters were subsequently rinsed with 30 mL deionized water. Material from the polycarbonate filters was gently scraped and rinsed from filters with deionized water into acid-washed, deionized water-rinsed bottles. Samples were frozen and held at −20 °C until they were lyophilized prior to isotopic analysis. The GF/F filters that retained the POM fraction were folded, placed in Al foil, and stored frozen at −20 °C. Prior to isotopic and elemental analysis filters were completely dried at 50 °C under N_2. The 0.2 μm filtrate, referred to as the DOM fraction, was collected in acid-washed, deionized water-rinsed bottles, immediately frozen, and later lyophilized in precombusted glass freeze drier flasks.

In addition to the seasonal sampling, samples were collected over a diel period on June 3–4, 1999. Whole water samples (30 L) were collected approximately every 3 hours, then filtered and processed in the same manner as described above for the seasonal sampling. Whole water subsamples were collected for bacterial enumeration, ammonium concentration, TOC, DOC, and the carbon isotopic composition of DIC. Additional samples during the diel sampling on June 4, 1999 were collected from the main river channel of the Patuxent River up river from the marsh study site. These samples were processed in the same manner as those for the marsh site and were used for comparison with the wetland samples.

Isotope measurements

For bulk isotope analysis the lyophilized DOM samples were acidified with 6 N HCl (Pierce Chemical), in precombusted glass vials with teflon lined caps, to remove inorganic carbon. Once acidification was complete, the samples were dried under a stream of N_2 in silver boats at room temperature. After removing excess glass fiber filter, POM samples were loaded directly into tin boats for isotopic and elemental analyses. Both the bacterial and 10–35 μm fraction were directly weighed out into tin boats for isotopic analyses. The $\delta^{13}C$ and $\delta^{15}N$ were determined for triplicate samples using a Carlo Erba elemental analyzer (Carlo Erba, Rodano, Italy) interfaced with an isotope ratio mass spectrometer (Finnigan Delta^{Plus}XL) via the Conflo II interface (Finnigan MAT, Bremen, Germany). Isotopic compositions were determined by measuring samples relative to high purity, calibrated, reference gas standards expressed relative to international standard PDB.

The carbon isotopic composition of dissolved inorganic carbon (DIC) was determined for water samples collected and fixed with $HgCl_2$ in gas-tight bottles fol-

lowing Hassan (1982). A subsample from each bottle was placed in one side of an acid washed two-legged reaction vessel opposite a solution of H_3PO_4. Once evacuated, the acid was introduced into the water sample and the resulting CO_2 was purified by cryogenic distillation. Each purified CO_2 sample was introduced via a dual inlet and measured using an isotope ratio mass spectrometer (Finnigan Delta XL-^{plus}).

The carbon isotopic composition of individual amino acids was measured by gas chromatography/combustion-isotope ratio mass spectrometry (GC/C-IRMS) (Silfer et al. 1991). Samples (1–12 mg) were weighed into precombusted hydrolysis tubes and hydrolyzed, under an atmosphere of N_2, in 1 mL of 6 N constant-boiling HCl (Pierce Chemical) for 20 h at 110 °C. Hydrolyzed samples were then derivatized to their isopropyl-N-TFA derivatives (Engel and Hare 1985; Silfer et al. 1991). A standard mixture of 12 amino acids (Sigma-Aldrich, St. Louis, Mo., USA) was derivatized and analyzed with each batch of samples. Hydrolysates were dried under a stream of N_2 at 100 °C, esterified with 1 mL of anhydrous acidified isopropanol (1 h at 110 °C), and acylated by 0.5 mL trifluoroacetic anhydride (TFAA) in 0.5 mL dichloromethane (10 min. at 110 °C). Derivatized samples were analyzed on a Varian 3400 gas chromatograph (splitless injection; Varian Inc. Palo Alto, CA, USA) coupled to an isotope ratio mass spectrometer (Finnigan Delta XL) via a combustion interface. Samples (1 μL) were injected onto an HP Ultra-1 column (0.32 mm ID, 50 m) with an injector temperature of 220 °C. Samples were analyzed in triplicate with a corresponding standard mixture of amino acids. The measured carbon isotopic compositions were corrected for carbon added during derivatization, using a mixture of standard amino acids of known isotopic composition. We were able to measure ten amino acids: serine (Ser), alanine (Ala), threonine (Thr), aspartic acid (Asp), glutamic acid (Glu), proline (Pro), isoleucine (Ile), phenylalanine (Phe), valine (Val), and leucine (Leu). Standard deviation for average carbon isotopic values of individual amino acids in the triplicate samples analyzed was typically 0.4‰ and ranged from < 0.01 to 1.6‰.

Other analyses

Water samples were analyzed for total and dissolved organic carbon content by Accutest, Dayton, NJ using high-temperature combustion (EPA method 415.1 SW8469060M). Chlorophyll *a* concentrations were estimated spectrophotometrically from nitrocellulose filters (0.45 μm) extracted for 24 h in 90% acetone (Jeffrey and Humphrey 1975; Lorenzen 1967). Ammonium concentration was determined on filtered samples using the phenol-hypochlorite method (Solorzano 1969). Bacterial abundance was determined on whole water samples preserved with 5% (final concentration) formalin. Enumeration of bacteria was conducted using epifluorescent microscopy of DAPI stained slides prepared within 3 days of collection (Porter and Feig 1980).

Table 1. Average water temperature (°C), dissolved oxygen (DO), pH, chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), particulate organic carbon (POC), and ammonium (NH_4^+) concentrations at the South Marsh site during sampling for organic matter fractions. Values are all reported as mean of 3 replicate samples taken around noon, except for June 3–4 and July 22–23, 1999.

Date	Water temp.	DO (mg L^{-1})	pH	Chl (μg L^{-1})	DOC (μM)	POC (μM)	NH_4^+ (μM)
Dec	12.1	7.8	6.5	ND	ND	ND	11.3
Mar	15.0	7.2	6.8	ND	258	ND	9.0
Jun*	22.9 ± 3.2	4.2 ± 2.5	7.2	52 ± 21	392 ± 31	150 ± 59	14.6 ± 6.5
Jun	27.2	7.3 ± 1.7	7.0	55 ± 5	400	ND	20.2
Jul*	29.1 ± 1.1	8.0	7.5	47 ± 12	ND	ND	ND
Sep	25.4	4.8	7.2	110 ± 3	408	100	2.3
Nov	6.1	8.6	7.0	21 ± 4	300	117	3.43

* Mean ± standard deviation of 8 samples taken over 24 hour period on June 3–4, 1999 and July 22–23, 1999.

Results

Seasonal changes in the isotopic composition of organic matter

Seasonal water column parameters

Water temperature ranged from 6.1 to 29.1 °C for the different sample dates, with lowest temperatures recorded in November, and highest recorded in July (Table 1). Dissolved oxygen (DO) concentrations ranged from 4.18 to 8.58 mg O_2 L^{-1} for dates sampled (Table 1), and ranged from 1.3 to 8.2 mg O_2 L^{-1} over the 24 hour period sampled June 3rd. Total organic carbon (TOC) concentrations tended to be highest during the more productive times of the year. Throughout the year, dissolved organic carbon (DOC) represented about 70–80% of the TOC with concentrations ranging from 300 to 408 μM. Highest concentrations of DOC occurred in September and lowest concentrations in November and March. Particulate organic carbon (POC) ranged from 100 to 150 μM with highest concentrations measured in June. Chlorophyll *a* concentrations were highest at the end of the summer (September; 110 μg L^{-1}) and lowest in the fall (November; 21 μg L^{-1}). Ammonium concentrations ranged from 2.3 to 20.2 μM at the study site for the dates sampled with similar ranges having been measured during diel sampling in the summer months (Table 1).

Seasonal change in bulk ^{13}C and ^{15}N content of organic matter

The bulk $\delta^{13}C$ composition of DOM over the 11 month sampling period of the study ranged from −27.2 to −25.4‰ and was generally depleted in summer and fall relative to the spring (Table 2). Similarly, the bacterial fraction ranged from −27.0 to −25.4‰. The DOM and bacterial fractions were isotopically similar throughout the seasons and were usually about 2–4‰ enriched in ^{13}C relative to POM. The

Table 2. Seasonal values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition for dissolved organic matter (DOM), bacterial fraction 0.2 to 0.7 μm (BF), particulate organic matter 0.7 to 10 μm (POM), and 10–35 μm organic matter fractions collected at South Marsh December 1998 through November 1999. Average values (n = 3) ± 1 standard deviation.

$\delta^{13}C$ Date	DOM	BF	POM	10–35 mm
Dec	−26.7 ± 0.4		−35.5 ± 0.2	
Mar	−25.4 ± 0.4	−25.1 ± 0.4	−27.8 ± 0.2	−24.9 ± 0.4
*Jun	−25.9 ± 1.6		−27.9 ± 0.9	
Jul	−26.6 ± 0.3	−27.0 ± 0.4	−30.0 ± 0.4	−28.7 ± 0.1
Sep	−26.2 ± 0.3	−26.8 ± 0.0	−30.0 ± 0.4	−29.9 ± 0.0
Nov	−27.2 ± 0.2	−26.4 ± 0.1	−29.0 ± 0.2	−26.4 ± 0.1
$\delta^{15}N$				
Dec			11.0 ± 0.4	
Mar		7.1 ± 0.5	6.8 ± 0.5	6.1 ± 0.7
*Jun			8.2 ± 2.2	
Jul		6.5 ± 0.8	9.9 ± 0.2	7.3 ± 0.4
Sep		6.4 ± 0.3	7.9 ± 0.7	7.5 ± 0.2
Nov		5.3 ± 0.2	8.7 ± 0.7	4.5 ± 0.1

POM became enriched in ^{13}C during the spring when both primary production rates and temperatures increased.

The POM fraction was usually the most enriched fraction, with $\delta^{15}N$ values ranging from 6.8 to 11.0‰, whereas both the bacterial fraction and the 10–35 μm fraction were more depleted in ^{15}N, with values ranging from 5.3 to 7.1‰ and 4.5 to 7.3‰, respectively. In March all three particulate fractions had similar $\delta^{15}N$ composition but differed in $\delta^{13}C$ composition. In July, the bacterial fraction was more depleted in ^{15}N relative to the POM. The POM was most enriched in ^{15}N in December and became more enriched again during the summer months when inorganic nutrients were at their lowest concentrations relative to the other sampling periods.

Seasonal change in the $\delta^{13}C$ composition of amino acids

The pattern of the isotopic composition of amino acids in each organic matter fraction was similar among the fractions. Greater isotopic variability, however, occurred within the DOM fraction (Figure 1). The stable carbon isotopic composition of the hydrolyzable amino acids in DOM and the bacterial fraction were more enriched relative to the other fractions. The $\delta^{13}C$ of 10 amino acids analyzed ranged from −36.7 to −1.9‰ in the DOM, −38.4 to −9.8‰ in the bacterial fraction, −42.7 to −10.7‰ in the POM, and −38.2 to −16.7‰ in the 10–35 μm fraction (Table 3). For each fraction collected, Ser and Ala were typically the most enriched in ^{13}C. Asp,

Glu, and Pro were similar in isotopic composition, and Ile, Phe, Val, and Leu tended to be the most depleted in ^{13}C relative to the bulk $\delta^{13}C$ signature (Figure 1).

To evaluate the relationships among different organic matter fractions, least-squares regressions of all amino acids in individual samples were determined assuming that some dependency of smaller fractions on larger organic matter fractions existed. The compositions of POM and the bacterial fractions were found to be similar based upon the linear regression of $\delta^{13}C$ of amino acids for each fraction ($r^2 = 0.75$; $p = 0.0001$; $df = 27$). Linear regression of the $\delta^{13}C$ composition of individual amino acids in DOM versus the bacterial fraction, although significant, indicated that variation in the bacterial fraction could only explain 40% ($r^2 = 0.40$) of the variability in the amino acid composition of DOM (Figure 2a). The same comparison between the DOM and POM fractions was also significant ($p < 0.003$), but the POM fraction could only explain 30% ($r^2 = 0.30$) of the variability in the ^{13}C composition of the DOM pool (Figure 2b). Based upon these comparisons, amino acids of DOM were isotopically enriched and more variable relative to the larger organic matter fractions throughout the year.

The $\delta^{13}C$ of amino acids in the DOM fraction were more positive than amino acids in the particulate fractions during each season (Figure 3). The degree of similarity among the organic matter fractions varied among seasons. In March and November only amino acids Glu, Asp, Ala, Ser, and Leu were measured within the DOM fraction. In March, the $\delta^{13}C$ of Ser became more positive along with the bulk composition of each fraction (Table 2). During June and September the $\delta^{13}C$ of amino acids in all fractions were similar to one another in terms of relative carbon isotope composition of individual amino acids (Figure 3). In June amino acids in DOM had more positive isotopic compositions relative to the other fractions. In general, the $\delta^{13}C$ of amino acids became progressively more negative with increasing organic matter fraction size (Figure 3). During September these isotopic shifts were less evident, and the DOM was more isotopically similar to the larger fractions. In November, Asp and Glu were the most abundant amino acids and had the highest $\delta^{13}C$ of all the amino acids in the DOM fraction.

Diel changes in the isotopic composition of organic matter

Diel water column parameters

During the 24-hour sampling period in 1999, highest water levels at the site occurred at 21:00 on June 3 and 09:10 on June 4, and lowest water levels occurred at 15:49 on June 3 and 03:55 June 4 (Figure 4c). Chlorophyll *a* , ammonium, and DOC concentrations ranged from 26.7 to 74.1 μg L^{-1}, 5.3 to 22.5 μM, and 350 to 433 μM, respectively, over the 24 h period (Figure 4). A peak in DOC concentrations was detected just prior to sunset on June 3 following elevated DO concentrations (Figure 4). Bacterioplankton abundance increased during the course of the night and ranged from 1.5×10^6 to 6.7×10^6 cells mL^{-1} over the 24 hour period sampled.

The $\delta^{13}C$ of POM remained relatively constant during daylight hours and became slightly more positive during the night. More dramatic changes in $\delta^{13}C$ were

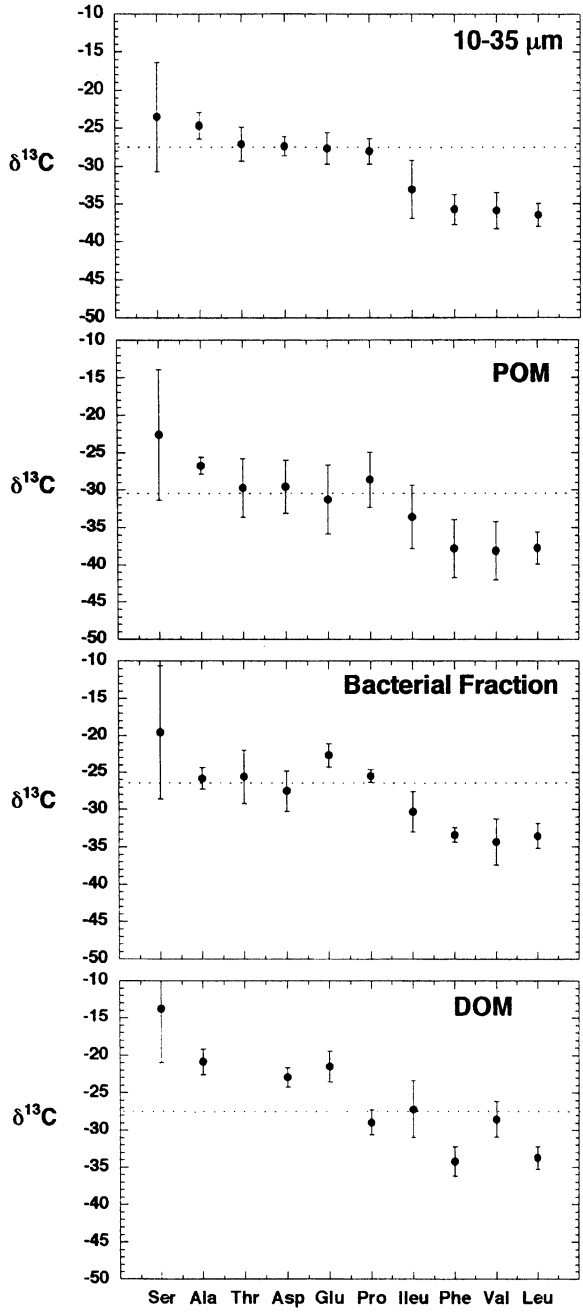

Figure 1. Plot of the annual average $\delta^{13}C$ composition of individual amino acids in the (a) 10–35 μm organic matter fraction, (b) particulate organic matter (POM), (c) 0.7 to 0.2 μm bacterial fraction, and dissolved organic matter (DOM) fraction. Error bars represent one standard deviation of the mean of values of samples from December, March, June, July, and November. Dotted line is the mean bulk $\delta^{13}C$ value of the sample.

Table 3. Stable carbon isotopic composition ($\delta^{13}C$) of 10 amino acids in dissolved organic matter (DOM), bacterial fraction (0.2–0.7 μm), particulate organic matter (POM), and the 10–35 μm organic matter fraction collected at the study site during December 1998 through November 1999. Additionally the $\delta^{13}C$ composition of the total organic carbon (OC) for each fraction is given at the bottom of the table. All values are reported as mean ± 1 standard deviation (std) for 3 replicate analyses. Samples where a listed amino acid was either undetected or not distinguishable from other components in the sample is given as ND. Samples that were not available because they were not collected or were not able to be analyzed are given as NA.

	Dec		Mar		Jun		Sep		Nov	
DOM	$\delta^{13}C$	Std	$\delta^{13}C$	Std	$\delta^{13}C$	Std	$\delta^{13}C$	Std	$\delta^{13}C$	Std
Ser	NA		−10.8	0.2	−1.87	0.0	−28.7	0.2	ND	
Ala	NA		−16.6		−22.5	0.4	−23.4	0.1	ND	
Thr	NA		ND		ND		ND		ND	
Asp	NA		−20.0	0.5	−21.7	0.4	−28.8	0.0	−21.0	0.3
Glu	NA		ND		−20.7	0.3	−22.7		−21.0	0.6
Pro	NA		−36.7	0.6	−23.0	1.8	−29.3	0.5	−26.7	4.0
Ileu	NA		ND		−23.6	0.2	−30.8	0.4	ND	
Phe	NA		ND		−32.9	0.6	−35.6	0.2	ND	
Val	NA		ND		−26.4	1.9	−30.7	0.2	ND	
Leu	NA		−34.7	1.7	−34.0	0.2	−33.6	0.5	−32.7	1.1
OC	−26.7	0.4	−25.4	0.4	−26.5	0.4	−26.2	0.3	−27.2	0.2
Bacterial Fraction										
Ser	NA		−9.77	0.0	−16.4	0.2	−21.1	0.0	−31.1	0.2
Ala	NA		−24.6	0.0	−27.1	0.1	−27.1	0.7	−24.6	0.5
Thr	NA		−20.4	0.4	−26.3	0.2	−26.9	0.2	−28.7	0.3
Asp	NA		−26.2	0.8	−31.4	0.4	−25.3	0.7	−27.1	0.3
Glu	NA		−20.8	0.6	−22.0	0.7	−23.9	0.2	−24.1	0.3
Pro	NA		−26.4	0.8	−24.3	0.8	−25.6	0.3	−25.7	0.5
Ileu	NA		−27.0	0.5	−30.6	0.7	−30.0	1.1	−33.6	0.3
Phe	NA		−32.0	0.1	−33.6	0.4	−34.2	0.2	−33.8	0.6
Val	NA		−30.9	0.6	−33.5	0.3	−38.4	0.1	−34.6	0.5
Leu	NA		−31.1	0.2	−34.0	0.2	−34.2	0.1	−34.9	0.4
OC	NA		−25.1	0.3	−26.6	0.5	−26.9	0.1	−26.4	0.1
POM										
Ser	−23.1	1.3	−10.7	0.0	−22.0	0.5	−21.8	0.2	−35.3	0.9
Ala	−25.4	0.1	−25.7	0.1	−27.7	0.4	−27.8	0.7	−27.1	0.4
Thr	−31.0	0.1	−32.6	0.2	−23.6	0.1	−28.3	0.3	−33.0	0.7
Asp	−34.3	0.2	−25.0	0.1	−30.2	0.1	−27.4	0.1	−30.9	0.4
Glu	−36.2	0.5	−26.9	0.0	−29.3	0.1	−27.6	0.8	−36.2	1.2
Pro	−33.9	0.8	−25.7	0.2	−28.8	0.2	−24.7	0.7	−30.0	0.8
Ileu	−34.1	0.3	−28.5	0.4	−31.5	0.8	−33.8	1.0	−40.0	0.2
Phe	−42.7	0.6	−32.5	0.1	−38.6	0.2	−35.7	0.4	−39.5	0.8
Val	−37.2	0.6	−34.7	0.3	−34.6	0.9	−43.8	0.5	−40.2	0.5
Leu	ND		−36.1	0.2	−37.6	0.2	−36.4	0.1	−40.8	0.4
OC	−35.5	0.2	−27.8	0.2	−30.0	0.4	−30.0	0.4	−29.0	0.2

Table 3. Continued

DOM	Dec		Mar		Jun		Sep		Nov	
	$\delta^{13}C$	Std	$\delta^{13}C$	Std	$\delta^{13}C$	Std	$\delta^{13}C$	Std	$\delta^{13}C$	Std
10–35 μm Fraction										
Ser	NA		−16.67	0.3	−18.03	0.3	−29.36	0.2	−30.10	0.3
Ala	NA		−23.68	0.2	ND		−27.12	0.6	−23.33	0.1
Thr	NA		−24.56	0.2	−26.08	0.2	−27.97	0.1	−29.67	0.3
Asp	NA		−27.96	0.2	−27.87	0.4	−28.17	0.6	−25.44	0.5
Glu	NA		−25.97	0.1	−29.94	0.3	ND		−26.99	0.7
Pro	NA		−28.33	0.7	−28.91	0.2	−29.33	0.6	−25.52	0.5
Ileu	NA		−27.85	0.4	−32.42	0.2	−35.87	0.2	−36.08	0.5
Phe	NA		−34.03	0.5	−38.16	0.8	−36.58	0.0	−34.19	0.3
Val	NA		−35.02	0.9	ND		−33.87	0.4	−35.20	0.2
Leu	NA		−34.93	0.1	−38.00	0.3	−37.54	0.1	−35.42	0.4
OC	NA		−24.87	0.4	−28.98	0.1	−29.90	0.0	−26.43	0.1

measured in DOM over this same period, with $\delta^{13}C$ values ranging from −28.2 to −22.7‰ and the most enriched DOM measured around sunset (Figure 5). A similar diel pattern was detected in the $\delta^{13}C$ composition of the DIC. The $\delta^{15}N$ of POM also dramatically changed over this diel in an opposite trend to the concentration of ammonium in the water column (Figure 5b). The $\delta^{15}N$ of POM ranged from 5.4 to 11.4‰, and the more deplete values coincided with higher ammonium concentrations.

We analyzed river water from the main channel immediately after high tide, when water was beginning to recede from the marsh surface to determine the importance of incoming river water on diel change in organic matter isotopic composition. Samples of each organic matter fraction were collected from the river concurrently with the last sample of the diel sample set from the marsh site. The $\delta^{13}C$ of POM from the riverine site was −28.8 ± 0.2 and the $\delta^{13}C$ of amino acids from this fraction were similar to the POM from the study site collected at the same time (Table 4; least squares regression of two data sets slope = 1.0 ± 0.3, $r^2 = 0.86$; p = 0.0001). This similarity indicated that direct tidal input from the river was not an important factor in determining organic matter composition at our study site. This comparison, however, does not take into account the potential influence tidal flushing of surface sediments, such as resuspension. These processes, which were beyond the scope of this study, may have some effect on organic matter fluxes in this system.

Diel change in the $\delta^{13}C$ composition of amino acids

The $\delta^{13}C$ composition of the hydrolyzable amino acids in the POM and DOM fractions was measured on samples collected for bulk isotope composition. Distribution of the isotopic composition of each of ten amino acids analyzed in both the DOM and POM was fairly similar to the distribution found in the seasonal samples

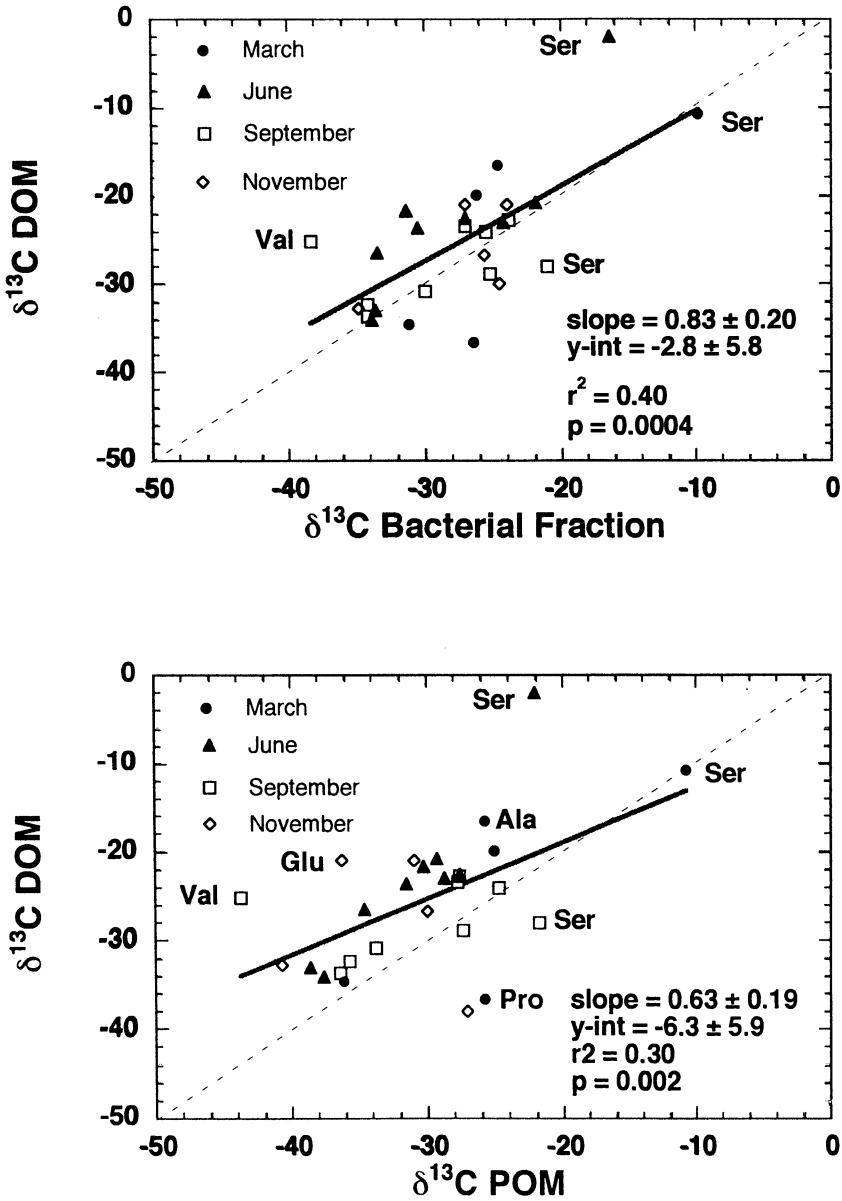

Figure 2. Least-squares regression of the $\delta^{13}C$ composition of individual amino acids in the dissolved organic matter (DOM) versus the (a) bacterial fraction (0.2 to 0.7 μm) and (b) particulate organic matter (POM) in samples from March, June, September, and November.

of DOM and POM (Tables 3 and 4). The isotopic composition of most of the amino acids in the POM became progressively enriched in ^{13}C from early or late afternoon into the early morning hours. The enrichment was then followed by depletion in the ^{13}C content prior to sunrise. A few amino acids, however, were found to

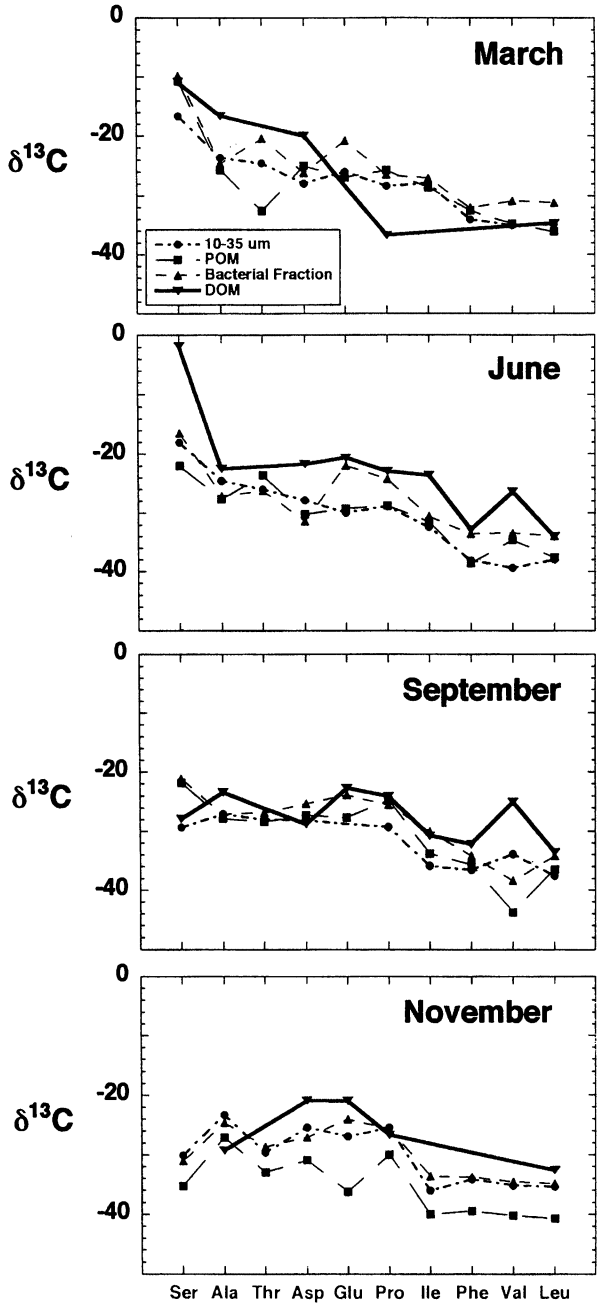

Figure 3. The distribution of the $\delta^{13}C$ composition of individual amino acids in the 10 to 35 μm organic matter fraction, particulate organic matter (POM), bacterial fraction (0.2 to 0.7 μm), and dissolved organic matter (DOM) from samples collected during (a) March, (b) June, (c) September, and (d) November.

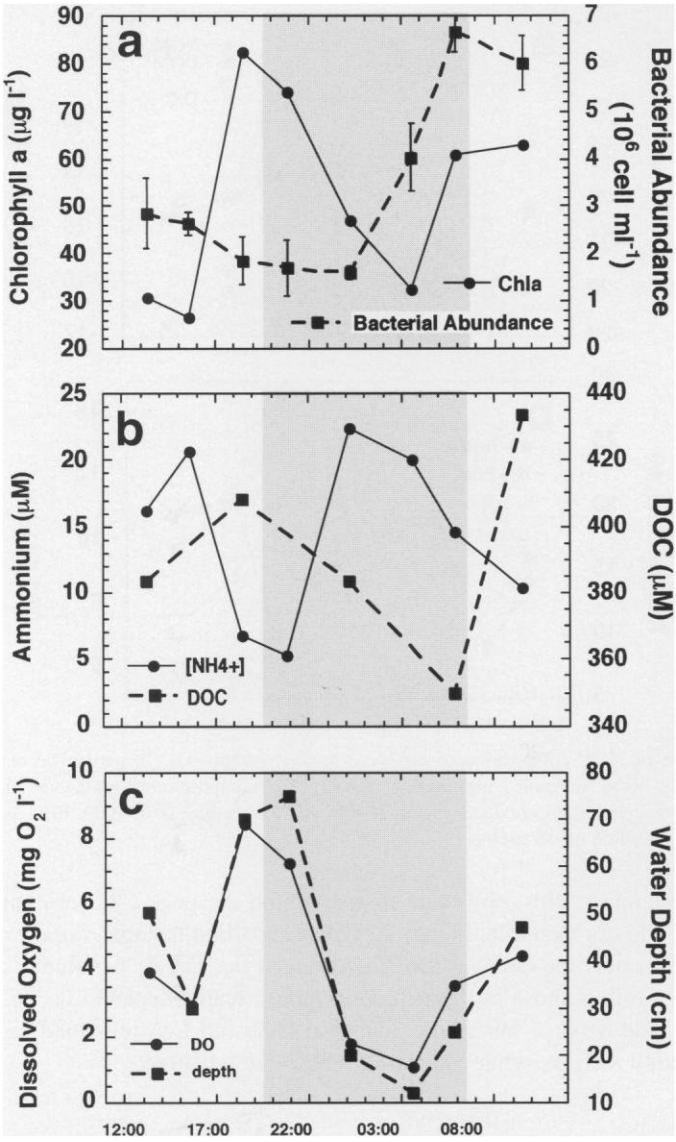

Figure 4. The change in (a) chlorophyll *a* concentration and bacterial abundance, (b) ammonium and dissolved organic carbon (DOC) concentration, and (c) dissolved oxygen concentration and water depth over a 24-h period at the study site during June 3–4, 1999. Error bars represent one standard deviation of the mean.

change substantially over the diel. For example, the $\delta^{13}C$ of Val and Ala changed the most dramatically in the POM fraction over the diel period (Figure 6).

The $\delta^{13}C$ of Glu, Pro, and Ile of the DOM fraction increased overnight and mirrored the enrichment of the bulk $\delta^{13}C$ of the DOM pool. The ^{13}C enrichment of

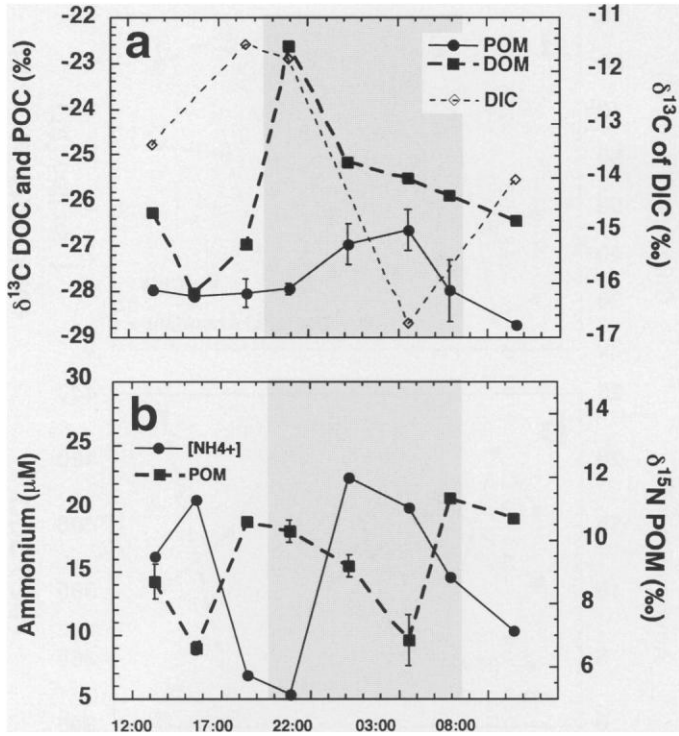

Figure 5. The (a) $\delta^{13}C$ composition of dissolved organic carbon (DOC), particulate organic carbon (POC), and dissolved inorganic carbon (DIC), and (b) $\delta^{15}N$ composition of particulate organic matter (POM) and ammonium concentration over a 24 h period during June 3–4, 1999. Error bars represent one standard deviation of the mean.

most of the amino acids, however, lagged behind the peak ^{13}C enrichment of the bulk DOM (Ile, for example; Figure 7). Greatest carbon isotopic variation of amino acids in DOM over the diel was found in Asp and Ile. The $\delta^{13}C$ value of Asp, however, did not follow those of the bulk DOM and became depleted in ^{13}C overnight (Figure 7). The $\delta^{13}C$ of the amino acids Val, Ser, and Leu remained fairly steady overnight until sunrise, when values became more negative.

Discussion

Isotopic indicators of seasonal changes in DOM sources

The seasonal pattern in the $\delta^{13}C$ composition of bulk organic matter and individual amino acids and how they compare among organic matter fractions is a useful indicator of change in DOM source. The bulk isotopic composition and the similarity in the seasonal change in this composition among the organic matter fractions sug-

Table 4. Average $\delta^{13}C$ value for hydrolyzable amino acids in particulate organic matter (POM) and dissolved organic matter (DOM) collected at study site for each sample collected during June 3–4, 1999 as well as samples collected from the adjacent river site (r). Values are reported as an average of 3 replicate analyses with 1 standard deviation from the mean (sd). The bulk $\delta^{13}C$ composition of each sample (OC) is also provided as an average of 3 replicate analyses with 1 sd except for the DOM samples which were not analyzed in triplicate due to sample size constraints. Amino acids either undetected or not distinguishable from other components in the sample are given as ND. Samples not available because they were not collected or were not able to be analyzed are given as NA.

	13:20 POM		16:35 POM		19:28 POM		21:52 POM		01:13 POM		04:36 POM		06:55 POM		10:30 POM		10:30 POM (r)	
	$\delta^{13}C$	sd	$\delta^{13}C$	sd	$\delta^{13}C$	sd	$\delta^{13}C$	sd	$\delta^{13}C$	sd	$\delta^{13}C$	sd	$\delta^{13}C$	sd	$\delta^{13}C$	sd	$\delta^{13}C$	sd
Ser	−22.1	0.2	−19.7	0.3			−20.3	0.1	−17.8	0.1	−18.5	0.1	−18.2	0.2	−18.2	0.0	−16.0	0.0
Ala	−29.7		−29.1	0.4	−21.9	0.3	−23.5	0.2	−24.9	0.9	−24.4	0.7	−27.9	0.3	−29.8	0.3	−27.6	0.2
Thr	−24.7	0.1	−26.3	0.2	−26.0	0.2	−24.5	0.1	−23.7	0.1	−26.8	0.2	−25.8	0.2	−25.9	0.0	−23.1	0.1
Asp	−26.4	0.4	−27.4	0.4	−25.7	0.1	−26.5	0.2	−24.7	0.4	−22.3	0.9	−28.8	0.1	−30.4	0.3	−27.9	0.2
Glu	−27.7	0.1	−27.0	0.9	−27.0	0.1	−25.9	0.1	−26.3	0.2	−24.7	0.7	−27.6	0.3	−26.8	0.5	−27.7	0.1
Pro	−25.4	0.3	−27.7	0.4	−26.6	0.4	−25.2	0.1	−23.5	0.2	−26.6	0.0	−25.6	0.3	−25.9	0.6	−29.3	0.3
Ileu	−29.0	1.0	−30.9	0.9	−29.6	0.2	−28.8	0.2	−29.9	0.5	−27.3	0.5	−28.2	0.5	−27.6	0.3	−30.7	0.6
Phe	−36.1	0.2	−35.0	0.3	−35.1	0.1	−35.6	0.2	−34.7	0.3	−33.8	0.7	−35.1	0.0	−37.1	0.2	−36.3	0.2
Val	−35.5	0.6	−33.0	0.9	−31.9	0.4	−33.8	0.5	−33.2	0.2	−34.9	0.8	−32.3	0.3	−32.7	0.0	−34.0	0.4
Leu	−35.7	0.1	−35.8	0.4	−36.2	0.2	−34.5	0.2	−34.1	0.2	−33.6	0.5	−35.6	0.3	−36.5	0.1	−37.0	0.3
OC	−28.0	0.1	−28.1	0.0	−28.0	0.3	−28.0	0.1	−27.2	0.5	−27.0	0.5	−28.5	0.7	−28.8	0.0	−28.8	0.2

	DOM $\delta^{13}C$	sd	DOM $\delta^{13}C$	sd	DOM $\delta^{13}C$	sd	DOM $\delta^{13}C$	sd	DOM $\delta^{13}C$	sd	DOM $\delta^{13}C$	sd	DOM $\delta^{13}C$	sd	DOM $\delta^{13}C$	sd	DOM (r) $\delta^{13}C$	sd
Ser	NA		−15.5	0.3	NA		−15.9	0.3	NA		−15.7	0.0	NA		−18.2	0.4	NA	
Ala	NA		−25.4	0.5	NA		−25.8	0.7	NA		−22.8	0.0	NA		−24.4	0.4	NA	
Thr	NA		ND		NA		ND		NA		ND		NA		ND		NA	
Asp	NA		−23.5	0.5	NA		−23.9	0.0	NA		−25.3	0.1	NA		−29.2	0.4	NA	
Glu	NA		−20.3	0.1	NA		−19.5	0.2	NA		−18.6	0.1	NA		−21.3	0.3	NA	
Pro	NA		−23.4	0.3	NA		−20.7	0.6	NA		−19.6	0.2	NA		−21.0	0.1	NA	
Ileu	NA		−31.1	0.8	NA		−28.2	1.1	NA		−25.8	0.0	NA		−30.6	0.4	NA	
Phe	NA		−30.8	0.8	NA		−31.0	0.3	NA		−28.3	0.6	NA		−31.3	0.3	NA	
Val	NA		−31.1	0.2	NA		−31.5	0.6	NA		−31.1	0.3	NA		−33.5	0.9	NA	
Leu	NA		−30.9	1.0	NA		−32.5	0.6	NA		−31.4	0.0	NA		−34.7	0.6	NA	
OC	−26.3		−28.1		−27.0		−22.6		−25.2		−25.5		−25.9		−26.4		−26.8	

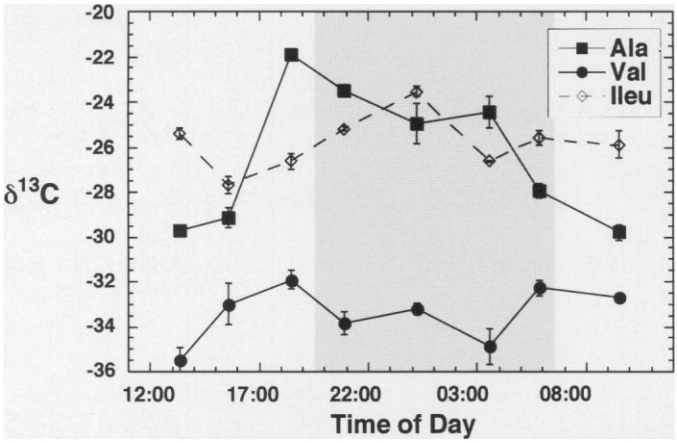

Figure 6. The change in the $\delta^{13}C$ composition of alanine (ala), valine (val), and isoleucine (ile) in particulate organic matter (POM) collected from the study site over a 24 h period during June 3–4, 1999.

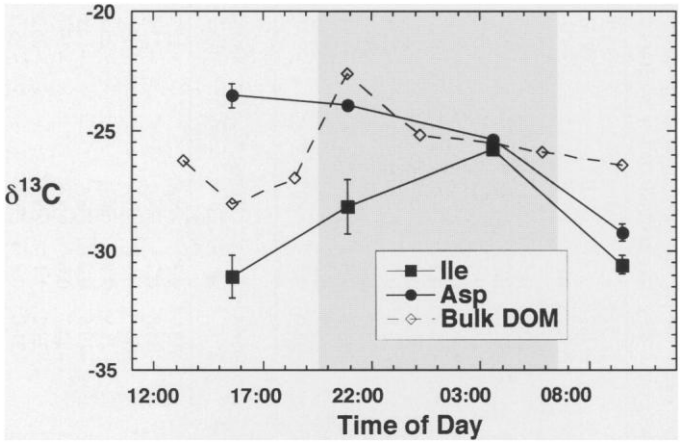

Figure 7. The change in the $\delta^{13}C$ composition of isoleucine (ile) and aspartic acid (asp) in dissolved organic matter (DOM), and bulk $\delta^{13}C$ of DOM collected from the study site over a 24 h period during June 3–4, 1999.

gests DOM is in part derived from the larger organic matter fractions in the marsh. The concept that DOM is derived from the breakdown of larger fractions of organic matter (Amon and Benner 1996) is supported in this study by the more positive $\delta^{13}C$ of the bulk DOM and its amino acids relative to POM, and the similarity between DOM and the bacterial fractions. Each fraction, including the DOM, was more enriched in ^{13}C during spring. A rapid increase in phytoplankton production without substantial organic matter mineralization, due to the low temperatures, likely caused a draw down and ^{13}C enrichment of the DIC in March. This enrichment was detected within all the organic matter fractions. Primary productivity in the water column, as indicated by chlorophyll concentrations, increased throughout

the summer and peaked in September. Each fraction became more depleted throughout the summer, indicating that some component of the phytoplankton biomass production made its way into the smaller organic matter fractions, including the DOM. Similarity in the $\delta^{13}C$ composition of amino acids among the organic matter fractions increased through the summer, with the greatest similarity detected in September when chlorophyll concentration and water temperatures were high. This suggests that planktonic contributions to DOM were likely greatest during the more productive times of the year.

Isotopic composition of organic matter has suggested the importance of microbial sources of organic matter in wetlands (Kracht and Gleixner 2000). The importance of microbial sources to the composition of the organic matter fractions investigated in this study changed seasonally, as evidenced by the change in stable isotopic composition of N. The bacterial fraction became more depleted in ^{15}N from summer into the fall. Microbial utilization of inorganic N and production of biomass can lead to depletion in the ^{15}N of organic matter due to fractionation during microbial growth (Hoch et al. 1992, 1994). Greater fractionation associated with the microbial utilization of ammonium will occur at times when concentrations of ammonium are highest. The diel changes in the $\delta^{15}N$ of POM in June were consistent with this, where the POM was more depleted in ^{15}N following peaks in ammonium concentration. These patterns in $\delta^{15}N$ suggest that the composition of POM in this marsh was largely the result of phytoplankton and bacterial biomass in June.

The seasonal variability in the $\delta^{13}C$ of Val in DOM, provided more detailed information regarding the microbial sources of DOM, and indicated that the importance of bacterial versus algal or plant sources of DOM changed seasonally in this marsh. Unique amino acid biosynthetic pathways among different groups of organisms, such as eukaryotic phytoplankton versus bacteria, can be used to decipher the importance of these organisms as sources of organic matter. Different pathways employing unique enzymes in plants and other eukaryotes (acetolactetate mutase) versus bacteria (acetohydroxy acid synthase), for example, are used to catalyze the first step in the biosynthesis of Val (Gottschalk 1988; Voet and Voet 1990). Val in the DOM became progressively depleted throughout the summer months and into the fall, indicating that bacterial synthesis of this amino acid was important during the warmer months.

The comparison of the $\delta^{13}C$ of amino acids in each organic matter fraction suggests seasonal change in the importance of phytoplankton versus macrophytes and terrestrial sources of organic matter in this freshwater marsh. The POM and bacterial fractions were related throughout the year. A large component of the bacterial fraction may be derived from POM (least squares regression; $r^2 = 0.75$, $p < 0.0001$), possibly through the utilization of phytoplankton-derived organic matter by bacteria collected in the 0.2 to 0.7 μm fraction. The contribution of live bacteria to the POM fraction, however, cannot be ruled out in this comparison given the technique used. Deviations from the 1:1 line suggest that some alternative source, as well as degradation products, contributed to these organic matter fractions throughout the year. The isotopic compositions of central metabolic amino acids (Glu, Asp, Pro, Gly, and Ser) were the primary source of variation in the isotopic composition of

Table 5. Least squares regression analysis parameters for the $\delta^{13}C$ of individual amino acids in dissolved organic matter (DOM) versus other organic matter fractions given as independent variables. Least squares regressions for DOM versus bacterial fraction and POM are based upon all individual comparisons for each season measured, and wild rice comparisons are based upon annual average values.

Independent Variable	r^2	p-value	df
Bacterial Fraction	0.40	0.0004	26
POM	0.30	0.0028	27
Wild rice (fresh)*	0.18	0.2950	7
Wild rice (rotted)*	0.69	0.0110	7

*Wild rice $\delta^{13}C$ amino acid data from Fogel and Tuross (1999).

POM and the bacterial fraction. Although the amino acid data support the bulk $\delta^{13}C$ results, suggesting that DOM is more closely related to the bacterial fraction, substantial variability exists in the least squares analyses of DOM versus either POM or the bacterial fraction (Table 5). The outlying amino acids contributing to this variability were Ala, Ser, Pro, Asp, and Glu, common amino acids indicating potential inputs from zooplankton, macrophytes or terrestrial organic matter.

Comparison of compound specific amino acid data from fresh and microbially-decomposed plants (see Fogel and Tuross (1999)) with that of the DOM from the study site reveals that on a seasonal and diel basis, macrophytes are another important source to the DOM pool in freshwater marshes (Table 5). Based on biochemical information, Fogel and Tuross found that decomposed plant material from the sediments of the marsh was composed of some of the original plant proteins, but microbial overprinting was substantial. Isotopic signals from fresh wild rice material were important to the DOM pool at the end of a warm summer day during our diel sampling (16:35), but decreased throughout the night. Photosynthetic input from POM phytoplankton and marsh plants, therefore, influenced the DOM isotopic signals during daylight hours (Table 6). There was, however, almost no relationship between the amino acid isotopic compositions of fresh marsh plants and the yearly-averaged values of amino acids from water column DOM (Table 5). Yearly-averaged values of amino acids from DOM were more related to the decomposed wild rice, but by no means more so than amino acids found in the water column's bacterial and POM fractions.

Seasonal importance of microbial reworking to the isotopic composition of organic matter

Microbial reworking, or the contribution of new amino acids via microbial synthesis from other compounds, is an important and seasonally variable process affecting organic matter composition in the freshwater wetland studied. Microbial cycling of fresh organic matter results in substantial changes in the isotopic composition of amino acids (Fogel and Tuross 1999; Keil and Fogel 2001) and other compounds (Kracht and Gleixner 2000). The pattern of isotopic composition of amino acids in POM at the study site during November was more variable then

Table 6. Least squares regression analysis parameters for the $\delta^{13}C$ of individual amino acids from dissolved organic matter (DOM) collected over a diel period on June 3–4, 1999 versus other organic matter sources given as independent variables.

Independent variable	r^2	p-value	df	r^2	p-value	df
		04:36			**10:30**	
POM	0.64	0.0054	9	0.69	0.0030	9
Wild rice (fresh)	0.42	0.0833	7	0.43	0.0762	7
Wild rice (rotted)	0.42	0.0826	7	0.45	0.0667	7
		16:35			**21:52**	
POM	0.89	0.0002	8	0.81	0.0004	9
Wild rice (fresh)	0.67	0.0131	7	0.52	0.0431	7
Wild rice (rotted)	0.76	0.0050	7	0.59	0.0263	7

*Wild rice $\delta^{13}C$ amino acid data from Fogel and Tuross (1999).

other times of the year, providing evidence of increased microbial reworking of POM in the fall. Although the bulk isotopic composition of DOM did not change substantially over the course of the year, the $\delta^{13}C$ of individual amino acids did vary, demonstrating the important role of microbial reworking in determining DOM composition. The annual average $\delta^{13}C$ of each individual amino acid in DOM was more variable than those of the larger organic matter fractions. Additionally, microbial degradation influenced the composition of DOM during the year as indicated by the $\delta^{13}C$ of amino acids in DOM, which did not conform to the overall pattern found in the particulates. This "rearrangement" in the isotopic composition of amino acids is consistent with the isotopic changes in amino acids detected in degraded plant tissues, caused by microbial reworking of the fresh plant material (Fogel and Tuross 1999). In fact, nearly 70% of the variation in the annual average amino acid data from DOM may be explained by the isotopic signature of degraded, not fresh, wild rice, one of the dominant vascular plants in the study site (Table 5). The stable isotopic composition of the hydrolysable amino acids in DOM may, therefore, serve as an indicator for microbial sources of the proteinaceous component of DOM.

Seasonal change in the isotopic composition of amino acids provided an assessment of the relative importance of fresh versus degraded sources of DOM at different times of the year. The bacterial fraction, for example, was more directly derived from POM in late summer, when a tighter relationship between $\delta^{13}C$ of amino acids in the POM and the bacterial fraction was measured. When the comparison of the $\delta^{13}C$ of amino acids in POM and the bacterial fraction is broken down by season, it is evident that the two pools were most closely related in September (slope = 1.2 ± 0.3, intercept = 3.1 ± 9, $r^2 = 0.94$, $p < 0.0001$). This was additionally supported by the overall pattern of the isotopic composition of the amino acids in these two fractions. The $\delta^{13}C$ of amino acids in DOM, however, were most similar to those of the larger fractions in November when fewer amino acids were detected

in the DOM fraction. Remnants of the microbial degradation of the larger organic matter fractions were probably an important source of DOM in the fall, further evidenced by the elevated concentrations of nonprotein amino acids β-Ala and γABA (data not shown) in DOM at that time.

The isotopic composition of Ser, Asp and Ala has been instructive in elucidating the biosynthetic pathways of different microorganisms, demonstrating how variation in microbial community and its activity can contribute to variation in isotopic composition of individual amino acids. (Scott et al. 2001). The proteinaceous components of each organic matter fraction at Jug Bay were subjected to microbial reworking during periods of high primary production and temperature as indicated by the δ^{13}C of Ser and Val. In March when chlorophyll *a* levels increased at the study site, the δ^{13}C of Ser and Val in POM were more positive by 12‰ and 4‰, respectively, relative to values in December. Ser in each organic matter fraction, however, was more enriched in ^{13}C relative to all other amino acids in the spring and summer. The δ^{13}C of Ser and Val caused the majority of the variability in DOM during the more productive times of the year, providing further evidence that the remains of particulate fractions resulting from microbial reworking are an important source of DOM. As with Gly, Ser may be subjected to enrichment in ^{13}C when utilized during the biosynthesis of other amino acids such as Gly or Cysteine. The unusual δ^{13}C of Ser (–1.87‰) in DOM during June indicates that Ser was serving the role as a carbon donor for other amino acids as a result of substantial levels of microbial reworking of the DOM pool during this productive time of year.

Phytoplankton exudation and microbial reworking are important determinants of organic matter composition on diel time scales

Selective processes such as phytoplankton exudation and/or microbial reworking were likely the cause of the dramatic changes in the stable isotopic composition of DOM detected on diel time scales. Changes in the bulk stable isotopic composition of DOM, POM, and DIC that occurred over the 24 hour period sampled conformed to the change in daylight and less so with change in water level at the site (Figures 4c & 5). The diel change in the bulk δ^{13}C of DOM in June was large, and likely the result of macrophyte or phytoplankton exudation of carbohydrates, which are usually enriched in ^{13}C relative to other biomolecules. The lack of a similar diel pattern in the δ^{13}C composition of any amino acid analyzed in the DOM samples rules out amino acids or proteinaceous components of DOM as contributors to the enrichment in ^{13}C of the bulk DOM. The rapid depletion in the ^{13}C of DOM overnight was likely the result of the utilization of the more enriched exudates as evidenced by the increase in bacterial abundance and decrease in DOC concentration overnight. Selective bacterial utilization of carbohydrates derived from phytoplankton or macrophytes could explain the enriched composition of the DOM and bacterial fraction relative to POM throughout the year (Macko et al. 1991; Teece et al. 1999). The slight depletion of ^{13}C of DOM that occurred from spring to fall may also have been due to some loss of the heavier carbohydrate component, but the small change in isotopic composition suggests exudation was probably not impor-

tant on seasonal time scales. Tidal flushing has been determined to influence organic matter on diel time scales (Goni and Thomas 2000), and further study of sediment organic matter fluxes at our study site are required to distinguish water column versus sediment algal exudation and microbial processing.

Much of the protein component of DOM was the remnant of the microbial reworking of fresh organic matter rather than direct input from primary or secondary microbial production in the wetland studied. Unlike the bulk isotopic composition of DOM, the $\delta^{13}C$ of amino acids in DOM did not follow the same diel pattern as that of the Ala in POM. The carbon isotopic composition of Ala in the POM changed synchronously with chlorophyll *a*, DIC, and DOC concentrations indicating the fresh input of proteinaceous materials into POM. Additionally, the dramatic change in the $\delta^{13}C$ of Ala demonstrated how primary production occurring in the water column impacted the isotopic composition of the POM, and subsequently the DOM. The majority of the amino acids in DOM, like Ile, lagged behind the $\delta^{13}C$ of the bulk DOM until well after sunset. This lag in the enrichment of most of the amino acids could be attributed to algal production of ^{13}C-enriched carbohydrates, their utilization by microbes, and the subsequent release as amino acids. Differences in the diel change in the isotopic composition of the bulk versus individual amino acids of DOM demonstrated how chemical components of DOM can differ in both their source and temporal nature.

Relative importance of autotrophic versus heterotrophic activity to organic matter composition on diel time scales

Diel variation in the $\delta^{13}C$ of specific amino acids within the organic matter fractions sampled reveals how changes in autotrophic and heterotrophic activity influenced the composition of organic matter. The isotopic composition of Ala and Val of POM were the most indicative of the diel changes in autotrophy and heterotrophy. Because Ala is synthesized directly from transamination of pyruvate, it can serve as an important metabolic indicator (Voet and Voet 1990). The $\delta^{13}C$ of Ala in POM changed dramatically over a 24 h period in June, illustrating the direct photosynthetic input of the ^{13}C enriched DIC available in late afternoon. A similar, but diminished, change in $\delta^{13}C$ was found with both Val and Ile in POM, further evidence of isotopically enriched DIC fixed by phytoplankton.

Changes in the contribution of bacterial versus phytoplankton sources of DOM could be tracked by examining differences in the $\delta^{13}C$ of Val or Ile. Ile is synthesized using the same unique bacterial enzyme used in the synthesis of Val and is therefore subjected to different levels of fractionation when synthesized by bacteria versus plants or algae. The carbon isotopic composition of those amino acids, such as Phe, synthesized by similar pathways in bacteria, algae, and plants, varied in a similar way in both DOM and POM over the diel period sampled (Figure 8a). Different levels of heterotrophic and autotrophic activity contributing to these two fractions of organic matter on short time scales should result in amino acids synthesized by different pathways. For example, Val and Ile were isotopically decoupled over the diel in the DOM and POM fractions (Figure 8b) and hold the highest

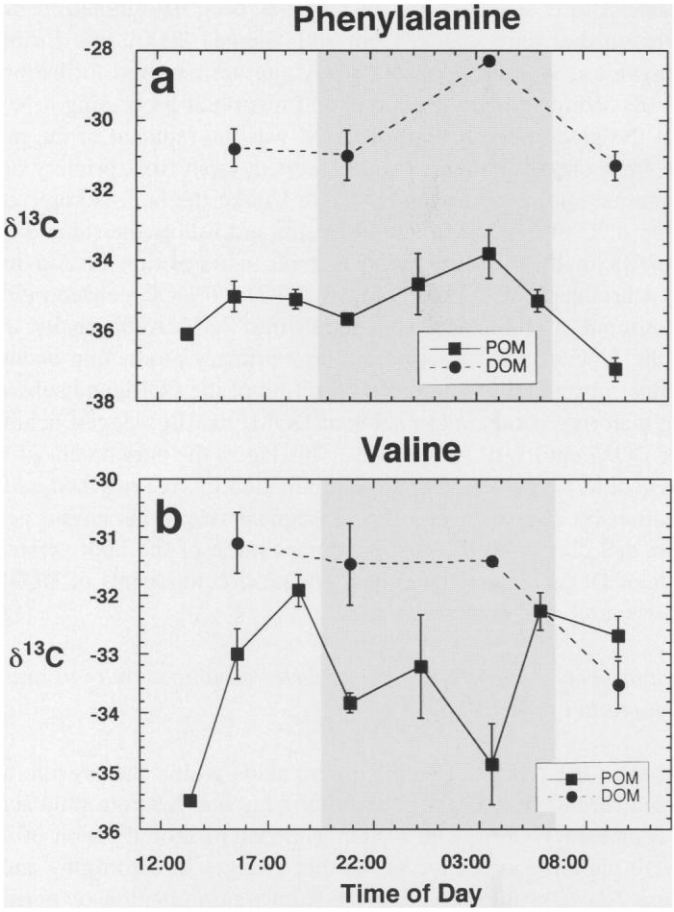

Figure 8. The change in the $\delta^{13}C$ composition of (a) phenylalanine and (b) valine in both the particulate organic matter (POM) and dissolved organic matter (DOM) collected from the study site over a 24 h period during June 3–4, 1999.

potential for distinguishing between heterotrophic and autotrophic biogeochemical cycling.

Conclusions

This study, in addition to evaluating the seasonal and diel variation in the source of DOM in a freshwater wetland, provides links between biological processing and stable isotopic composition of organic matter. Results from this study reveal that specific amino acids and their isotopic composition hold important information regarding the significance of (1) microbial reworking and (2) autotrophic versus heterotrophic sources of DOM (Figure 9). More specifically, the diel changes detected

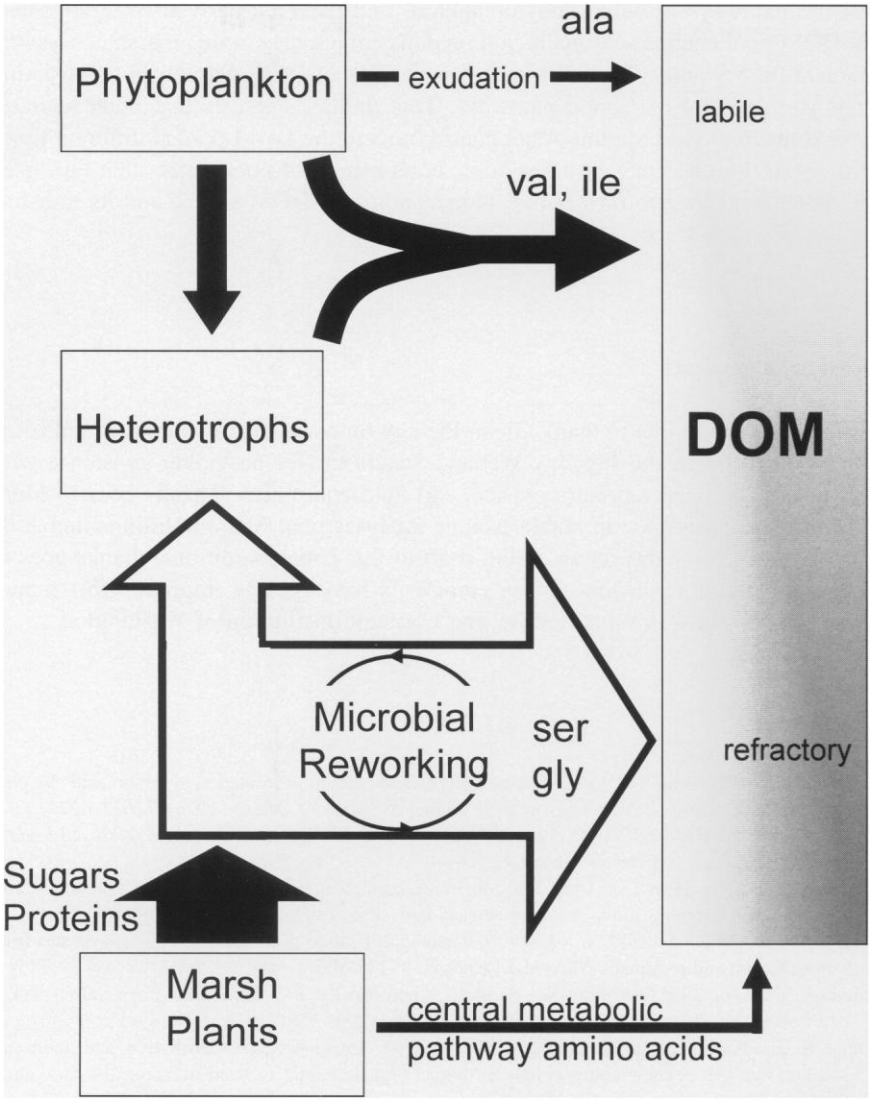

Figure 9. Conceptual diagram of the flow of organic matter in a freshwater marsh, indicating those amino acids whose isotopic composition can reveal the importance of pathways indicated. Size of arrows are indicative of importance of the processes within the site studied.

in the isotopic composition of bulk versus individual amino acids of DOM demonstrate how chemical components of DOM can differ in both their source and temporal nature. The changes in the isotopic composition of amino acids, from organic matter fractions in the wetland studied, suggest that seasonal and diel changes in DOM are attributed to both autotrophic and heterotrophic processes. Alterations in the isotopic composition of DOM and larger fractions of organic matter indicate

that the pathways by which phytoplankton- and bacterial-derived material enters the DOM pool change seasonally and on daily time scales in this marsh ecosystem. Much of the variability in the isotopic composition of DOM was attributed to amino acids from central metabolic pathways. This finding suggests that other sources, such as macrophytes, are important contributors to the DOM pool at different times of the year. Future work on the isotopic composition of pore waters that flux from sediments is needed to further our understanding of DOM source and its transformation in wetland ecosystems.

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