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Shallow ponds are biogeochemically distinct habitats in salt marsh ecosystems

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Abstract

Runaway expansion of shallow ponds can catalyze the conversion of vegetated marshes into open water environments. Predicting how this transition affects ecosystem functioning is difficult because little is known about pond biogeochemistry. We characterized sediment organic matter sources and transformations in three ponds with different plant communities, over alternating periods of tidal isolation and flushing, during summer and fall, using a combination of stable isotopes, lipid biomarkers, and benthic fluxes. Sediment respiration rates (1.66 ± 0.09 mmol C m^{-2} d^{-1} to 28.53 ± 7.76 mmol C m^{-2} d^{-1}) were comparable to shallow estuaries and driven by sulfate reduction. Rates varied across ponds, reflecting differences in summertime *Ruppia maritima* and macroalgae abundances, but were similar between seasons. Interactions between aboveground plant and sediment bacterial communities translated into distinct biogeochemical processes across the three ponds. Tidal isolation and summer weather intensified plant and bacterial community effects on pond carbon dynamics, resulting in algal biomass and lipid $\delta^{13}C$ values that were 3–12‰ enriched, compared to nearby habitats. Surface sediment organic matter mainly derived from pond microalgae and was compositionally distinct from tidal creeks and marshes. Surprisingly, sediment bacteria were not tightly coupled to benthic microalgae but decomposed multiple carbon sources in surface sediments and became increasingly reliant on buried peat at deeper horizons. Pond development over time could largely be explained by sediment respiration and the simultaneous accretion of the surrounding marsh platform. The role of decomposition in pond expansion is consistent with previous assessments based on whole-pond metabolism rates. Consequently, future pond expansion could alter ecosystem biogeochemistry and reduce carbon storage.

Salt marshes are disappearing from coastlines across the globe (Nicholls et al. 2007; Gedan et al. 2009). In many regions, disturbances that cause soil waterlogging, such as rising sea levels and certain land management practices, catalyze the conversion of marsh habitat into unvegetated mudflats or open water environments (Craft et al. 2008; Kirwan et al. 2010; Temmerman et al. 2012). As part of this transition, the shallow ponds that dot the landscape expand at the expense of productive marsh grasses. This process occurs over decades and likely affects the ecosystem functions that underlie valuable services (Hartig et al. 2002; Schepers et al. 2017; Watson et al. 2017). Incorporating ponds into landscape-scale biogeochemical assessments may therefore be important for refining our current understanding of marsh ecosystem functioning and predicting changes under future scenarios. A major impediment, however, is that the biogeochemistry of marsh ponds is largely unknown.

Shallow ponds occur naturally in marsh landscapes and have been described as pools, potholes, and rotten spots (Harshberger 1909, 1916; Miller and Egler 1950). These permanently inundated systems occupy as much as 60% of marsh platforms and can form suddenly during episodic events (e.g., ice rafting) or progressively through physical (e.g., compaction, erosion) and biogeochemical processes (Miller and Egler 1950; Adamowicz and Roman 2005; Mariotti and Fagherazzi 2013; Schepers et al. 2017). Ponds often exist for decades before intersecting with a tidal channel and draining. This can facilitate re-establishment of emergent grasses and rapid vertical accretion of organic matter (OM), so that the elevation of the former pond may eventually become level with the surrounding marsh platform (Wilson et al. 2010; Wilson et al. 2014; Mariotti 2016). However, ponds can contribute to permanent marsh loss when disturbances that waterlog soils and reduce drainage accelerate rates of formation and expansion (Reed 1995; Kennish 2001; Hartig et al. 2002; Kearney et al. 2002; Ortiz et al. 2017;

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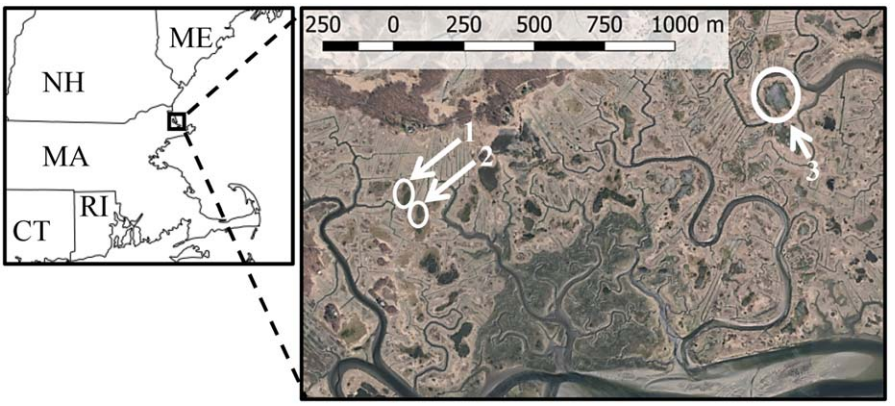

Fig. 1. Ponds 1, 2, and 3 are located in the high marshes of the PIE-LTER site (Massachusetts, U.S.A.).

Raposa et al. 2017). Over time, pond expansion may create landscape mosaics that represent the gradual conversion of vegetated marshes into open water environments (Turner and Rao 1990; Fagherazzi et al. 2006; Marani et al. 2010; Wang and Temmerman 2013).

Developing ponds are colonized by estuarine plant communities and become habitat for invertebrates, fish, and water birds (Heck et al. 1995; Layman et al. 2000; Smith and Able 2003; Bolduc and Afton 2004). However, unlike estuaries, high marsh ponds are shallow (20–30 cm) and only intermittently flushed by spring and storm tides (Miller and Egler 1950; Adamowicz and Roman 2005). Long stretches of hydrologic isolation facilitate the accumulation of metabolites and depletion of critical resources (e.g., inorganic nutrients), thereby altering pond biogeochemistry. Tidal flushing may only partially alleviate these effects because exchange is often incomplete (Spivak et al. 2017). Ponds also differ from other coastal habitats because they are embedded in the marsh platform and surrounded by organic-rich peat. This combination of characteristics makes it likely that ponds have biogeochemical properties that are distinct from emergent marshes and nearby intertidal and subtidal environments.

Pond expansion may not only increase landscape heterogeneity but also reduce ecosystem-level carbon storage. Respiration measurements suggest that decomposition of marsh peat is an important process contributing to progressive pond development (Johnston et al. 2003; Spivak et al. 2017). However, respiration data are scarce and reflect the combined metabolism of the animal, plant, and bacterial communities that inhabit ponds (Johnston et al. 2003; Moseman-Valtierra et al. 2016; Spivak et al. 2017). Moreover, bacteria can assimilate OM from multiple sources and decomposition rates and pathways are affected by local plant communities. Submerged grasses, macroalgae, and microalgae contribute directly to whole-pond respiration rates and influence sediment bacteria by producing labile OM and altering redox conditions (Holmer and Nielsen 1997; Koop-Jakobsen and Wenzhöfer 2015; Spivak and Reeve 2015). In addition, marine algae and marsh grass detritus washed into ponds can be deposited to sediments and stimulate respiration. Determining whether sediment bacteria decompose carbon that was produced *in situ*, imported from nearby habitats, or buried in peat is important for evaluating how ponds affect marsh carbon storage.

Through this study, we aimed to characterize pond biogeochemistry by identifying the sources of OM decomposed by sediment bacteria and determining how pond carbon dynamics change over tidal stages (flushing vs. isolation) and seasons (summer vs. fall) and with primary producer abundances. We focused on three high marsh ponds, where we had previously characterized surface water chemistry, plant communities, and oxygen-based metabolism rates (Fig. 1; Spivak et al. 2017). We predicted that carbon dynamics would vary across ponds due to interactions between aboveground primary producers and sediment bacteria, which would be intensified by tidal isolation and summer weather. In particular, sediment respiration rates would be higher in ponds where the submerged grass *Ruppia maritima* and macroalgae *Ulva* were more abundant. Similarly, the OM composition of surface sediments would reflect the plant communities that had colonized the ponds, rather than marsh grasses or buried peat. We further expected that sediment bacteria would decompose a mixture of OM from pond primary producers and marsh peat and that sediment respiration rates would provide a mechanism for pond deepening and expansion. Finally, different biogeochemical processes in ponds compared to tidal creeks, estuaries, and the marsh platform would indicate that ponds are unique habitats and future expansion could result in a transitional landscape that is distinct from endpoint ecosystems.

Materials

The three ponds are located in the high marsh (1.43–1.46 m above North American Vertical Datum of 1988) of the Plum Island Ecosystems—Long Term Ecological Research

(PIE-LTER) site, where the emergent vegetation is dominated by *Spartina patens*, short-form *S. alterniflora*, and *Distichlis spicata* (Fig. 1; Millette et al. 2010). Because the ponds are situated in the high marsh, they are hydrologically isolated for days-to-weeks of the tidal cycle and only connected to tidal creeks during spring and storm tides.

We previously established that the ponds are permanently inundated and have similar depths but differ in surface area and volume (Pond 1: 0.26–0.30 m deep, 962 m^2, 288.6 m^3; Pond 2: 0.24–0.29 m deep, 643 m^2, 186.5 m^3; Pond 3: 0.24–0.29 m deep, 7149 m^2, 2073.2 m^3) (Spivak et al. 2017). Intermittent tidal flushing exported dissolved organic carbon (DOC) but did not affect pond salinities, algal abundances, or levels of suspended particulate organic carbon (POC). There was considerable heterogeneity in plant communities across the ponds as abundances of macroalgae and *R. maritima* were highest in pond 1, slightly lower in pond 2, and considerably lower in pond 3. Differences in plant abundances were reflected in whole-pond oxygen metabolism rates, as pond 1 was net autotrophic, pond 2 switched from autotrophic to heterotrophic between summer and fall, and pond 3 was heterotrophic. Plant communities likely affected a range of biogeochemical processes by driving surface water dissolved oxygen (DO) to supersaturation during the day and hypoxia at night ($DO < 2$ mg L^{-1}; 59% of nights in ponds 1 and 2, 29% in pond 3). Additional details on surface water chemistry (temperature, salinity, DOC), plant communities (benthic and suspended chlorophyll, macroalgae, *R. maritima*), and whole-pond oxygen dynamics are reported in Spivak et al. (2017).

In this study, we characterized surface water and sediment carbon pools, pore water chemistry, and benthic fluxes on a weekly basis between 25 June 2014–13 August 2014 and 11–25 November 2014. Samples were collected between 07:00 h and 13:30 h during the 11 sampling events, which captured alternating periods of tidal flushing and isolation; 3 weeks of flushing were interspersed between 5 weeks of isolation in the summer, while 2 weeks of flushing bookended 1 week of isolation in the fall. Flushing periods were defined as high tides that overtopped the marsh platform and connected with the ponds, and were based on observations and predicted tide heights.

Primary producers and pond sediments

The elemental (total organic carbon [TOC] and total nitrogen [TN]) and isotopic ($\delta^{13}C$) composition of the dominant primary producers were measured to evaluate the sources of OM in pond sediments and used by bacteria. Leaves from *S. alterniflora*, *S. patens*, *R. maritima*, and *Ulva* were collected once (August 2014) from across the marsh platform and all three ponds; tissues were combined in combusted (450°C) glass jars to form species-specific composite samples. POC suspended in pond surface waters was collected weekly in 1 L combusted glass bottles and filtered through combusted glass fiber filters (25 June 2014–13 August 2014, 11–25 November 2014). Benthic microalgae (BMA) were collected from ponds 1 and 2, as part of a separate study (July 2016), by placing combusted glass slides on surface sediments for 1 week and scraping attached material onto combusted glass fiber filters. All samples were frozen (−20°C) until analysis.

Surface sediments were collected weekly for TOC, TN, $\delta^{13}C$, and lipid biomarker composition (25 June 2014–13 August 2014 and 11–25 November 2014). Cores (5 cm diameter × 2 cm deep) were collected from three 1 m^2 quadrats placed at random locations along two crisscrossing transects in each pond. Sediments were combined in combusted glass vials to form composite samples and stored (−80°C) until analysis.

Surface and pore water

Fluxes across the sediment-water interface were calculated from weekly profiles of dissolved inorganic carbon (DIC), $\delta^{13}C$-DIC, sulfate (SO_4^{2-}), and ammonium (NH_4^+; 25 June–13 August, 11–25 November). Surface water samples were collected from the same 1 L bottle as POC, while pore waters were collected from cores (10 cm diameter × 21 cm deep) that were extracted from one of the three sediment organic matter (SOM) quadrats. Upon collection, cores were capped and placed on ice for transport to WHOI, where pore waters were extracted from six horizons (0–3 cm, 3–6 cm, 6–9 cm, 10–13 cm, 14–17 cm, and 18–21 cm). The polycarbonate core liners had previously drilled holes at the mid-point of each sampling horizon that were sealed with electrical tape until the core was under a N_2 atmosphere. Then, the tape was peeled back and Rhizon samplers (pore size 0.12–0.18 μm, Rhizosphere research products) were inserted through the holes to collect and filter pore water (Seeberg-Elverfeldt et al. 2005). Both surface and pore waters were filtered through Rhizon samplers for consistency. Rhizons were rinsed with 60 mL of 18 MΩ Milli-Q water prior to use and the first 5 mL of filtered water was discarded before samples were collected. Additional cores were collected once, in triplicate, from each pond for bulk density and porosity measurements.

Bulk OM analyses

Plant tissues, POC filters, and sediment samples were prepared for elemental and isotopic analyses by drying to constant mass (60°C), homogenizing with a Retsch Mixer Mill 200 (plants and sediments), and fuming with hydrochloric (HCl) acid to remove carbonates (Hedges and Stern 1984). Analyses were performed by the Stable Isotope Laboratory at the Marine Biological Laboratory (Woods Hole, Massachusetts). Isotopic data are reported in the conventional δ-notation in units of per mil (‰).

Lipid biomarker analyses

Lipid biomarker compounds were extracted from SOM cores using a modified Bligh and Dyer (1959) method (Spivak and Reeve 2015). Sediments were extracted with a methanol : chloroform : phosphate buffer saline mixture (2 : 1 : 0.8, v : v : v) using a microwave-accelerated reaction system (MARS6); samples were heated to 80°C for 10 min with continuous stirring. Samples were then partitioned and the organic phase removed. The total lipid extract was concentrated under N_2 and samples were eluted on silica gel columns with chloroform, acetone (F1/2), and methanol (F3) (Guckert et al. 1985). The F3 (phospholipids) was dried under N_2 and saponified with 0.5 M NaOH at 70°C for 4 h. Saponified samples were acidified and extracted three times with hexane. The extract was methylated with acidic methanol (95 : 5, methanol : HCl) and heated overnight at 70°C to form fatty acid methyl esters (FAME). Samples were analyzed with an Agilent 7890 gas chromatograph with the effluent split $\sim$ 70 : 30 between a 5975C mass spectrometer and a flame ionization detector. Compounds were separated on an Agilent DB-5 ms column (60 m, 0.25 mm inner diameter, 0.25 μm film). FAME concentrations were quantified using methyl heneicosanoate as an internal standard. FAs are designated A : BωC, where A is the number of carbon atoms, B is the number of double bonds, and C is the position of the first double bond from the aliphatic "ω" end of the molecule. Iso- and anteiso refer to whether the methyl group of branched compounds is attached to the penultimate or antepenultimate carbon atom.

Stable carbon isotope ratios of FAMEs were determined by the WHOI Organic Mass Spectrometry Facility with a Hewlett-Packard 6890 GC coupled to a DeltaPlus isotope-ratio-monitoring gas chromatography-mass spectrometer (IRM-GCMS) via a GCCIII combustion interface held at 850°C with a constant oxygen trickle. Isotopic values of phospholipid-linked fatty acids (PLFAs) were derived from the isotopic composition of FAMEs and corrected for the δ^{13}C of the carbon added during methylation using a mass balance approach as well as for a $-3‰$ fractionation during lipid synthesis (Hayes 2001; Bouillon and Boschker 2006). The δ^{13}C values of PLFA subclasses were calculated as concentration weighted averages (Spivak and Ossolinski 2016).

Surface and pore water analyses

Filtered surface and pore waters were aliquoted into specific vials for each analyte. Salinity was measured using a handheld refractometer. DIC concentrations were determined with a UIC coulometer 5510 and quantified relative to a seawater certified reference material, as described by Pohlman et al. (2008). Samples for δ^{13}C-DIC were acidified with phosphoric acid and vigorously shaken over several hours to convert DIC to CO_2 and transfer the gas to the vial headspace; isotopic composition was determined by the WHOI IRMS Facility using a Thermo Finnigan Delta^{plus} XL IRM-GCMS coupled to an Agilent 6890 GC (Pohlman et al. 2008). Sulfate samples were preserved with a saturated zinc solution to bind sulfides as ZnS. Sulfate samples were diluted prior to analysis by ion chromatography with suppressed conductivity detection (Dionex DX-120 or DX-500, dependent on availability) and peak areas were quantified against similarly diluted International Association for the Physical Sciences of the Oceans standard seawater. Ammonium concentrations were determined by flow injection analysis, as described by Hall and Aller (1992). We determined sediment bulk density and porosity gravimetrically after drying samples to constant mass (60°C).

Data analyses

Diffusive fluxes of DIC, SO_4^{2-}, and NH_4^+ were calculated from pore water concentration profiles according Fick's first law of diffusion (Berner 1980),

$$J = -\phi D_s \frac{dC}{dz} \quad (1)$$

where J is the diffusive flux per unit area, ϕ is sediment porosity, D_s is the analyte-specific diffusion coefficient in sediments, C is the concentration, and z is the depth below the sediment-water interface. Values for D_s were calculated by correcting diffusion coefficients at infinite dilution, D, for tortuosity (Boudreau 1996). We used temperature-dependent D values reported by Li and Gregory (1974) for HCO_3^-, SO_4^{2-}, and NH_4^+. Fluxes were calculated as described by Kalnejais et al. (2015).

To detect differences in OM composition and benthic fluxes between tidal stages (flushing vs. isolated) and seasons (summer vs. fall), we constructed linear mixed effect models using the nlme package for R (Pinheiro et al. 2016). The mixed models evaluated the fixed effects of pond, tidal stage, and season. We specified a first-order autoregressive correlation structure to account for our repeated measures sampling design. By modeling covariances among data points sampled within ponds, a mixed model approach allowed us to meet the assumption of independence of errors. For each model, we calculated the marginal (variance of fixed effects only) and conditional (variance of both fixed and random effects) r^2, (sensu Nakagawa and Schielzeth 2013), using the piecewiseSEM package in R (Lefcheck 2016). Elemental and isotopic data and benthic fluxes were analyzed for all 11 weeks between 25 June–13 August and 11–25 November (five flushing, six isolated periods). Lipid biomarker results were evaluated from five summer weeks (6/26, 7/10, 7/25, 8/01, 8/13) and three fall weeks (11/11, 11/17, 11/24), and included four flushing and four isolated tidal periods. The absence of seasonal changes in biomarker composition validated the more selective analytical approach.

To evaluate the factors influencing benthic DIC, SO_4^{2-}, and NH_4^+ fluxes, we constructed linear mixed models as described above, but with potential drivers as fixed effects

Table 1. Organic carbon and nitrogen content of primary producers, suspended particles, and surface sediments. (a) The most abundant primary producers had distinct %TOC and nitrogen (TN) compositions and molar carbon : nitrogen ratios (C : N). (b) The bulk OM content of suspended particles and surface sediments varied across ponds and occasionally by season, but did not change across tidal stages. SE is standard error of the mean. See Table 2 for statistical results.

	%TOC		%TN		C : N (molar)	
a.	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Primary producers						
BMA	3.73	1.35	0.48	0.16	8.55	0.38
Macroalgae	22.84	0.89	2.14	0.11	12.57	1.10
R. maritima	24.05	1.63	2.56	0.16	10.97	0.64
S. alterniflora	40.67	0.12	1.55	0.07	30.66	1.29
S. patens	41.41	0.12	1.12	0.01	43.09	0.53

b.

		%TOC		%TN		C : N (molar)	
Season	**Pond**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Suspended particles							
Summer	1	5.36	1.26	0.66	0.17	10.04	0.48
Summer	2	4.89	0.52	0.59	0.07	9.85	0.49
Summer	3	2.63	0.58	0.41	0.08	7.37	0.41
Fall	1	1.56	0.12	0.19	0.02	9.73	0.45
Fall	2	1.94	0.37	0.24	0.05	9.67	0.21
Fall	3	1.37	0.36	0.19	0.04	8.53	0.25
Sediment OM							
Summer	1	14.54	0.92	1.58	0.07	10.69	0.38
Summer	2	14.13	0.63	1.56	0.04	10.56	0.51
Summer	3	8.48	0.51	0.83	0.02	11.92	0.58
Fall	1	13.73	0.13	1.83	0.01	8.77	0.11
Fall	2	12.77	0.38	1.64	0.03	9.09	0.15
Fall	3	8.64	1.20	0.87	0.03	11.45	1.18

and pond identity as a random effect. The potential drivers of benthic respiration included surface water temperature and salinity, sediment chlorophyll, macroalgae, *R. maritima*, suspended POC, and SOM. Data used in these models were collected concurrently, but surface water properties and plant abundances are reported in Spivak et al. (2017). For each model, we calculated the marginal and conditional r^2 (as detailed above).

We estimated the relative contributions of plant and algal OM to pond sediments by constructing mixing models, with the MixSIAR package for R (Stock and Semmens 2013), that included isotopic (δ^{13}C, δ^{15}N), elemental (C : N), and PLFA biomarker (ΣC18:1, C18:2, C18:4, C20:4ω6, C20:5ω3, C22:5, C22:6) data. We used literature data to describe plant and algal lipid profiles, basing *S. patens* and *S. alterniflora* on Sessions (2006) and Canuel et al. (1997), *R. maritima* on Henninger et al. (2009) and Jeffries (1972), microalgae on Volkman et al. (1989), and *Ulva* on Fleurence et al. (1994) and Dembitsky et al. (1991). *S. alterniflora* and *S. patens* were grouped as emergent grasses because they are compositionally similar (Spivak and Ossolinski 2016). We assumed that diatoms were the dominant microalgae in suspended POC and BMA, based on field observations (Tobias et al. 2003; Spivak and Ossolinski 2016). Mixing models were calculated for summer and fall only, because sediment markers did not vary with tidal stage. Including additional metrics (e.g., δ^{13}C of PLFAs) did not improve model estimates.

Data were $\log_{10}$-transformed as necessary to maintain homogeneity of variance and values are presented as means ± SE, unless noted otherwise. Analyses were performed using R open-source software (R Development Core Team 2016).

Results

The dominant primary producers had distinct elemental and δ^{13}C compositions (Table 1a; Fig. 2a). %TOC and C : N were highest in the emergent grasses, lowest in BMA, and intermediate in macroalgae and *R. maritima* (Table 1a). Macroalgae (-10.3 ± 0.3‰) had the highest δ^{13}C values while *R. maritima* (-11.6 ± 0.1‰) and BMA (-14.2 ± 0.9‰) were lower. The δ^{13}C of emergent grasses (-13.3 ± 0.1‰) was typical of the PIE-LTER marshes (Galván et al. 2011; Spivak and Ossolinski 2016).

Particles suspended in pond surface waters had a similar elemental composition to BMA and a wide range δ^{13}C values (-13.2 ± 0.5‰ to -21.2 ± 0.5‰; Table 1b; Fig. 2b). %TOC and C : N were similar in ponds 1 and 2 but lower in pond 3 (Tables 1b, 2). The seasonal transition from summer to fall resulted in lower %TOC, %TN, and δ^{13}C values (Tables 1b, 2; Fig. 2b). Only δ^{13}C changed with tidal stage, as values were highest in ponds 1 and 2 during isolated tides but were lower and more similar across ponds during flushing tides, particularly in the fall (Fig. 2b). For simplicity, %TOC, %TN, and C : N are only presented by pond and season because these values did not change with tidal stage (Tables 1b, 2).

We used bulk and molecular markers to characterize SOM sources. Sediment C : N ratios (8.77 ± 0.11 to 11.92 ± 0.58) were similar to suspended particles, BMA, macroalgae, and *R. maritima*, but lower than emergent marsh grasses (Table 1). SOM δ^{13}C values (-15.4 ± 0.3‰ to -16.5 ± 0.2‰) were lower than emergent grasses, macroalgae, and *R. maritima* but were similar to BMA and suspended POC (Fig. 2). SOM elemental and δ^{13}C composition differed across ponds, but did not change with season or tidal stage (Tables 1b, 2; Fig. 2c). Sediments in ponds 1 and 2 had higher %TOC, %TN, and δ^{13}C, but lower C : N ratios than pond 3 (Tables 1b, 2; Fig. 2c).

We further evaluated SOM composition using PLFAs, which are source-specific lipid biomarkers that are rapidly

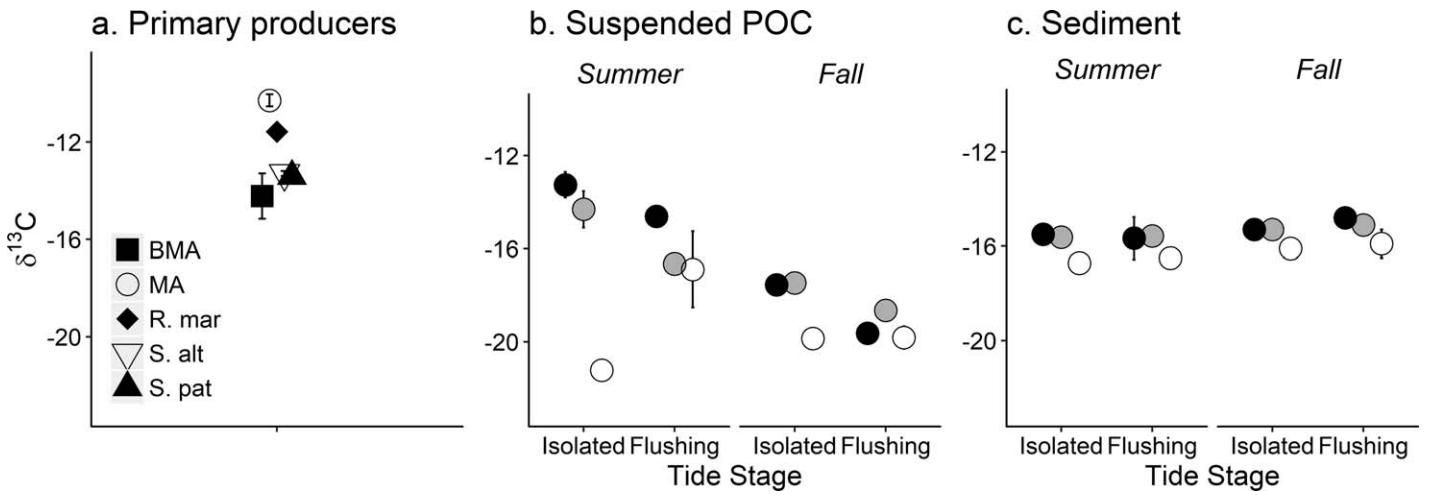

Fig. 2. The δ^{13}C of primary producers (**a**), suspended POC (**b**), and surface sediments (**c**). The main primary producers include BMA, macroalgae (MA), *R. maritima* (R. mar), *S. alterniflora* (S. alt), and *S. patens* (S. pat). Suspended POC and SOM δ^{13}C values were characterized in ponds 1 (black), 2 (gray), and 3 (white) during isolated and flushing tide stages, in summer and fall. Error bars are SE of the means. See Tables 1, 2 for elemental data and statistical results, respectively.

degraded after cell death and represent viable, or recently viable, cells (Bianchi and Canuel 2011). Similar to sediment %TOC, total PLFA concentrations were higher in ponds 1 and 2 than pond 3 and did not change between seasons or tidal stages (Fig. 3a; Tables 1b, 2). We examined several PLFA sub-classes to better resolve SOM sources and found that, for the most part, abundance patterns mirrored total PLFAs.

Short chain fatty acids [SCFA, Σ(C12:0, C14:0, C16:0, C18:0)] are produced by microbes, algae, and some vascular plants, and comprised 25.16% ± 0.62% (pond 3) to 27.84% ± 0.51% (pond 1) of total PLFAs (Supporting Information Table S1). Levels of SCFA did not change between seasons or tidal stages and were lowest in pond 3 (Fig. 3b; Table 2). Monounsaturated C18 PLFAs (Σ(C18:1ω7, C18:1ω9)) are produced by bacteria (Volkman et al. 1980; Kaneda 1991), microalgae and macroalgae (Volkman et al. 1989; Dembitsky et al. 1991; Viso and Marty 1993; Fleurence et al. 1994) and at low levels by *S. alterniflora* (Canuel et al. 1997; Sessions 2006). Abundances of ΣC18:1 accounted for 23.33% ± 1.32% (pond 3) to 29.97% ± 0.64% (pond 2) of total PLFAs, were highest in ponds 1 and 2, did not change with tidal stage, but decreased slightly in the fall in pond 3 (Fig. 3c; Table 2, Supporting Information Table S1). C18:2 and C18:3 are synthesized by microalgae (Volkman et al. 1989; Viso and Marty 1993), *R. maritima* (Jeffries 1972; Henninger et al. 2009), *S. alterniflora* (Canuel et al. 1997; Sessions 2006), and *Ulva* (C18:3 only; Dembitsky et al. 1991; Fleurence et al. 1994) but were either minor components of the total PLFAs (C18:2: 0.74% ± 0.07% to 1.27% ± 0.15%) or below detection (C18:3; Supporting Information Table S1). Concentrations of C18:2 were highest in pond 1 and lowest in pond 3 and did not change between seasons or tidal stages (Fig. 3d). C18:4 is mainly produced by microalgae but occurs at low levels in *Ulva* (Volkman et al. 1989; Dembitsky et al. 1991; Fleurence et al. 1994). This compound was a small fraction of the total PLFAs and concentrations were lowest in pond 3 (Fig. 3e). Polyunsaturated PLFAs represent microalgae (polyunsaturated fatty acids [PUFA], Σ(C20:4, C20:5, C22:5, C22:6)) (Volkman et al. 1989; Volkman et al. 1998) and were 4.78% ± 0.33% to 8.71% ± 0.67% of the total PLFAs. Unlike other markers, PUFA did not vary across ponds, seasons, or tidal stages (Fig. 3f). Bacterial lipids, including branched PLFAs [BrFA, Σ(iso-, anteiso- C13, C15, C17)] and 10-methyl C16:0 (Kaneda 1991; Viso and Marty 1993) were more abundant in ponds 1 and 2 than pond 3 and during tidally isolated periods (Fig. 3g,h; Table 2). The effects of tidal stages were clearest in the summer in ponds 1 and 2. Overall, bulk and lipid biomarkers indicate there were greater differences in SOM composition across ponds, with sediments in ponds 1 and 2 being more organic-rich than pond 3, than between tidal stages or seasons (Figs. 2, 3). However, bacterial communities in the surface sediments of ponds 1 and 2 were sensitive to alternating tidal stages.

We measured the δ^{13}C of ΣC18:1, C18:2, and C18:4 in surface sediments to better resolve their OM sources (Fig. 4a–c). The δ^{13}C of ΣC18:1 PLFAs (−15.1‰ to −21.2‰) was lowest in pond 2 and similar to SOM and suspended POC, particularly in the fall (Table 2; Figs. 2b,c, 4a). The δ^{13}C of C18:2 (−13.6‰ to −19.6‰) and C18:4 (−8.7‰ to −16.1‰) did not vary consistently across ponds, tidal stages, or seasons (Table 2; Fig. 4b,c). The δ^{13}C of C18:2 was generally lower than *S. alterniflora* and *R. maritima* leaves but similar to POC, while C18:4 values overlapped macroalgae, BMA, and POC (Figs. 2a, 4b,c).

We measured the δ^{13}C of PUFA, BrFA, and 10-methyl C16 to determine whether sediment bacteria relied on BMA-

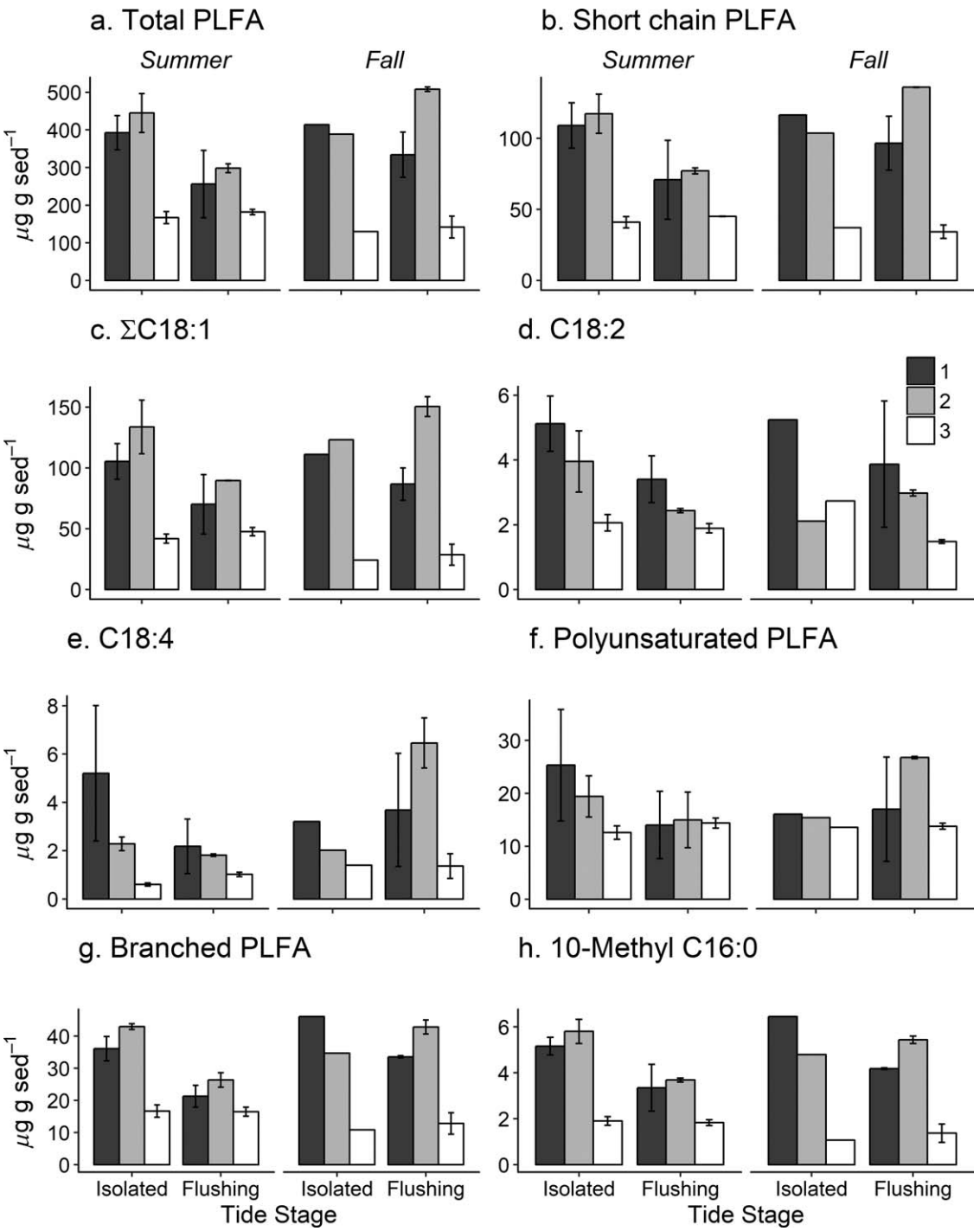

Fig. 3. Sediment PLFA composition. Concentrations of total PLFAs (**a**) and sub-classes representing microbes, macroalgae, vascular plants (**b–e**), microalgae (**f**), heterotrophic bacteria (**g**), and sulfate-reducing bacteria (**h**) were measured in the ponds during periods of tidal isolation and flushing in summer and fall. Error bars are SE of the mean. See Table 2 for statistical results and the text for the identity of PLFAs in the sub-classes.

derived carbon or assimilated multiple sources of OM (Fig. 4d–f). PUFA δ^{13}C values (−8.5‰ to −14.9‰) were lowest in pond 3 and similar to BMA and suspended POC (Table 2; Figs. 2a,b, 4d). BrFA δ^{13}C (−13.7‰ to −17.6‰) did not vary across ponds, tidal stages, or seasons (Table 2; Fig. 4e). The δ^{13}C of 10-Methyl C16 (−8.8‰ to −16.1‰) tended to be highest in pond 1, particularly during flushing tides, (Table 2; Fig. 4f). Both bacterial markers were slightly depleted

Table 2. Statistical results for the OM composition of suspended particles and surface sediments. Differences in the TOC and TN content, carbon : nitrogen ratio (C : N), and stable isotope and PLFA composition of pond OM pools, across seasons and tidal stages, were detected using linear mixed effect models. The marginal (mar) and conditional (cond) r^2 reflect the variance explained by fixed effects alone or by fixed and random effects combined, respectively. Data were transformed as indicated. Significant p values ($<$ 0.05) are in bold. See text for PLFA source information.

	Pond		Season		Tide		Pond × Season		Pond × Tide		Season × Tide		P × S × T		r^2	
Response	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	**cond.**	**marg.**
Suspended particles																
$\log_{10}$%TOC	9.90	**0.002**	8.71	**0.021**	0.04	0.846	1.13	0.350	0.51	0.612	0.22	0.655	1.80	0.210	0.78	0.48
$\log_{10}$%TN	3.07	0.078	7.94	**0.026**	0.00	0.997	0.40	0.676	0.50	0.618	0.18	0.688	1.44	0.271	0.78	0.40
C : N (molar)	21.11	**<0.001**	0.10	0.764	1.03	0.345	1.54	0.209	1.47	0.262	0.00	0.957	0.76	0.488	0.71	0.49
δ^{13}C (‰)	36.95	**<0.001**	54.66	**<0.001**	1.04	0.341	8.36	**0.004**	11.21	**0.001**	1.43	0.272	1.68	0.222	0.80	0.80
Sediment OM																
%TOC	38.75	**<0.001**	0.66	0.444	1.40	0.321	0.41	0.668	0.84	0.452	0.36	0.566	0.76	0.484	0.75	0.68
$\log_{10}$%TN	242.17	**<0.001**	3.87	0.090	0.09	0.768	1.06	0.374	0.76	0.484	0.08	0.788	0.72	0.506	0.94	0.92
C : N (molar)	5.17	**0.021**	3.47	0.105	0.68	0.438	0.63	0.549	0.04	0.961	0.04	0.854	0.23	0.800	0.42	0.33
$\log_{10}$ δ^{13}C (‰)	11.35	**0.001**	4.77	0.065	0.23	0.644	0.07	0.934	0.05	0.947	0.24	0.638	0.17	0.842	0.53	0.45
Sediment PLFA (μg g dry sediment^{-1})																
$\log_{10}$ Total PLFA	56.19	**<0.001**	0.08	0.793	1.70	0.283	2.87	0.115	3.08	0.102	1.54	0.304	1.34	0.314	0.87	0.79
$\log_{10}$ SCFA‡	63.04	**<0.001**	0.20	0.688	1.59	0.296	2.54	0.140	2.26	0.166	0.77	0.444	2.29	0.170	0.87	0.80
$\log_{10}$ ΣC18:1	91.08	**<0.001**	0.01	0.939	1.37	0.325	8.03	**0.012**	2.83	0.118	0.82	0.433	1.25	0.337	0.88	0.77
$\log_{10}$ C18:2	14.82	**0.002**	0.40	0.571	7.92	0.067	0.05	0.947	0.50	0.627	0.48	0.537	2.61	0.134	0.80	0.45
$\log_{10}$ C18:4	16.51	**0.001**	3.88	0.143	0.04	0.846	1.13	0.370	1.97	0.201	1.24	0.347	1.62	0.257	0.70	0.60
$\log_{10}$ PUFA*	1.29	0.326	0.22	0.668	0.01	0.938	0.31	0.745	0.40	0.681	1.31	0.335	0.51	0.619	0.22	0.22
BrFA†	80.96	**<0.001**	1.66	0.288	16.17	**0.028**	4.70	**0.045**	6.61	**0.020**	6.44	0.085	5.41	**0.033**	0.90	0.90
10-methyl C16:0	62.86	**<0.001**	0.01	0.929	19.37	**0.022**	1.55	0.270	4.92	**0.041**	5.14	0.108	2.27	0.166	0.88	0.86
Sediment PLFA (concentration-based δ^{13}C)																
ΣC18:1	21.24	**0.001**	0.48	0.526	0.53	0.508	1.31	0.330	2.10	0.193	0.13	0.734	1.46	0.295	0.70	0.70
C18:2	0.33	0.732	0.07	0.804	0.08	0.792	0.63	0.563	0.86	0.470	0.07	0.799	0.48	0.641	0.14	0.14
C18:4	2.99	0.125	0.22	0.666	0.94	0.387	1.40	0.317	0.06	0.944	0.39	0.565	0.23	0.801	0.34	0.34
PUFA*	57.09	**<0.001**	0.01	0.926	0.11	0.753	0.92	0.442	2.51	0.151	5.27	0.083	0.92	0.441	0.79	0.79
BrFA†	2.09	0.194	0.69	0.453	0.93	0.390	1.05	0.398	1.41	0.305	0.04	0.860	1.17	0.364	0.33	0.33
10-methyl C16:0	116.19	**<0.001**	3.41	0.139	1.82	0.249	3.79	0.077	39.53	**<0.001**	0.75	0.436	3.66	0.095	0.86	0.62

BrFA, branched PLFA; PUFA, polyunsaturated PLFA; SCFA, short chain PLFA.
* PUFA = Σ(C20:4, C20:5, C22:5, C22:6).
† BrFA = Σ(iso-, anteiso- C13, C15, C17).
‡ SCFA = Σ(C12:0, C14:0, C16:0, C18:0).

relative to PUFAs, particularly in ponds 1 and 2, enriched relative to marsh soils (-17.3 ± 1.5‰ to -18.9 ± 0.3‰; Wang et al. 2003; Spivak and Reeve 2015), and overlapped with emergent grasses, BMA biomass, and suspended POC (Figs. 2a,b, 4d–f).

Despite differences in elemental, isotopic, and PLFA composition across the ponds, BMA were the main source of surface sediment OM in the summer while both BMA and suspended POC were important in the fall (Fig. 5). In both seasons, $<5\%$ of SOM derived from emergent grasses, *R. maritima*, or macroalgae. Contributions from suspended POC were lowest in pond 2 (mean ± SD, $10.5\% \pm 9.0\%$, $10.2\% \pm 12.9\%$) and highest in pond 3 ($19.9\% \pm 12.1\%$, $63.3\% \pm 13.0\%$) in the summer and fall, respectively. BMA contributions were similar across ponds in the summer ($77.5\% \pm 12.6\%$ to $86.9\% \pm 9.6\%$) but were more variable in the fall, ranging from $31.8\% \pm 14.1\%$ in pond 3 to $75.9\% \pm 13.9\%$ in pond 2. The seasonal shift in the relative importance of BMA vs. suspended POC was sharpest in pond 3.

There were larger differences in benthic DIC, SO_4^{2-}, and NH_4^+ fluxes across ponds than between tidal stages or seasons (Fig. 6; Table 3). In each pond, DIC and NH_4^+ concentrations increased from the surface waters to depth in the sediments (Supporting Information Figs. S1, S2). Consequently, diffusive fluxes were positive, with sediments releasing DIC and NH_4^+ into the surface water (Fig. 6a,c). DIC fluxes were

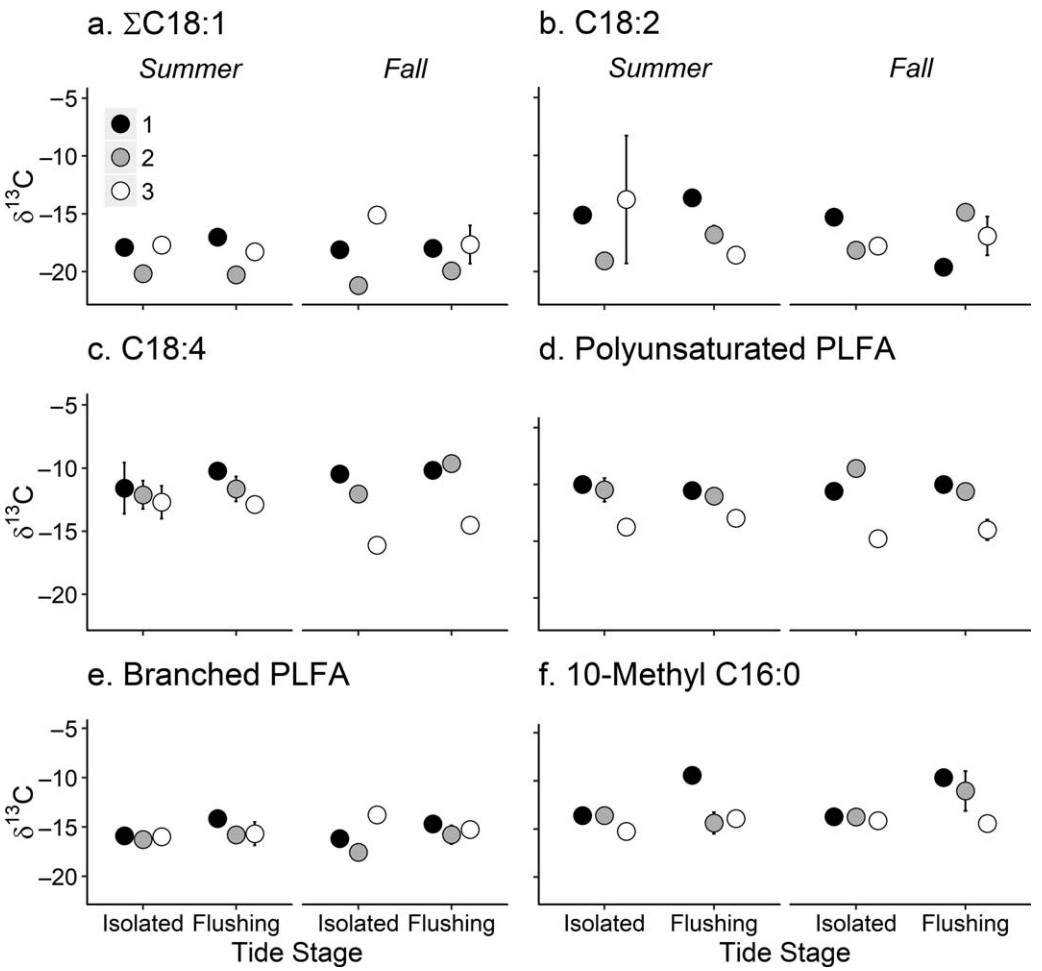

Fig. 4. The δ^{13}C (‰) of PLFAs in pond surface sediments during periods of tidal isolation and flushing, in summer and fall. PLFA δ^{13}C values are useful in identifying the sources of compounds synthesized by multiple organisms (**a–c**), evaluating microalgal dynamics (**d**), and characterizing bacterial carbon sources (**e, f**). Error bars are SE of the mean. See Table 2 for statistical results.

highest in pond 1 and lowest in pond 3, and were greater during tidal isolation (Table 3; Fig. 6a). Fluxes of NH_4^+ were also highest in pond 1, but did not change between tidal stages or seasons (Table 3; Fig. 6c). Profiles of SO_4^{2-} decreased from the surface waters into the sediments in ponds 1 and 2, reflecting sulfate reduction (Fig. 6b, Supporting Information Fig. S3). In pond 3, however, SO_4^{2-} concentrations either decreased slightly or increased between surface and pore waters, which suggested slower rates of sulfate reduction and/or sulfide oxidation (Fig. 6b, Supporting Information Fig. S3). Surface-to-pore water profiles of δ^{13}C-DIC were more complex, indicating that multiple processes contributed to DIC composition (Supporting Information Fig. S4). Plots of salinity vs. DIC, NH_4^+, or SO_4^{2-} indicated that downcore profiles reflected biological processes, not mixing (data not shown). Sediment properties, including bulk density (pond 1: 0.13 ± 0.04 g cm^{-3}; pond 2: 0.09 ± 0.01 g cm^{-3}; pond 3: 0.19 ± 0.01 g cm^{-3}) and porosity (pond 1: 0.83 ± 0.03; pond 2: 0.82 ± 0.03; pond 3: 0.82 ± 0.05), were similar across ponds. Estimated D_{sed} (cm^2 s^{-1}) for HCO_3^- (8.48×10^{-6}–8.51×10^{-6}), SO_4^{2-} (6.40×10^{-6}–7.71×10^{-6}), and NH_4^+ (1.21×10^{-5}–1.43×10^{-5}) were similar to values in salt marsh and estuarine sediments (Lord and Church 1983; Iversen and Jørgensen 1993).

Discussion

Characterizing pond biogeochemistry is critical for predicting how the expansion of open water habitats at the expense of emergent grasses will affect marsh ecosystem functioning. Benthic respiration rates were similar to shallow coastal systems but SOM composition and carbon isotope systematics differed from emergent marshes and estuaries. Heterogeneity in benthic respiration rates and surface OM composition across ponds largely reflected differences in summertime primary producer communities. However, while plants senesced from summer to fall, benthic fluxes and SOM were similar in both seasons. Interactions between

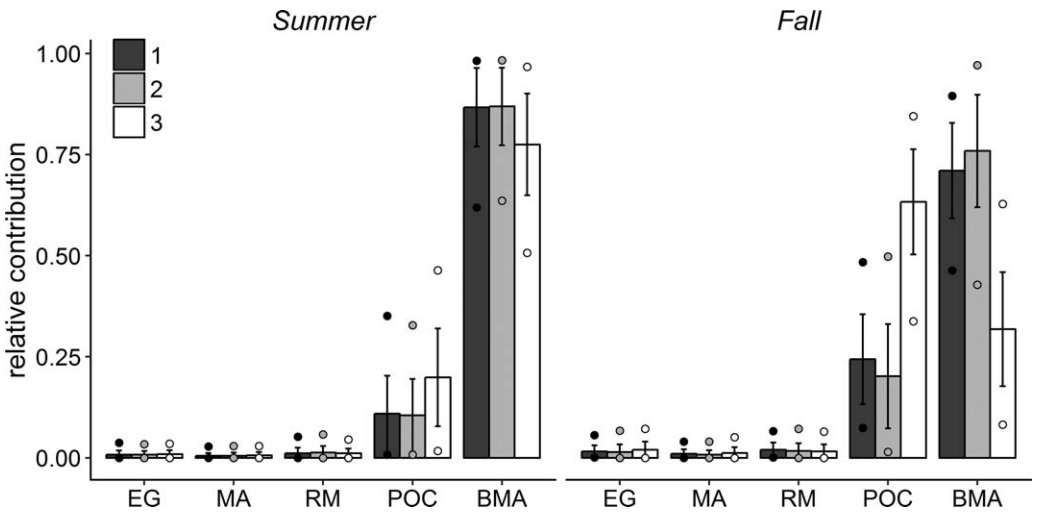

Fig. 5. Sources of pond surface SOM. We used mixing models to estimate OM contributions from emergent grasses (EG), macroalgae (MA), *R. maritima* (RM), suspended POC, and BMA to pond surface sediments in summer and fall. Error bars are standard deviation of the mean and dots represent 2.5% and 97.5% credible intervals. See text for the biomarkers included in the models.

aboveground plant communities and sediment bacteria affected biogeochemical processes and resulted in carbon dynamics that differed across ponds and from other nearby habitats. Consequently, ponds are distinct but heterogeneous habitats, and future expansion will likely impact both marsh ecosystem metabolism and carbon dynamics.

Ponds are heterogeneous and biogeochemically distinct habitats in salt marsh ecosystems

Like shallow estuaries, ponds are permanently inundated and colonized by brackish water plants. Yet, ponds are irregularly flushed by tides and embedded in carbon-rich marsh peat. Pond biogeochemistry reflects this unique set of characteristics as respiration rates were similar to subtidal and intertidal habitats, but SOM composition was distinct from marshes and other coastal environments. Pond DIC fluxes were comparable to lower estimates of benthic respiration in shallow estuaries, mudflats, and tidal creeks, but much slower than vegetated marshes (Fig. 6a; Hopkinson et al. 1999; Hamersley and Howes 2003; Cook et al. 2004; Forbrich and Giblin 2015). Lower rates in pond sediments, compared to other submerged systems may be real, but could also reflect differences in estimating fluxes from pore water profiles vs. benthic chambers (e.g., Morford et al. 2007, 2009). For instance, sediment cores were collected during the day when BMA would have been actively assimilating pore water DIC, and possibly changing the concentration profiles used to calculate fluxes. The ratio of DIC: SO_4^{2-} fluxes (1.9, $r^2 = 0.71$) indicates that sulfate reduction was the dominant metabolic pathway in pond sediments, as in most organic-rich benthic systems (Howarth and Teal 1979; Canfield et al. 1993; Weston et al. 2006). Benthic NH_4^+ fluxes were comparable to tidal creeks and temperate, shallow estuaries (Fig. 6c; Banta et al. 1995; Sundbäck et al. 2000; Vieillard and Fulweiler 2012) and generally met (ponds 2 and 3) or occasionally exceeded (pond 1) BMA demand (Supporting Information Fig. S5), estimated from chlorophyll concentrations (Spivak et al. 2017), algal turnover rates (Spivak and Ossolinski 2016), a chlorophyll : carbon ratio of 40 (de Jonge and Colijn 1994), and Redfield stoichiometry. This suggests that nitrogen was tightly recycled in surface sediments and that NH_4^+ released by decomposition is not likely to be exported and contribute to coastal eutrophication.

Despite similar respiration rates, differences in SOM composition indicate that pond biogeochemistry and microbial communities were distinct from other intertidal and subtidal environments. Pond sediments had a higher %TOC and lower C : N than tidal creeks and vegetated marshes (Wang et al. 2003; Deegan et al. 2012; Spivak and Ossolinski 2016). Algal and microbial (PUFA, SCFA) contributions to SOM were higher in ponds than marshes, but lower than tidal creeks (Pascal et al. 2013; Spivak and Reeve 2015; Spivak and Ossolinski 2016). Bacterial lipids (BrFA, 10-methyl C16) made up a similar fraction of total PLFAs in pond and tidal creek sediments, but were relatively more abundant in marsh sediments (Pascal et al. 2013; Spivak and Reeve 2015; Spivak and Ossolinski 2016). Unlike many shallow systems, pond surface SOM composition and fluxes were similar in summer and fall (Figs. 3, 5, 6; Tables 1b, 2, 3; Kristensen 1993; Hopkinson et al. 1999; Spivak 2015). This likely reflects the steady availability of carbon-rich peat to decomposers and highlights a key difference between ponds and shallow estuaries, where benthic respiration rates often track seasonal deposition of phytodetritus (Kelly et al. 1985; Grenz et al. 2000). However, our data represent processes occurring during the summer and late fall, and seasonal changes may have been more apparent over a longer study period.

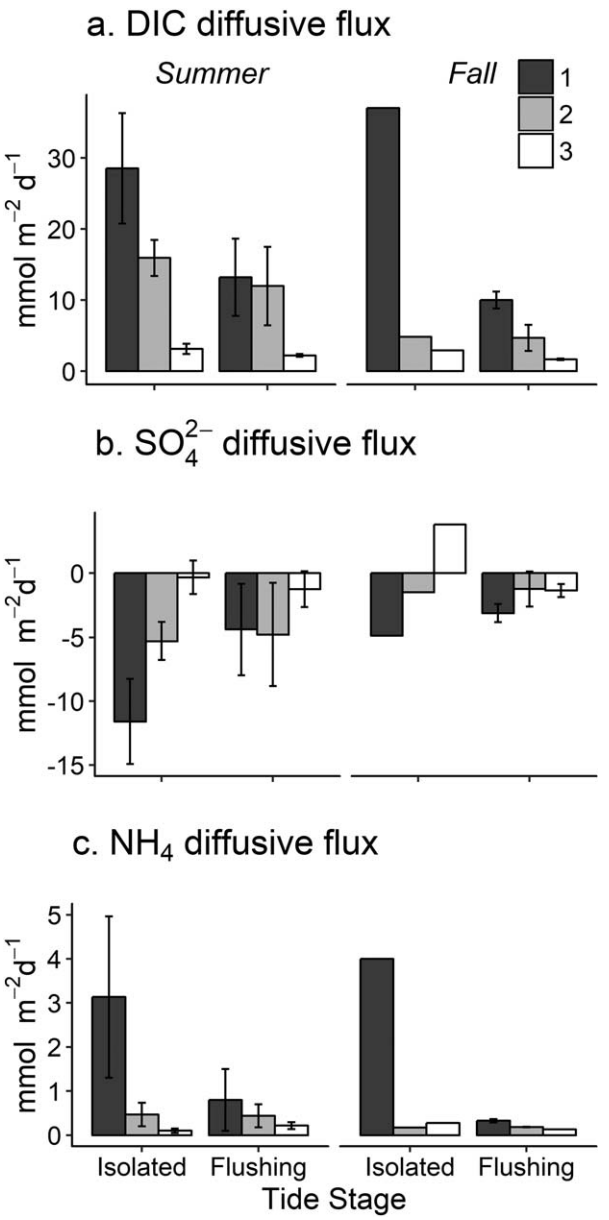

Fig. 6. Diffusive benthic fluxes. Pore water profiles were used to calculate fluxes of DIC (**a**), sulfate (SO_4^{2-}, **b**), and ammonium (NH_4^+, **c**) during periods of tidal isolation and flushing, in summer and fall. Positive values indicate efflux from the sediment and negative rates indicate uptake into the sediments. Error bars are SE of the mean. See Table 3 for statistical results.

Sediment processes not only differed between ponds and other coastal systems, but also across ponds. Benthic respiration rates, %TOC, and total PLFAs largely mirrored summertime macrophyte abundances, which were highest in pond 1 and lowest in pond 3 (Figs. 3a, 6; Table 1b, Supporting Information Table S2; Spivak et al. 2017). Yet, *R. maritima* and macroalgae died back in the fall and accounted for small fractions of surface SOM in both seasons (Fig. 5). Low concentrations of C18:2 and C18:4 and undetectable levels of C18:3 indicate that macrophytes were not important sources of surface SOM (Fig. 3d,e; Supporting Information Table S1). This is further supported by δ^{13}C values of ΣC18:1 and C18:2 that were more depleted than would be expected if macroalgae and *R. maritima* were the primary sources, respectively (Figs. 2a, 4a,b). Moreover, the δ^{13}C of SOM was lower than macroalgae, *R. maritima*, and emergent grasses (Fig. 2a,c). Instead, multiple biomarkers indicate that BMA and, to a lesser extent, suspended POC were the main OM sources in pond surface sediments (Fig. 5). Microalgal contributions to SOM were likely produced *in situ* since flushing tides did not import phytoplankton and δ^{13}C values of BMA and suspended POC were higher than in tidal creeks (Figs. 2, 4d; Galván et al. 2011; Spivak and Ossolinski 2016; Spivak et al. 2017). Despite being the dominant sources of surface SOM, suspended POC and BMA either weakly enhanced SO_4^{2-} and NH_4^+ fluxes or had no effect (Supporting Information Table S2). In contrast, *R. maritima* and macroalgae were more strongly correlated with DIC effluxes (Supporting Information Table S2). Relationships between macrophytes and sediment processes may be due, in part, to indirect interactions since microbial and bacterial markers (SCFA, ΣC18:1, BrFA, 10-Methyl C16) accounted for 58.9% ± 1.1% to 66.7% ± 0.6% of total PLFAs and concentrations generally tracked across-pond differences in summertime *R. maritima* and macroalgae abundances (Fig. 3; Supporting Information Table S1).

Macrophytes may have affected benthic fluxes by altering resource availability to sediment microbial communities and through root respiration. For instance, macroalgae could have contributed to higher DIC fluxes by shading the sediments and inhibiting BMA (Supporting Information Table S2; McGlathery et al. 2001; Hardison et al. 2011). *R. maritima* may have contributed to DIC fluxes directly, through root respiration, and indirectly, by releasing oxygen and labile substrates into the rhizosphere (Supporting Information Table S2; Thursby 1984; Azzoni et al. 2001; Kaldy et al. 2006). The belowground effects of *R. maritima* are somewhat unclear because sulfide toxicity inhibits root production in organic-rich sediments and pore water profiles indicate that DIC was produced below the rooting zone (Supporting Information Fig. S1; Pulich 1989; Kantrud 1991). However, root-derived oxygen can alter sediment redox conditions while labile exudates may promote decomposition of marsh peat (Azzoni et al. 2001; Kaldy et al. 2006; Bianchi 2011; Schmidt et al. 2011). A greater understanding of seasonal changes in *R. maritima* root respiration and rhizodeposition could provide valuable insight into the processes contributing to benthic fluxes and microbial lipid abundances. Macrophytes may have also affected benthic respiration by pushing surface water oxygen concentrations to super-saturation during the day and contributing to nighttime hypoxia (Spivak et al. 2017). The magnitude of day-night swings varied across

Table 3. Statistical results for benthic respiration rates. Differences in respiratory fluxes between ponds, seasons, and tidal stages were detected using linear mixed effect models. The marginal (mar) and conditional (cond) r^2 reflect the variance explained by fixed effects alone or by fixed and random effects combined, respectively. Data were transformed as indicated. Significant p values (< 0.05) are in bold.

	Pond		Season		Tide		Pond × Season		Pond × Tide		Season × Tide		P × S × T		r^2	
Response	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	**cond.**	**marg.**
$\log_{10}$ DIC (mmol m^{-2} d^{-1})	56.71	**<0.001**	4.88	0.063	6.06	**0.043**	0.90	0.427	1.66	0.225	0.00	0.997	0.18	0.840	0.76	0.68
SO_4^{2-} (mmol m^{-2} d^{-1})	6.33	**0.011**	4.70	0.067	0.83	0.393	0.44	0.653	1.83	0.197	0.88	0.379	0.11	0.892	0.39	0.39
$\log_{10}$ NH_4 (mmol m^{-2} d^{-1})	4.51	**0.031**	0.20	0.668	0.25	0.630	0.31	0.736	2.24	0.143	0.53	0.491	0.38	0.692	0.33	0.33

ponds and likely affected oxygen penetration depths. This, in turn, could have contributed to higher benthic fluxes and abundances of bacterial lipids in ponds 1 and 2, since oscillating redox conditions can enhance decomposition and bacterial turnover (Figs. 3g,h, 6; Aller 1994a; Sun et al. 2002; Abril et al. 2010). It is unlikely that bioturbation affected benthic fluxes since many animals cannot tolerate frequent nighttime hypoxia and we did not observe grazing effects on plants (Diaz and Rosenberg 1995; Layman et al. 2000; Spivak et al. 2017).

Similar respiration rates but distinct biogeochemical processes in ponds compared to nearby environments suggest that the expansion of open water habitats will create patchy landscapes that function differently than marshes or estuaries. Integrating ponds into ecosystem-scale assessments will be complicated by heterogeneity across ponds and require consideration of how ponds interact with the landscape. Characterizing of a broader suite of ponds and developing a better understanding of the processes driving spatial and temporal heterogeneity will be critical for predicting how ecosystem biogeochemistry will change as these systems expand.

Natural abundance carbon isotopes provide insight into pond functioning

Stable isotopes have provided foundational information about OM transformations and energy flows in salt marsh ecosystems (Peterson and Howarth 1987; Currin et al. 1995; Cloern et al. 2002). Pond expansion could complicate the interpretation of isotope-based models in marsh landscapes because algal δ^{13}C values were more enriched in ponds compared to nearby habitats. For instance, the δ^{13}C of *Ulva*, BMA biomass, and microalgal lipids (PUFA) were 6–11‰, 3–12‰, and 5–11‰, respectively, more enriched in the ponds than in adjacent intertidal and subtidal habitats (Figs. 2a, 4d; Deegan and Garritt 1997; Galván et al. 2011; Spivak and Ossolinski 2016). Suspended POC was enriched in the ponds (Fig. 2b) compared to tidal creeks (−18‰ to −21‰) and the larger estuary (−20‰ to −22‰), particularly during the summer and isolated tidal stages (Deegan and Garritt 1997; Galván et al. 2011; Spivak and Ossolinski 2016).The wider range of pond surface water δ^{13}C-DIC (−7.11‰ to +6.41‰; Supporting Information Fig. S4) compared to the nearby Parker River (1.4‰–1.6‰, 28–29 PSU) indicates that tidal flushing was only one factor affecting DIC composition (Hopkinson et al. 2015). Incorporation of pond OM into estuarine sediments or animals could therefore bias mixing models, if across-habitat differences in isotope values are not accounted for. This could be particularly important in landscapes where ponds are prominent and expanding as well as in food web models that include fish and invertebrates that move between habitats.

Anomalous δ^{13}C values also hint that carbon dynamics differ across ponds and with the wider ecosystem. Two characteristics of high marsh ponds—shallow depths and irregular tidal flushing—have the potential to intensify the effects of plant and bacterial communities on biogeochemical processes, including carbon dynamics and isotope systematics. The shallow water column places autotrophic and heterotrophic communities in close proximity while long stretches of tidal isolation make the ponds semi-closed systems. Under these conditions, primary production depletes DIC concentrations, thereby forcing algae to fractionation less against ^{13}C and shifting both algae and DIC to more enriched δ^{13}C values (Fry 1996; Finlay 2004; Boschker et al. 2005). At the same time, surface water δ^{13}C-DIC is affected by *in situ* respiration as well as atmospheric gas exchange. To better understand pond carbon dynamics, we examined microalgal fractionation rates and controls on surface and pore water δ^{13}C-DIC.

Fractionation rates (ε) of suspended (POC) and benthic (BMA, PUFA) microalgae were calculated according to Freeman and Hayes (1992) and Finlay (2004),

$$\varepsilon(‰) = \frac{\delta^{13}C_{DIC} - \delta^{13}C_{algae}}{1 + \delta^{13}C_{algae}/1000}, \quad (2)$$

relative to surface and pore waters (0–3 cm), because benthic respiration releases DIC into overlying waters and BMA can assimilate DIC from both sources. Relative to surface water,

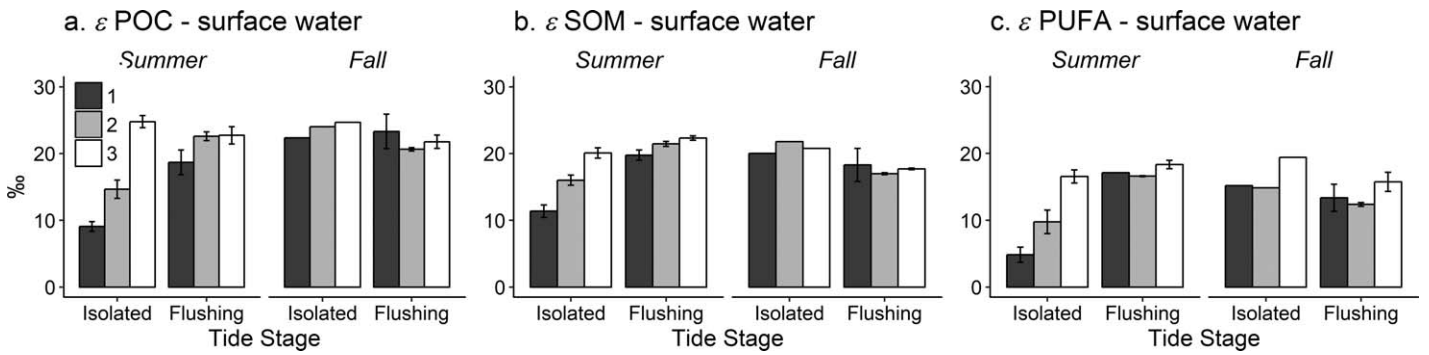

Fig. 7. Microalgal fractionation factors (ε, ‰) and environmental conditions. Fractionation rates for suspended (POC; **a**) and benthic (SOM, PUFA; **b, c**) microalgae, relative to surface water DIC, varied across ponds, tide stages, and seasons. Error bars are SE of the mean. See Table 4 for statistical results.

microalgal fractionation rates decreased when primary production and tidal isolation synergistically resulted in low DIC concentrations (Figs. 7, 8). Fractionation rates were lowest in ponds 1 and 2 during isolated tides in the summer, when primary producers removed 26.4% ± 6.2% and 7.9% ± 4.7% of surface-water DIC per day, respectively (Fig. 7; Table 4; Spivak et al. 2017). Fractionation was more typical of aquatic microalgae when macrophyte abundances were low (pond 3) and when flushing tides replenished DIC concentrations and primary production slowed in the fall (Fig. 7; Table 4). Microalgae were more reliant on surface water DIC, since fractionation relative to pore water DIC was not concentration dependent (Supporting Information Fig. S6). This was unexpected, since autotrophic and heterotrophic communities can be tightly coupled in shallow sediments and DIC fluxes generally exceeded BMA demand (Supporting Information Fig. S5), but may reflect microalgal affinities for the different carbonate species in surface and pore waters (Hinga et al. 1994; Burkhardt et al. 2001; Finlay 2004). Our results provide useful insight into pond carbon dynamics and isotope systematics, but an important caveat is that diurnal changes in δ^{13}C-DIC would affect fractionation estimates. Consequently, higher resolution data are needed to better resolve the mechanisms contributing to the unusually enriched and variable algal δ^{13}C values.

The composition of surface and pore water δ^{13}C-DIC could be influenced by several processes, including photosynthesis, atmospheric gas exchange, and bacterial respiration. High rates of primary production that depleted surface water DIC and led to lower algal fractionation rates should have increasingly enriched δ^{13}C-DIC. Yet, during isolated tides in the summer, when the effects of production would have been strongest, δ^{13}C-DIC values were lowest in pond 1, where *R. maritima* and macroalgae were most abundant (Supporting Information Fig. S4; Spivak et al. 2017). Depleted surface water δ^{13}C-DIC values in pond 1 could reflect carbonate disequilibrium and incomplete dissolution of isotopically light CO_2(g) (approximately −7‰; Emerson and Hedges 2008). However, whole-pond respiration could turn over surface water DIC more quickly than atmospheric exchange (Spivak et al. 2017).

Correlations between benthic fluxes and δ^{13}C-DIC suggest that bacterial respiration influenced the composition of surface and pore water DIC (Fig. 9). Higher fluxes were associated with more depleted δ^{13}C-DIC, reflecting decomposition of comparatively light OM. Surprisingly, lower rates of sulfate reduction, or even sulfide oxidation, correlated to enriched δ^{13}C-DIC values, that were often higher than Parker River water (Fig. 9c,d). These relationships, while significant, had low explanatory power, indicating that multiple processes affect δ^{13}C-DIC composition. This is reinforced by ambiguous results from mixing models assessing OM sources supporting respiration (Sayles and Curry 1988; Pataki et al. 2003; Aller et al. 2008). Similarly enriched δ^{13}C-DIC in coastal pore waters have been attributed to selective decomposition, fractionation during OM oxidation, carbonate dissolution and recrystallization, and different diffusion rates of carbonate species with distinct isotopic values, but the mechanisms remain unclear (McNichol et al. 1991; Hu and Burdige 2007; Walter et al. 2007; Komada et al. 2012). Our data provide new insight into unexpectedly enriched δ^{13}C-DIC values by hinting at interactions between sulfur cycling and carbon isotope systematics.

The mix of processes potentially contributing to the isotopic composition of inorganic and organic carbon pools highlight the dynamic biogeochemistry of marsh ponds and could complicate the use of δ^{13}C to characterize OM dynamics. More clearly elucidating how interacting autotrophic, heterotrophic, and abiotic processes affect carbon cycling over short diurnal cycles and longer annual time scales will be key for predicting the impact of pond expansion on ecosystem functioning and isotope systematics. Although the mechanisms remain uncertain, our data demonstrate that differences in aboveground plant and sediment bacterial communities resulted in carbon dynamics that were distinct across ponds and from nearby tidal creeks and the wider estuary.

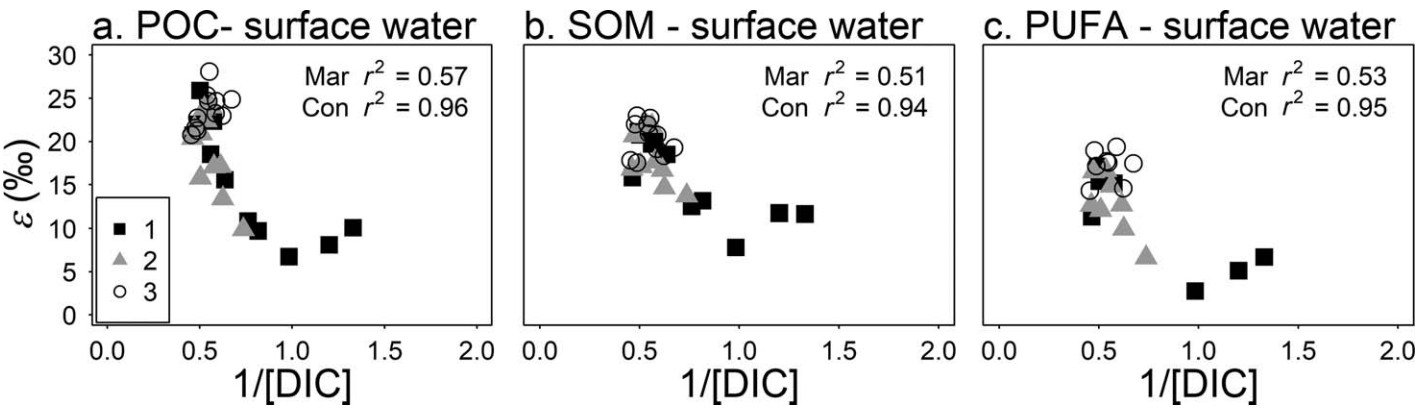

Fig. 8. Microalgal fractionation (ε, ‰) relative to surface water DIC concentrations. Fractionation factors for suspended (**a**) and benthic (**b, c**) microalgae varied with surface water DIC concentrations (1/DIC, mmol). Correlations were estimated using linear mixed effect models, with 1/[DIC] as the fixed effect and pond identity and sampling week as random effects. Marginal (Mar) and conditional (Con) r^2 calculations are described in "Data analyses" section.

Table 4. Statistical results for microalgal fractionation factors relative to surface waters. Differences in fractionation factors across ponds and between seasons and tide stages were detected using linear mixed effect models. The marginal (mar) and conditional (cond) r^2 reflect the variance explained by fixed effects alone or by fixed and random effects combined, respectively. Significant p values (< 0.05) are in bold.

	Pond		Season		Tide		Pond × Season		Pond × Tide		Season × Tide		P × S × T		r^2	
Response	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	**cond.**	**marg.**
Fractionation relative to surface water DIC (‰)																
POC	34.58	**<0.001**	20.73	**0.003**	13.49	**0.008**	13.61	**0.001**	11.96	**0.001**	11.17	**0.012**	2.84	0.092	0.85	0.85
SOM	34.55	**<0.001**	1.00	0.352	13.14	**0.009**	13.97	**0.001**	9.20	**0.003**	17.54	**0.004**	2.64	0.107	0.87	0.81
PUFA*	216.89	**<0.001**	2.21	0.212	15.45	**0.017**	46.95	**<0.001**	38.88	**<0.001**	21.95	**0.009**	16.53	**0.002**	0.89	0.83

* PUFA = Σ(C20:4, C20:5, C22:5, C22:6).

Pond development and marsh carbon storage

Assessing the potential role of decomposition in contributing to pond development is key for refining ecosystem biogeochemical budgets as well as our understanding of marshes as long-term carbon sinks. One clue that sediment processes were not tightly coupled to aboveground plant communities was that bacterial PLFAs and benthic fluxes were similar between seasons, even though lower temperatures and light levels in the fall resulted in the senescence of suspended algae and macrophytes and slower whole-pond metabolism rates (Figs. 3g,h, 6; Tables 2, 3; Spivak et al. 2017). This suggests that bacteria may have supported similar fluxes in summer and fall by decomposing multiple carbon sources.

The two main carbon sources available to bacteria in surface sediments were BMA and the underlying marsh peat (Fig. 5). We expected bacteria to be tightly coupled to BMA, yet BrFA and 10-methyl C16 were isotopically depleted relative to PUFAs and did not track across-pond variations in the δ^{13}C of suspended POC, SOM, or PUFA (Figs. 2b,c, 4d–f). Bacteria were not entirely reliant on the underlying peat either, as δ^{13}C of BrFA and 10-methyl C16 were slightly more enriched than marsh soils (-17.3 ± 1.5‰ to -18.9 ± 0.3‰) (Wang et al. 2003; Spivak and Reeve 2015). Isotopic offsets between bacterial lipids and OM sources suggest that communities may have assimilated more carbon from peat ($\Delta\delta$ BrFA vs. soil: 2.77 ± 0.5‰, 1.91 ± 0.4‰) than microalgae ($\Delta\delta$ BrFA vs. PUFA: 5.17 ± 0.5‰, 5.77 ± 0.5‰) in ponds 1 and 2, respectively, but decomposed both OM sources in similar proportions in pond 3 ($\Delta\delta$ BrFA vs. soil and PUFA: 2.65 ± 0.4‰ and 1.70 ± 0.6‰; Fig. 4d,e). A smaller offset between ΣC18:1 and marsh soils ($\Delta\delta$: 0.27 ± 0.2‰ to 2.17 ± 0.2‰) than PUFAs ($\Delta\delta$: 3.76 ± 0.8‰ to 9.85 ± 0.6‰) also suggests that buried peat was an important carbon source to microbes in all three ponds (Fig. 4a,d). These results were surprising because peat-derived compounds are generally thought to be more resistant to degradation than BMA. Yet, changes in pore water DIC, NH_4^+, and SO_4^{2-} below

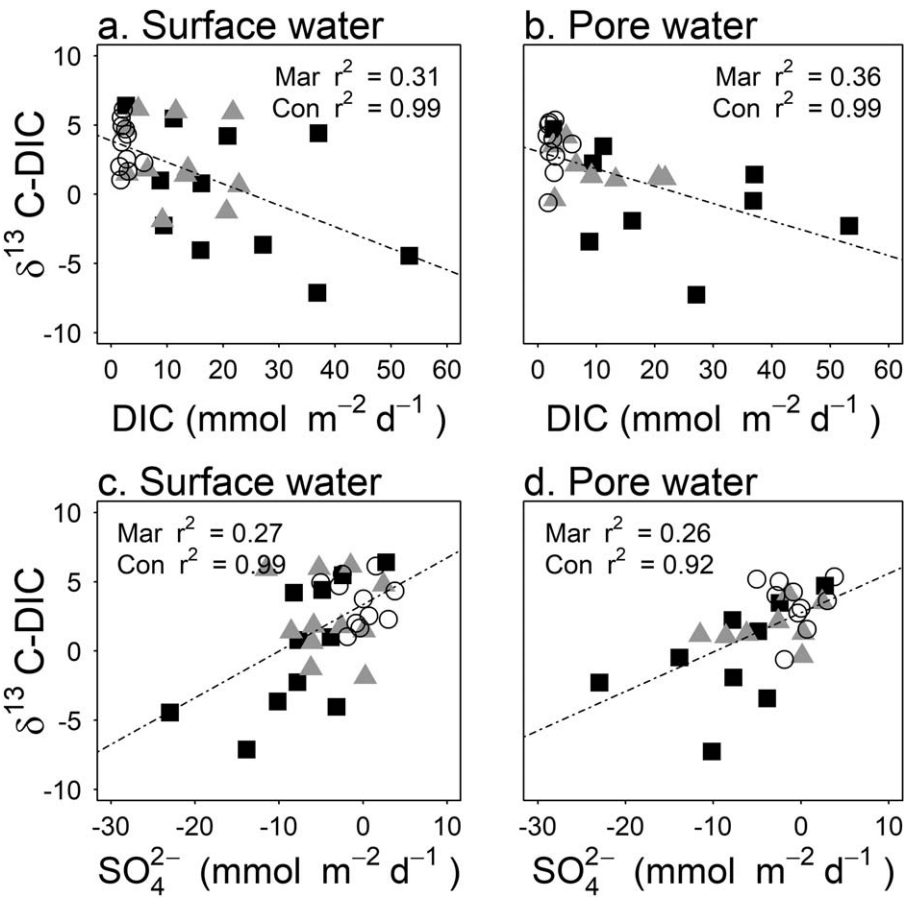

Fig. 9. Benthic respiration rates and δ^{13}C-DIC. The δ^{13}C-DIC (‰) of surface (**a, c**) and pore (**b, d**) waters decreased at higher rates of DIC respiration (top row) and sulfate reduction (bottom row). However, low sulfate reduction rates and sulfide oxidation were associated with more enriched δ^{13}C-DIC values. Pond 1 = square; pond 2 = triangle; pond 3 = circle. Correlations were estimated using linear mixed effect models, with respiration rates as the fixed effect and pond identity and sampling week as random effects. Marginal (Mar) and conditional (Con) r^2 values were calculated as described in "Data analyses" section.

depths where *R. maritima* roots and BMA are found (0–10 cm and 0–2 cm, respectively) indicate that mineralization was occurring in horizons where marsh peat would be the dominant OM source (Supporting Information Figs. S1–S3; Kantrud 1991; MacIntyre et al. 1996). Consequently, bacteria assimilated a mixture of OM sources in surface sediments but increasingly relied on buried carbon at deeper horizons.

Two key processes contributing to the development of ponds include accretion of the surrounding platform and loss of buried OM. Since the ponds began to form in 1978 (ponds 1 and 2) and 1965 (pond 3), the marsh platform has gained 9–12 cm of vertical elevation, which represents 30–42% of pond depths (Spivak et al. 2017). In order for the ponds to reach their current dimensions, we previously estimated that decomposition of the underlying peat would need to occur at average rates of 43–73 g C m^{-2} yr^{-1} (Spivak et al. 2017). This agrees well with seasonally averaged DIC fluxes, extrapolated over an entire year (11–92 g C m^{-2} yr^{-1}). Moreover, respiration rates based on pore water profiles are likely underestimates because they reflect the net result of processes driving concentration gradients and may miss contributions from metabolisms that rapidly recycle electron acceptors (e.g., Fig. 6b; Burdige 1993; Aller 1994b). The role of respiration in pond deepening and expansion is further supported by net metabolism estimates indicating that, over an annual cycle, ponds 2 and 3 were heterotrophic (68–172 g C m^{-2} yr^{-1}) (Spivak et al. 2017). This is also consistent with observations of net heterotrophy and carbon gas emissions from other ponds in PIE and New England (Johnston et al. 2003; Moseman-Valtierra et al. 2016). In combination, bacterial δ^{13}C composition, sediment pore water profiles and diffusive fluxes, and whole-system metabolism rates point to decomposition as an important mechanism contributing to pond development. Future pond expansion, in response to rising sea levels and certain land management strategies, therefore has the potential to reduce the effectiveness of marshes as long-term OM sinks by converting marsh peat back into inorganic carbon.

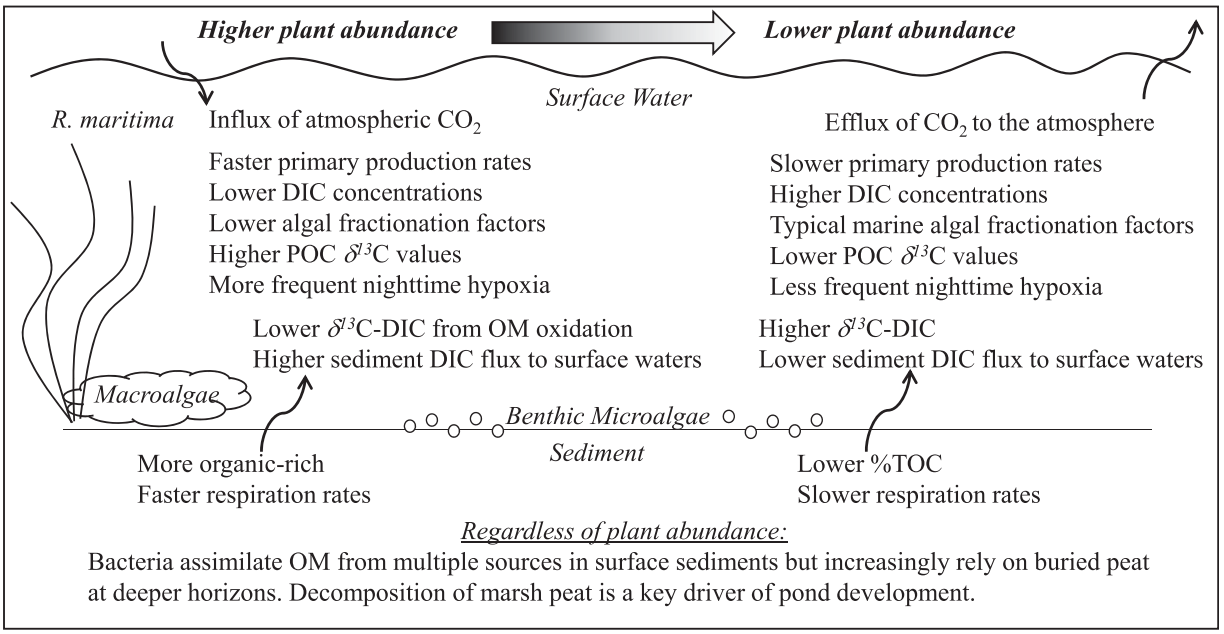

Fig. 10. Conceptual diagram of pond carbon dynamics. Pond biogeochemistry was more strongly affected by plant communities than tides or seasons. Interactions between aboveground plant and sediment bacterial communities affected biogeochemical processes and resulted in carbon dynamics and isotope systematics that differed across ponds and from the wider estuary. Regardless of plant abundances, however, several lines of evidence including the δ^{13}C of bacterial lipids, benthic fluxes, and whole-pond metabolism rates indicate that decomposition contributes to pond development.

Conclusions

Ponds are heterogeneous and biogeochemically distinct habitats within salt marsh ecosystems. Across-pond differences in SOM composition and benthic metabolism did not scale with physical dimensions, but rather reflected summertime plant abundances (Fig. 10). Because plant communities strongly influenced pond biogeochemistry, characterizing the factors leading to differences in species composition and abundances (e.g., dispersal, seed banks) could be useful in integrating these small systems into ecosystem-scale models. Interactions between aboveground plant communities and sediment bacteria affected a range of biogeochemical processes and resulted in carbon dynamics and isotope systematics that differed across ponds and with the larger ecosystem. Further elucidating the processes driving the variable δ^{13}C compositions of DIC and algae will inform the use of carbon isotopes in landscapes where ponds are rapidly expanding. Finally, several lines of evidence, including the isotopic composition of bacterial lipids, pore water concentration profiles, and benthic and whole-pond metabolism rates, provide support for the role of decomposition in contributing to pond deepening and expansion. The impact of ponds on marsh carbon storage may be mitigated by management practices, such as draining, but only if the OM contributing to infilling derives from new production. Ponds may still represent a net loss of buried carbon if the OM deposited in revegetated ponds comes from elsewhere in the ecosystem, such as eroding creek banks. Thus, refining marsh carbon budgets requires careful accounting for the effects of current and past ponds.

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Conflict of Interest

None declared.

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