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GENERAL WETLAND SCIENCE

Methane Cycling Contributes to Distinct Patterns in Carbon Stable Isotopes of Wetland Detritus

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Abstract

Increasing global temperatures are changing the balance between carbon sequestration and its microbial processing in wetlands, making the tracking of these processes important. We used detrital carbon stable isotopes ($\delta^{13}C$) to trace aerobic decomposition and CH_4 production in two experiments conducted in Alaskan wetlands. In laboratory bottle incubations, larger decreases in detritus $\delta^{13}C$ corresponded to higher net CH_4 and CO_2 production rates. Because net CH_4 production was the stronger predictor and its effect was negative, we hypothesize that decreases in $\delta^{13}C$ trace concurrent CH_4 production and oxidation. In a field experiment, decreases in detritus $\delta^{13}C$ were not correlated with aerobic decomposition rates, but were positively correlated with CH_4 production potentials as estimated from bottle incubations. We hypothesize that the positive relationship reflects only CH_4 production, rather than concurrent production and oxidation. Although CH_4 production rates were correlated with changes in detrital $\delta^{13}C$ in both experiments, the direction of this relationship differed between laboratory and field with important consequences for the scale of ecological experiments. Our study demonstrates that CH_4 cycling can create distinct patterns in $\delta^{13}C$ of wetland detritus. Future studies should conduct explicit mass balance experiments to clarify mechanisms and determine the importance of scale in shaping isotopic patterns.

Keywords Methane · Stable isotopes · Wetlands · Decomposition · Food webs

Introduction

Wetlands play a critical role in carbon processing, acting as hotspots for microbial decomposition and methane (CH_4) production (Sierszen et al. 2012; Melton et al. 2013). Due to high primary production, wetlands sequester an estimated 49 Tg of carbon per year (Bridgham et al. 2006). Microbial decomposition of this characteristically high primary production results in detritus-based food webs that support extraordinary biodiversity of flora and fauna (Bryant 1991; Kwak and Zedler 1997; Junk et al. 2006; Sierszen et al. 2012). In addition, anaerobic microbial decomposition in wetlands accounts for an estimated 60% of all natural CH_4 emissions (Wuebbles and Hayhoe 2002; Kirschke et al. 2013), supplying between 140 and 260 Tg of CH_4 to the atmosphere annually (Melton et al. 2013).

Increasing global temperatures may alter the relative rates of carbon sequestration and microbial processing of carbon, specifically by enhancing CH_4 production or methanogenesis. Longer growing seasons, particularly at northern latitudes (Walther et al. 2002), and carbon dioxide (CO_2) fertilization increase primary production and accelerate decomposition (Matthews 2007; Ringeval et al. 2011). Oxygen depletion resulting from higher aerobic decomposition may stimulate anaerobic processes including methanogenesis and lead to greater CH_4 emissions, which upon release would trap additional heat in the atmosphere (Myhre et al. 2013). As a result, increasing global temperatures might create a positive feedback loop involving CH_4 production (Whiting and Chanton 2001; Bridgham et al. 2013). As rates of decomposition processes (i.e., methanogenesis and aerobic decomposition) shift with increasing global temperatures, being able to trace these processes in wetland ecosystems will become more important.

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Carbon stable isotope ratios ($\delta^{13}C$, or the ratio of $^{13}C/^{12}C$ relative to a standard, expressed in ppt) offer a potential tool for tracing both aerobic and anaerobic decomposition in wetland ecosystems. Previous research has used $\delta^{13}C$ to identify energy sources in aquatic food webs (Hamilton et al. 1992; Peterson 1999; Vander Zanden and Rasmussen 1999). For example, consumers from detritus-based food webs exhibit lower $\delta^{13}C$, whereas consumers from periphyton-based food webs tend to have higher $\delta^{13}C$ due to the photosynthetic uptake of dissolved inorganic carbon (Kwak and Zedler 1997; Sierszen et al. 2004). Recently, $\delta^{13}C$ has also demonstrated that CH_4-derived carbon can provide a subsidy to upper trophic levels in aquatic ecosystems (Bastviken et al. 2003; Kohzu et al. 2004; Eller et al. 2005; Jones et al. 2008; Hershey et al. 2015; DelVecchia et al. 2016).

Carbon stable isotopes can also be used to trace the process of aerobic decomposition in wetland ecosystems. Previous studies suggest that $\delta^{13}C$ of organic material should decrease during aerobic decomposition since the chemical fractions of plant tissue differ in $\delta^{13}C$ initially and often decay at different rates (Benner et al. 1987; Wedin et al. 1995). Differential decomposition of organic compounds results in the preferential removal of labile carbon first, which tends to be enriched with the heavier ^{13}C (Lehmann et al. 2002). This process allows lighter ^{12}C to accumulate in detritus, thereby decreasing detrital $\delta^{13}C$ (Benner et al. 1987; Wedin et al. 1995; Lehmann et al. 2002).

Methanogenesis, an anaerobic process, can also be reflected in the $\delta^{13}C$ of organic matter in aquatic ecosystems (Bastviken et al. 2008). Biogenic CH_4 has a distinctly low $\delta^{13}C$ (~ − 110 to −60 ‰) compared to that of phytoplankton (~ − 30 to −15 ‰), C3 plants (~ − 27 ‰), or C4 plants (~ − 13 ‰; Wedin et al. 1995) due to the preferential incorporation of the lighter ^{12}C isotope by methanogens, or the microbial organisms responsible for CH_4 production (Conrad 2005). Therefore, anaerobic decomposition of organic matter could result in the accumulation of the heavier ^{13}C isotope and a higher residual detrital $\delta^{13}C$ as CH_4 is released.

By contrast, when CH_4 production is followed by oxidation by methane-oxidizing bacteria (MOB), CH_4-derived carbon (with its low $\delta^{13}C$) can be incorporated back into the food web (Grey 2016). The MOB consume CH_4 in aerobic zones at the sediment-water interface or in the water column, which supports the microbial loop and mitigates total CH_4 emission (Bastviken et al. 2004; Hershey et al. 2015). During oxidation, MOB assimilate the lighter ^{12}C isotope from CH_4. When MOB are associated with detritus, simultaneous CH_4 production and oxidation could result in an accumulation of the lighter ^{12}C isotope and lower detrital $\delta^{13}C$ (Bunn and Boon 1993).

As aerobic decomposition, methanogenesis, and CH_4 oxidation are all processes that can affect $\delta^{13}C$ and whose rates likely vary with environmental change, we were interested in determining whether we could measure and potentially tease apart the effects of these processes on detritus. Specifically, we wanted to examine whether any of these processes would have predictable effects on the $\delta^{13}C$ of decomposing plant matter and whether the scale of the experiment altered the patterns observed. Therefore, our overall study objective was to determine how aerobic decomposition and methanogenesis are related to the $\delta^{13}C$ of decomposing organic matter using laboratory and field experiments in Alaskan wetlands. We chose ecosystems which are particularly susceptible to environmental change (Vizza et al. 2017b) where it will be critical to trace decomposition processes in the future.

Methods

Study Site

The Copper River Delta (CRD) is a vast area of coastal wetlands, ponds, and braided river channels located in south-central Alaska, USA. Covering 283,000 ha, the CRD is the largest contiguous wetland on the Pacific Coast of North America and supports an impressive array of biodiversity (Bryant 1991). The Great Alaskan Earthquake of 1964 modified this heterogeneous landscape, elevating parts of the delta by 1–4 m (Thilenius 1990). As a result, the ponds span a gradient of environmental characteristics such as primary production, sediment organic matter, and water temperature (Vizza et al. 2017a).

Study Design

In laboratory experiments using bottle incubations, we measured net CH_4 and CO_2 production potential while simultaneously determining the change in $\delta^{13}C$ of decomposing aquatic macrophyte tissue included in bottles. We hypothesized that the $\delta^{13}C$ of this detritus would decrease during laboratory incubation, reflecting the processes of both aerobic decomposition and concurrent CH_4 production and oxidation. We also hypothesized that changes in $\delta^{13}C$ of the detritus would be more closely correlated with CH_4 production rates over CO_2 production rates because of the disproportionately large effect of CH_4-derived carbon on $\delta^{13}C$.

In field experiments using mesh litterbags, we estimated aerobic decomposition rates using a standard in situ cotton strip assay (Tiegs et al. 2013) while simultaneously determining the change in $\delta^{13}C$ of detritus deployed in situ. Laboratory bottle incubations were again used to estimate CH_4 production potential, but without the addition of detritus. We hypothesized that the $\delta^{13}C$ of detritus would decrease during in situ aerobic decomposition due to the preferential degradation of heavier, labile carbon. We also hypothesized that the change in $\delta^{13}C$ of detritus would be more closely correlated with aerobic decomposition rates than CH_4 cycle processes due to

abundant oxygen availability and because we measured CH_4 production at a smaller scale.

Laboratory Experiments: Net CH_4 Production, CO_2 Production, and $\Delta\delta^{13}C$

Sample Collection In July of 2014, sediment and hypolimnetic water samples were collected from each pond. Sediment samples (~250 mL) were collected from locations representative of different habitats (e.g., vegetation dominated, open water) at each of the nine ponds using a handheld bucket auger. Live tissue samples of yellow pond lily (*Nuphar lutea* (L.) Sm. ssp. *polysepala* (Engelm.) E.O. Beal), a floating macrophyte commonly found in ponds across the CRD, were collected from the same plant in a single pond (Tiedeman North) to ensure identical initial carbon isotope signatures of the tissue samples.

Bottle Incubations Sediment slurry bottle incubations (after Vizza et al. 2017b) were used to determine both net CH_4 and CO_2 production potential of nine ponds on the CRD and the change in $\delta^{13}C$ of simultaneously incubated macrophyte tissue. Three sediment incubations per pond were assembled using 250-mL serum bottles containing approximately 80 g of wet sediment, 0.7 g (wet mass) of *Nuphar* tissue, and 60 mL of hypolimnetic pond water. Hypolimnetic water was unfiltered to preserve the chemistry and microbial communities specific to each pond, and macrophyte tissue was consistently harvested from the leaf of the plant.

Following assembly, bottles were injected with 600 mL of ambient air and gently swirled for five minutes while an exit needle provided an outlet for the air initially held in the bottle (West et al. 2012). Ambient air was added to better mimic the natural atmosphere such that CH_4 production and oxidation could simultaneously occur within the bottle. Airtight serum bottle caps prevented further gas exchange with the atmosphere. A 10-mL gas sample was collected from the bottle headspace once per week for four weeks. The first sampling event occurred one week after incubation assembly. Samples were stored in individual 2-mL pre-evacuated glass vials and stored upside-down in water until analysis (Vizza et al. 2017b). To maintain atmospheric pressure, 10 mL of ambient air was injected into the incubation bottle following sample collection. Methane and CO_2 concentrations were analyzed using a gas chromatograph equipped with a flame ionization detector at the University of Notre Dame Environmental Research Center (UNDERC) in Land O'Lakes, WI, USA. Bottle-specific CH_4 and CO_2 production rate ($\mu mol\ day^{-1}$) were calculated by regressing gas concentrations over incubation time. All regressions were linear, suggesting that slurries did not undergo large changes in anoxia during the study period.

Oxygen was regularly replenished in these incubations after sampling using the 10-ml injections of ambient air, such that concurrent CH_4 production and oxidation could take place. Measured CH_4 concentrations, therefore, reflected net CH_4 production rates. Similarly, CO_2 concentrations also reflect multiple processes, including aerobic decomposition, acetoclastic methanogenesis, which forms both CH_4 and CO_2 as by-products, and CH_4 oxidation. Incubations were held at a constant temperature (14.2 ± 0.6 °C; mean ± sd) in the dark throughout the duration of the study. Since ambient incubation temperature was generally lower than average pond temperature (July: 17.3 ± 2.4 °C), estimated rates of net CH_4 and CO_2 production are assumed to be conservative.

Nuphar Stable Isotope Analysis Five random *Nuphar* tissue samples were frozen immediately upon field collection to measure the initial $\delta^{13}C$ of the tissue before incubation. Incubated *Nuphar* tissue was collected from the bottles following the fourth and final gas sampling event (after 28 days). Tissue samples were rinsed of sediment, dried at 60 °C, and pulverized using a mortar and pestle. Stable isotope ratios were determined using a Carlo Erba Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ, USA) coupled to a Finnigan Delta Plus Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) at the University of Notre Dame Center for Environmental Science and Technology (CEST). Stable isotope values were expressed in δ notation as per mille (‰) as defined by the following expression:

$$\delta^{13}C = \left[\left(R_{sample}/R_{standard}\right)-1\right] \times 1000,$$

where R is the isotope ratio $^{13}C/^{12}C$. Data were deemed acceptable if the standard deviation of acetanilide standards during the run was less than 0.2 ‰ for $\delta^{13}C$.

Field Experiments: In Situ Aerobic Decomposition, CH_4 Production, and $\Delta\delta^{13}C$

In Situ Aerobic Decomposition Cotton strips consisting of 95% cellulose were used to assess overall decomposition rates, as approximated by loss in tensile strength (Tiegs et al. 2013). According to Tiegs et al. (2013), the composition of the cotton strips makes its decomposition comparable to that of plant litter, the bulk of which is comprised of cellulose. Although the estimated decomposition rate encompasses both aerobic and anaerobic processes, we assume that the bulk of organic matter processing is due to aerobic activities as the dissolved oxygen levels in pond hypolimnia ranged from 2.2 ± 1.4 mg L^{-1} to 9.3 ± 0.3 mg L^{-1}.

In July 2013, two coarse-mesh bags with a pore size of 5 × 3 mm (Cady Bag Company, LLC, Pearson, GA, USA) were deployed at five sites representative of each pond's different habitats for nine study ponds: one bag contained three

standardized cotton strips to assess overall decomposition rate and one bag contained three strips of *Nuphar* tissue (0.7 g wet mass per *Nuphar* strip). Mesh bags were tethered to a metal rod and submerged to rest at the sediment-water interface (0.65 ± 0.1 m). One cotton strip and one *Nuphar* tissue sample from each site were retrieved at 21 and 35 days post-deployment in 2013. Decomposition bags and the final cotton strip were retrieved in June 2014 (average of 342 days post-deployment). After one year of deployment, *Nuphar* strips had completely decomposed. *Nuphar* tissue samples were analyzed for $\delta^{13}C$ as with the bottle incubation tissue samples. Estimating in situ aerobic decomposition was not possible from the change in mass of the *Nuphar* strips as they had often disintegrated such that it was not possible to collect the entire strip with confidence.

In contrast, tensile strength of all cotton strips was measured with confidence using a Mark-10 MG100 Tensiometer at 2 cm/s (after Tiegs et al. 2013). Percent loss in tensile strength of the cotton strip, a proxy for aerobic decomposition rate, was approximated using initial tensile strength and remaining tensile strength following one year of deployment. The percent loss in tensile strength was standardized over the number of degree-days accumulated in each pond, as measured with Hobo data loggers (Tiegs et al. 2013).

Bottle Incubations Potential CH_4 production rates for each pond were also determined in 2013 using bottle incubation sediment slurries, but without the addition of the *Nuphar* tissue sample. In 2013, sediment slurries were purged with nitrogen gas (N_2) so as to preclude the possibility of CH_4 oxidation. Bottles were sampled three times (one, four, and nine days after assembly). Bottle-specific CH_4 production rates were calculated in the same way as in 2014 and then converted to areal rates (μmol m^{-2} day^{-1}) assuming an active sediment layer depth of 20 cm (West et al. 2016). Bottle-specific CH_4 production rates were converted to areal rates in 2013 to better estimate the ecosystem CH_4 production potential, as opposed to the bottle-specific CH_4 production rates of the sediment slurries in 2014.

Pond Characterization: Water and Sediment Chemistry

Temperature and dissolved oxygen profiles were measured in situ at each pond with a YSI Pro Plus multi-parameter water quality meter monthly over the course of the season in 2014. Water column chlorophyll *a* was measured using buffered acetone extraction and fluorometry, and dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC-V total organic carbon analyzer (West et al. 2016).

A portion of sediment collected for incubations was frozen immediately upon field collection for later analysis. After thawing, sediment organic matter (OM) content was assessed by drying a portion of the sediments for at least 48 h at 60 °C and recording the dry weight. Sediments were then combusted at 500 °C for four hours, re-wetted, and dried at 60 °C for another 48 h before re-weighing. Sediment OM was estimated as the percent mass lost during combustion and scaled up to estimate the total OM in each incubation bottle.

To assess acetate, nitrate, and sulfate concentrations in sediment porewater, a portion of sediment (~50 mL) was centrifuged for 45 min at 4 °C at approximately 4000 rotations per minute. The total volume of supernatant was recorded and analyzed on a Dionex ICS-5000 for each analyte. Porewater concentrations were scaled to the total amount of sediment in each incubation bottle (μmol mL^{-1} of sediment). Major physicochemical characteristics of the nine study ponds are reported in Table 1.

Statistical Analyses

In the laboratory experiments, change in $\delta^{13}C$ ($\Delta\delta^{13}C$) was calculated as the difference in $\delta^{13}C$ of the *Nuphar* strip before and after incubation. In the field experiments, $\Delta\delta^{13}C$ was calculated as the difference in $\delta^{13}C$ of the *Nuphar* strips before and after 35 days of in situ deployment. We chose to calculate $\Delta\delta^{13}C$ of the *Nuphar* strips using $\delta^{13}C$ after 35 days of deployment rather than 21 days because the $\Delta\delta^{13}C$ following 21 days in situ was considerably lower in magnitude (−0.15 ± 0.6 ‰) than $\Delta\delta^{13}C$ following 35 days of deployment (−0.57 ± 0.6 ‰). We conducted paired t-tests (Zar 2010) to determine whether $\delta^{13}C$ of the *Nuphar* strip differed significantly before and after incubation in both the laboratory and field experiments; assumptions of normality were confirmed using Shapiro-Wilk tests (Zar 2010).

We used simple linear regression (Zar 2010) to assess the relationship between $\Delta\delta^{13}C$ of *Nuphar* tissue samples and CH_4 production and also between $\Delta\delta^{13}C$ and aerobic decomposition (CO_2 production rate or percent loss in tensile strength of the cotton strips) for both laboratory and field experiments. We performed Shapiro-Wilk tests on each model's residuals (Zar 2010); as a result, CH_4 production was log-transformed for field experiments only to meet assumptions of normality. Multiple linear regression (Zar 2010) was used to determine the relative strength of each $\Delta\delta^{13}C$ predictor for each experiment, if both factors were found to be significant. A significance level, or α, of 0.05 was used for all regression analyses. To compare models predicting changes in $\delta^{13}C$, this study used Akaike Information Criterion (AIC)-based model selection (Burnham and Anderson 2002), which identifies the most likely model given the data while also penalizing for model complexity. The model with the lowest AIC score is considered the most likely given the data. For example, a model with AIC value 4 units higher than another is considered to have relatively low support (Burnham and Anderson 2002). All

Table 1 Physico-chemical characteristics of the nine study ponds on the Copper River Delta. Mean temperature and dissolved oxygen are reported for the hypolimnion of each pond over the summer of 2014

Pond	Hypolimnion Temperature (°C)	Hypolimnion Dissolved Oxygen (mg L^{-1})	Chlorophyll *a* (μg L^{-1})	DOC (mg L^{-1})	% OM	PW Acetate (μmol mL^{-1} of sediment)	PW Nitrate	PW Sulfate
Eyak North	15.3 ± 0.9	7.4 ± 2.1	25.2 ± 45.3	6.7 ± 1.5	2.0 ± 0.5	8.7 ± 9.3	0.08 ± 0.06	7.7 ± 7.4
Eyak South	16.1 ± 1.3	6.9 ± 2.0	13.4 ± 16.3	5.2 ± 0.6	2.1 ± 0.7	1.4 ± 0.8	0.07 ± 0.07	4.1 ± 2.3
Lily	13.1 ± 0.8	3.2 ± 1.5	6.1 ± 9.0	3.8 ± 2.2	2.0 ± 0.6	3.7 ± 4.7	0.07 ± 0.03	0.4 ± 0.2
Rich Hate Me	11.6 ± 2.9	2.2 ± 1.4	1.9 ± 1.7	2.1 ± 0.5	2.2 ± 2.8	1.7 ± 2.8	0.11 ± 0.11	1.4 ± 3.1
Scott South	14.2 ± 0.9	8.6 ± 3.8	4.2 ± 2.1	2.0 ± 0.4	1.9 ± 3.6	2.5 ± 2.4	0.13 ± 0.13	0.7 ± 0.6
Storey North	16.8 ± 1.0	8.2 ± 0.3	8.8 ± 2.1	11.4 ± 1.0	1.8 ± 0.4	0.9 ± 1.1	0.09 ± 0.14	1.4 ± 0.8
Storey South	16.6 ± 2.4	9.3 ± 0.3	3.3 ± 1.2	4.4 ± 0.3	1.5 ± 2.2	1.2 ± 1.4	0.06 ± 0.06	2.0 ± 2.4
Tiedeman North	16.6 ± 1.1	8.4 ± 2.1	16.4 ± 26.2	6.6 ± 0.5	3.0 ± 3.0	1.9 ± 1.3	0.08 ± 0.07	2.8 ± 2.2
Tiedeman South	15.4 ± 1.4	8.6 ± 2.0	21.3 ± 38.6	5.1 ± 0.5	2.3 ± 0.6	1.4 ± 0.7	0.12 ± 0.12	3.7 ± 2.8

Water column chlorophyll *a*, dissolved organic carbon (DOC), percent sediment organic matter (% OM), porewater (PW) acetate, PW nitrate, and PW sulfate are also reported below. All values are expressed as summertime mean ± sd

statistical analyses were conducted in R statistical software version 3.2.2 (R Core Development Team 2015).

Results

Laboratory Experiments: Net CH_4 Production, CO_2 Production, and $\Delta\delta^{13}C$

The $\delta^{13}C$ of all *Nuphar* strips significantly decreased during incubation with an initial, pre-incubation $\delta^{13}C$ of −22.1 ± 0.1 ‰ and a post-incubation $\delta^{13}C$ of −23.2 ± 0.7 ‰ (paired t-test: $t = 7.44$; df = 26; $p < 0.001$). Mean net CH_4 and CO_2 production rates ranged from 5 to 110 μmol d^{-1} and 270 to 710 μmol d^{-1}, respectively. Net CH_4 production rate and the change in $\delta^{13}C$ ($\Delta\delta^{13}C$) were negatively related (Fig. 1; $R^2 = 0.54$, df = 7; $p = 0.015$); we observed a larger decrease in $\delta^{13}C$ with greater CH_4 production. Finally, CO_2 production rate and $\Delta\delta^{13}C$ exhibited a similar relationship to that of CH_4 and $\Delta\delta^{13}C$ (Fig. 2; $R^2 = 0.46$; df = 7; $p = 0.026$), such that incubations with higher CO_2 production rates also generated larger decreases in detrital $\delta^{13}C$.

Multiple linear regression was used to predict $\Delta\delta^{13}C$ using laboratory experiment estimates of net CH_4 and CO_2 production potential. Net CH_4 production was a significant predictor of $\Delta\delta^{13}C$ ($t = -2.57$; $p = 0.042$), but not CO_2 production ($t = -2.21$; $p = 0.070$). Although CO_2 production was not a statistically significant predictor of $\Delta\delta^{13}C$, the multiple linear regression including both factors ($F_{(2,6)} = 10.4$; $R^2 = 0.78$; AIC = 11.8; $p = 0.011$) was a stronger model than a simple linear regression using net CH_4 production alone (Fig. 1; AIC = 15.2). Because the AIC value of the simple regression

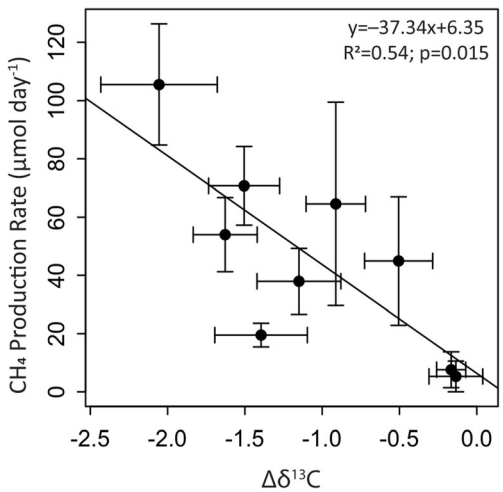

Fig. 1 Net CH_4 production rate (μmol day^{-1}) and change in *Nuphar* $\delta^{13}C$ ($\Delta\delta^{13}C$, ‰) in laboratory experiments were negatively related ($R^2 = 0.54$; df = 7; $p = 0.015$). Error bars depict standard error

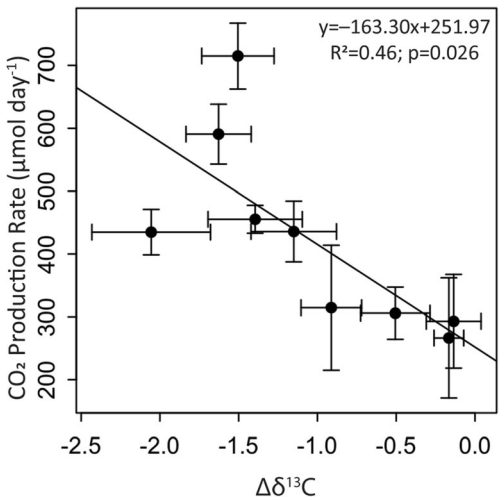

Fig. 2 Net CO_2 production rate (μmol day^{-1}) and change in *Nuphar* $\delta^{13}C$ ($\Delta\delta^{13}C$, ‰) in laboratory experiments were negatively related ($R^2 = 0.46$; df = 7; $p = 0.026$). Error bars depict standard error

model was 4.6 points higher than the multiple regression model, we can infer that the latter model, including both net CH_4 and CO_2 production as factors, was substantially stronger.

Field Experiments: In Situ Aerobic Decomposition, CH_4 Production, and $\Delta\delta^{13}C$

On average, the $\delta^{13}C$ of the *Nuphar* strips decreased slightly during in situ deployment from an initial, pre-deployment $\delta^{13}C$ of −25.3 ± 0.5 ‰ to a post-deployment $\delta^{13}C$ of −25.9 ± 0.8 ‰ (paired t-test: $t = 2.91$; df = 26; $p = 0.0073$), but the magnitude of change in $\delta^{13}C$ as well as the rate of change (−0.6 ‰, −0.04 ‰ per day) was about half of what we observed in laboratory experiments (−1.1 ‰, −0.02 ‰ per day). Aerobic decomposition rates (as indicated by loss in tensile strength of the cotton strips) varied from 0.014 to 0.047% of tensile strength loss by degree day, but was not significantly related to $\Delta\delta^{13}C$ of the in situ *Nuphar* strip (Fig. 3; $R^2 = -0.14$; df = 7; $p = 0.88$). In contrast, CH_4 production varied from 17 to 2400 μmol m^{-2} d^{-1}, and this factor (log-transformed) was positively related to $\Delta\delta^{13}C$ of the in situ *Nuphar* strip (Fig. 4; $R^2 = 0.65$; df = 7; $p = 0.0054$). As CH_4 production rate increased, $\Delta\delta^{13}C$ of the *Nuphar* strip approached zero, indicative of very little net change in the isotope signature.

Discussion

Our study examined how the $\delta^{13}C$ of decomposing organic matter could reflect the combined influence of aerobic decomposition and CH_4 production. The $\delta^{13}C$ of detritus decreased during incubation in both the laboratory and the field. Although the change in $\delta^{13}C$ was related to CH_4 production

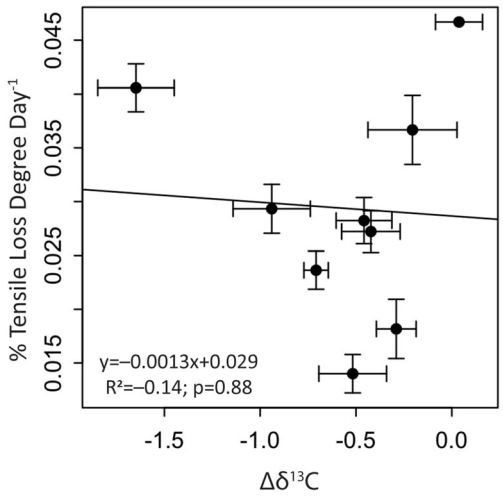

Fig. 3 Percent tensile loss per degree day (a proxy for aerobic decomposition rate) in cotton strips was not related to the change in *Nuphar* $\delta^{13}C$ ($\Delta\delta^{13}C$, ‰) in field experiments ($R^2 = -0.14$; df = 7; $p = 0.88$). Error bars depict standard error

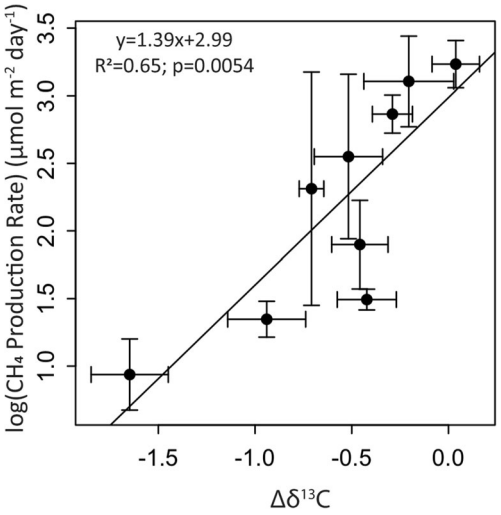

Fig. 4 Log CH_4 production rates (μmol m^{-2} day^{-1}) estimated from bottle incubations were positively related to changes in *Nuphar* $\delta^{13}C$ ($\Delta\delta^{13}C$, ‰) observed in field experiments ($R^2 = 0.65$; df = 7; $p = 0.0054$). Error bars depict standard error

potentials in both experiments, the direction (i.e., positive or negative) of this relationship depended upon the scale of study. Such differences could reflect the physical and chemical constraints under which the studies were operated, such as openness of the system, presence of wind, oxygen availability, etc. Because we did not directly measure CH_4 oxidation rates, it is challenging to identify the mechanisms responsible for isotopic changes. Nevertheless, the clear relationships between CH_4 production rates and detrital $\delta^{13}C$ suggest that CH_4 cycling influences detrital processes in wetlands.

Laboratory Experiments: CH_4 Production, CO_2 Production, and $\Delta\delta^{13}C$

The $\delta^{13}C$ of *Nuphar* tissue decreased during laboratory incubations with isotopic changes being related to both aerobic decomposition and net CH_4 production rates. However, the rates of these processes were not correlated with each other. As CO_2 production rates increased, we observed a decrease in the $\delta^{13}C$ of the *Nuphar* tissue, which suggests depletion of isotopically heavy, labile carbon during decomposition (Benner et al. 1987; Wedin et al. 1995; Lehmann et al. 2002). Since aerobic decomposition (Fig. 5b) and CH_4 production followed by oxidation (Fig. 5d) both produce CO_2 and result in similar isotopic patterns in detritus, it can be challenging to tease apart the mechanisms responsible for producing CO_2 and altering $\delta^{13}C$ of the *Nuphar* tissue.

Both net CH_4 and CO_2 production resulted in decreases in $\Delta\delta^{13}C$, but net CH_4 production was the stronger predictor of $\Delta\delta^{13}C$ in laboratory experiments. Thus, CH_4 production and oxidation were likely more important processes influencing $\Delta\delta^{13}C$ of the *Nuphar* tissue in the laboratory experiments even though CO_2 production rates were much greater,

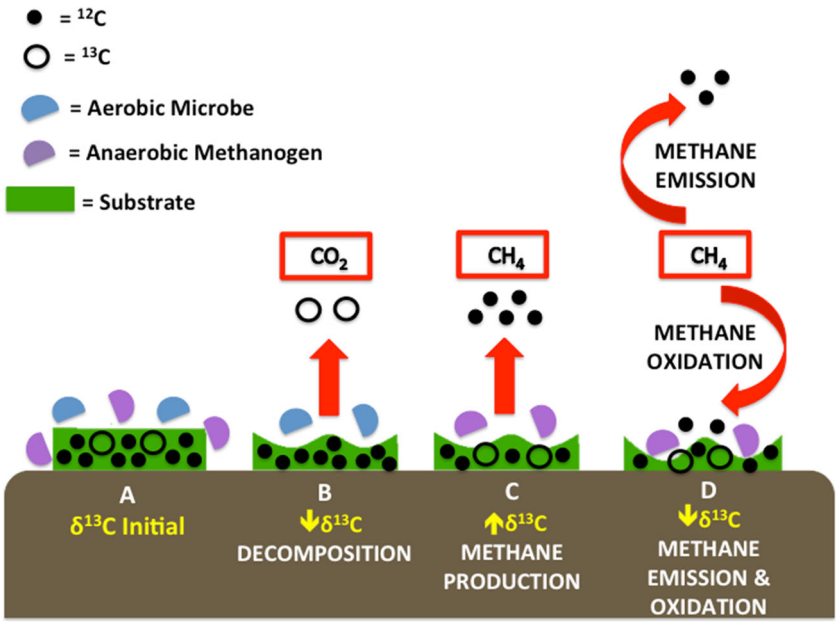

Fig. 5 Conceptual figure depicting the mechanisms by which anaerobic and aerobic processes could alter the $\delta^{13}C$ of decomposing organic matter. Panel A represents the initial $\delta^{13}C$ prior to decomposition. In panel B, aerobic microbial decomposers consume labile carbon enriched with the heavier ^{13}C, causing the $\delta^{13}C$ of the remaining organic matter to decrease. In panel C, methanogens preferentially incorporate the lighter ^{12}C, causing the $\delta^{13}C$ of the remaining organic matter to increase. Finally, in panel D, CH_4 production followed by oxidation causes a decrease in $\delta^{13}C$ as methane-oxidizing bacteria assimilate the lighter ^{12}C in CH_4

probably due to the distinctly low $\delta^{13}C$ of CH_4 (−110 to −60 ‰; Wedin et al. 1995; Conrad 2005). Although our study specifically looks at detritus, Grey et al. (2004) described the process of incorporating CH_4 into the food web as likely to increase intraspecific variability in the $\delta^{13}C$ values of consumers. Therefore, we might expect for concurrent CH_4 production and oxidation to have a more pronounced effect just because of the sheer magnitude of isotopic changes (Conrad 2005). Nonetheless, CO_2 production rates were also an important predictor of $\Delta\delta^{13}C$ after accounting for CH_4 production, which suggests that aerobic decomposition also influences detrital $\delta^{13}C$ even though it played a smaller role in our laboratory experiments.

The strong negative relationship between net CH_4 production rates and $\Delta\delta^{13}C$ of detritus supports the mechanism of concurrent CH_4 production and oxidation, through which CH_4 may be incorporated into the food web (Fig. 5d; Bastviken et al. 2003; Kohzu et al. 2004; Eller et al. 2005; Jones et al. 2008; Hershey et al. 2015; DelVecchia et al. 2016). Specifically, the more CH_4 that is produced, the more likely it is to be assimilated into detrital tissue by methane-oxidizing bacteria (MOB). Although we did not measure CH_4 oxidation in our incubations, CH_4 availability is often the limiting factor for MOB (Whalen 2005), particularly when oxygen is abundant. The assimilation of CH_4-derived carbon by MOB has previously been suggested as a potential mechanism for structuring $\delta^{13}C$ of organic carbon sources in wetlands (Bunn and Boon 1993), but we are unaware of any other studies that directly link isotopic changes to process rates in detritus. Although other studies have shown that CH_4 oxidation can alter consumer $\delta^{13}C$ (e.g., Bastviken et al. 2003; Hershey et al. 2015) or that of dissolved inorganic carbon (Corbett et al. 2013), we believe that our study is the first to demonstrate that CH_4 cycle processes are capable of altering detrital $\delta^{13}C$ in a predictable way.

Field Experiments: In Situ Aerobic Decomposition, CH_4 Production, and $\Delta\delta^{13}C$

We observed a small decrease in the $\Delta\delta^{13}C$ of the *Nuphar* tissue deployed in situ, which suggests that selective preservation of the isotopically light ^{12}C occurred during aerobic decomposition (Fig. 5b). Lignin compounds can decompose slowly and are usually rich in the lighter ^{12}C isotope, such that compounds rich in the heavier ^{13}C degrade first, which should result in a lower $\delta^{13}C$ during aerobic decomposition (Benner et al. 1987). However, we observed no significant relationship between $\Delta\delta^{13}C$ and aerobic decomposition rates as measured with cotton strips in the field experiments. Wedin et al. (1995) argued that the mixing of external C (e.g., from the sediments) with the original C of the plant tissue via fungal hyphae or microbes can affect $\Delta\delta^{13}C$ in unpredictable ways. In addition, ^{13}C enrichment of microbial biomass may counteract the loss of enriched labile compounds (Fellerhoff et al. 2003; Dai et al. 2005; Troxler and Richards 2009; Rossi et al. 2010). Microbial activity or mixing of other external carbon may have therefore confounded the ability of aerobic decomposition to alter $\Delta\delta^{13}C$ of *Nuphar* tissue in a predictable manner in our study.

Despite the decreasing trend in $\delta^{13}C$ during field incubation, the isotopic signature of *Nuphar* detritus appeared to be more predictably influenced by CH_4 production rates measured in bottle incubations than aerobic decomposition rates measured in field experiments. Even though CH_4 production was estimated at a different scale, it is possible that methanogenesis, because of its large effect on $\delta^{13}C$ (Conrad

2005) swamped the change due to other processes. For example, Grey et al. (2004) demonstrated that CH_4 as a carbon source can increase consumer variability in $\delta^{13}C$, thus confounding interpretation of food webs. Similarly, the process of starvation could disproportionately affect nitrogen stable isotope signatures, making it difficult to track an organism's typical food sources (Gannes et al. 1997). Although this "swamping" effect of certain processes could have a negative effect for studying food webs, it may be an advantage for tracing processes of the CH_4 cycle. Specifically, the positive relationship between $\Delta\delta^{13}C$ of *Nuphar* tissue and CH_4 production rates could reflect the isotopic pathway predicted if methanogens were playing even a small role in decomposing detritus at the sediment-water interface, but concurrent oxidation was not taking place (Fig. 5c).

CH_4 Processes Vary by Scale

CH_4 production potentials were strongly related to $\Delta\delta^{13}C$ and were the stronger predictor of $\Delta\delta^{13}C$ over aerobic decomposition in both laboratory and field experiments. However, the direction of the relationship differed depending on how the experiment was conducted. In the laboratory experiments, CH_4 production rate was negatively related to $\Delta\delta^{13}C$, but was positively related to $\Delta\delta^{13}C$ in field experiments. The differing trends we observed could be simply a product of ecological scale alone (Carpenter et al. 1995). More specifically, studies at smaller scales, such as a bottle experiments, can fail to predict the responses evident at the ecosystem-scale, such as a pond or lake (Schindler 1998).

Contrasting results of experiments conducted at different scales could be due to the way experimental scale affects physical, chemical, and biological processes (Schindler 1998). For example, in our bottle experiments, incubations were isolated from the atmosphere (i.e., closed systems), which meant that any CH_4 produced was largely available for oxidation. As CH_4 becomes supersaturated in the headspace of the bottles, we might expect that some would diffuse back to the sediment-water interface. Assimilation of isotopically light CH_4 by MOB associated with the *Nuphar* tissue might therefore be reflected in the lower detrital $\delta^{13}C$. In field experiments, the positive effect of CH_4 production on $\Delta\delta^{13}C$ suggests a lack of significant MOB activity at the sediment-water interface where detritus tissue was deployed. In contrast to the bottles, the sediment-water interface of the pond is continuously stirred by wind resulting in well-mixed CH_4 concentrations in the water column (Vizza et al. in review). Without stratification of the water column, which does not occur during the growing season (C. Vizza, unpublished data), CH_4 is either emitted to the atmosphere or temporarily stored in the water column. Therefore, it is less likely that concurrent CH_4 production and oxidation (Fig. 5d) takes place at the sediment-water interface of these ponds than in our bottle incubations.

Conclusions

As climate change modifies the relative rates of aerobic and anaerobic decomposition processes, tracing and identifying dominant carbon processes is essential to quantifying the contributions of wetlands to the global carbon budget, such as the 283,000 ha of the CRD. Our study demonstrates that CH_4 cycling can contribute to predictable patterns and may swamp the effect of other decomposition processes on the $\delta^{13}C$ of wetland detritus. The disproportionate effect that CH_4 production and oxidation appear to have on $\delta^{13}C$ may be an advantage for tracing these processes through detritus. Future studies should use mass balance experiments (e.g., Corbett et al. 2013) to attribute isotopic changes to specific processes and to determine the extent to which these processes differ due to experimental scale.

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Compliance with Ethical Standards

Conflict of Interest Statement The authors declare that they have no conflicts of interest.

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