Biogeochemistry (2014) 117:473–490
DOI 10.1007/s10533-013-9894-5

Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland

Ember M. Morrissey · David J. Berrier · Scott C. Neubauer · Rima B. Franklin

Received: 15 February 2013 / Accepted: 18 July 2013 / Published online: 3 August 2013

Abstract To gain a more mechanistic understanding of how soil organic matter (OM) characteristics can affect carbon mineralization in tidal freshwater wetlands, we conducted a long-term in situ field manipulation of OM type and monitored associated changes in carbon dioxide (CO_2) and methane (CH_4) production. In addition, we characterized microbial community structure and quantified the activity of several extracellular enzymes (EEA) involved in the acquisition of carbon, nitrogen, and phosphorus. Treatments included a plant litter addition, prepared using naturally-senescing vegetation from the site, and a compost amendment, designed to increase the concentration of aged, partially humified, OM. Both types of OM-amended soils had CO_2 production rates 40–50 % higher than unamended control soils, suggesting that the added OM had inherently higher quality and/or availability than the native soil OM. Rates of CO_2 production were not correlated with microbial community structure or EEA except a modest relationship with cellulose breakdown via the K_m of β-1,4-glucosidase. We interpret this lack of correlation to be a consequence of high functional redundancy of microorganisms that are capable of producing CO_2. Rates of CH_4 production were also influenced by OM quality, increasing by an order of magnitude with plant litter additions relative to compost-amended and control soils. Unlike CO_2, rates of CH_4 production were significantly correlated with the microbial community structure and with enzyme kinetic parameters (V_{max} and K_m) for both carbon (β-1,4-glucosidase, 1,4-β-cellobiosidase, and β-D-xylosidase) and nitrogen acquisition (leucyl aminopeptidase). The monophyletic nature of methanogenic archaea, combined with their reliance on a small select group of organic substrates produced via enzyme-mediated hydrolysis and subsequent bacterial fermentation, provides a basis for the strong links between microbial community structure, EEA, and CH_4 production. Our results suggest that incorporating microbial community structure and EEA into conceptual models of wetland OM decomposition may enhance our mechanistic understanding of, and predictive capacity for, biogeochemical process rates.

Responsible Editor: Matthew David Wallenstein

Electronic supplementary material The online version of this article (doi:10.1007/s10533-013-9894-5) contains supplementary material, which is available to authorized users.

Present Address:
E. M. Morrissey · D. J. Berrier · S. C. Neubauer · R. B. Franklin (✉)
Department of Biology, Virginia Commonwealth University, 1000 W Cary Street, Richmond, VA 23284, USA
e-mail: rbfranklin@vcu.edu

S. C. Neubauer
Baruch Marine Field Laboratory, University of South Carolina, P.O. Box 1630, Georgetown, SC 29442, USA

Keywords Microbial ecology · Decomposition · Anaerobic · Methanogenesis · Wetland restoration

Introduction

Though wetlands account for only ~10 % of terrestrial land area (Zedler and Kercher 2005), their influence on the global carbon (C) cycle is disproportionately large. For example, wetland soils store 45–70 % of terrestrial organic C (Mitra et al. 2005) and are responsible for nearly 25 % of global methane (CH_4) emissions (Conrad 2009). Carbon sequestration is enhanced in these environments due to a combination of high primary productivity and slow rates of decomposition. The quality and availability of organic material (OM), as well as an interacting suite of environmental factors (e.g., soil moisture and pH), determine the degree of OM storage versus mineralization to carbon dioxide (CO_2) and/or CH_4 (Segers 1998; Megonigal et al. 2004; Kayranli et al. 2010). Understanding how OM properties affect the balance between sequestration and mineralization is particularly relevant in the context of wetland restoration and creation, as these activities often involve OM amendments to hasten the development of organic-rich reduced soils (Davis 1995; Mitsch and Gosselink 2000).

The OM transformations that result in the production of CH_4 and/or CO_2 are driven by diverse microbial communities that depend on extracellular enzyme activity (EEA) to breakdown complex organic polymers into soluble compounds that can be transported into the cell and metabolized. Consequently, this depolymerization is the putative rate-limiting step in OM decomposition (Sinsabaugh et al. 1991) and enzyme depolymerization rates have been associated with microbial respiration in aquatic ecosystems (Arnosti and Holmer 2003; Arnosti and Jørgensen 2006; Baltar et al. 2009). In soils however, surprisingly few studies have explored the relationship between EEA and either C mineralization rates (Freeman et al. 1997, 2001; Allison and Vitousek 2005) or the composition of the associated microbial communities (Kourtev et al. 2003; Gallo et al. 2004; Costa et al. 2007; Kaiser et al. 2010). Even less consideration has been given to how these three components interact. Though some studies have found a strong relationship between microbial community composition and activity (Cleveland et al. 2007; Li et al. 2011; Goberna et al. 2012; Lazar et al. 2012), many others have not (Galand et al. 2003; Liu et al. 2011; Fromin et al. 2012). One common hypothesis for this inconsistency is that the high functional redundancy within microbial communities limits our detection of community structure–function relationships, especially when considering processes with a relatively ubiquitous distribution among taxa (e.g., respiration to CO_2, Griffiths et al. 2000; Nannipieri et al. 2003). Some have proposed that community structure only regulates "narrowly" distributed functions (Schimel 1995; McGuire and Treseder 2010), i.e., ones that are performed by only a small group of organisms with specialized physiological pathways such as methanogens.

In this study, we examined how microbial community structure and EEA regulate greenhouse gas (CO_2 and CH_4) production in wetlands receiving long-term in situ soil amendments of either plant litter or compost. This research was conducted in a recently restored tidal freshwater wetland, and the results have implications for recovering ecosystem services facilitated by OM-rich soils in impaired wetlands while simultaneously minimizing the production of CH_4.

Methods

Experimental design

In January 2011, soil (5–15 cm depth) was collected from the middle of a 30-ha tidal freshwater wetland at Virginia Commonwealth University's Walter and Inger Rice Center for Environmental Life Sciences (James River, Charles City County, VA, USA; 37°20′05″N, 77°12′27″W). This wetland was an impounded lake for nearly 70 years before a storm breached the dam and restored natural wetland hydrology to the site in 2006. The site was continually saturated, usually with standing water on the surface, and dominated by obligate wetland vegetation such as *Leersia oryzoides*, *Juncus effusus*, and *Nuphar luteum*. The soil had an OM content of 8 %, a C:N ratio of 10 (by mass), a pH of 6.4, and soil texture was 30 % sand, 55 % silt, and 15 % clay. Following extensive homogenization in the laboratory, one fraction of the collected soil was amended with plant litter (standing

dead material of the above mentioned species harvested from the field site in early December 2010; 99 % OM content, C:N = 72). A second fraction was amended with compost (Black Kow, Oxford, FL; organic blend containing 26 % OM, C:N = 18). The compost and litter amendments were similar in particle size (0.1–5 mm in diameter). Amendments were added to raise the soil OM content to approximately double ambient levels. A third soil fraction was unamended and served as an experimental control.

Litter bags (12 cm × 22 cm), constructed of 0.5-mm Nitex mesh (Wildlife Supply Company, Buffalo, NY, USA), were filled with 400 ml (~215 g dry weight) of control or amended soil for in situ incubation. In January 2011, 10 bags of each type were buried (5–15 cm depth) at random locations within a single 20 × 20 m experimental plot near the soil collection site. Samples were incubated for either 6 or 18 months (until July 2011 and 2012, respectively). At each sampling event, five replicate bags of each type were collected, as were five intact field cores. Samples were placed in airtight plastic bags, quickly transported back to the laboratory, and subdivided for soil characterization (200 g) and molecular genetic analyses (5 g, immediately archived at −20 °C). For the 18-month sampling, subsamples were also removed for determination of CO_2 and CH_4 production (40 g, stored for 7 days at 4 °C) and analysis of extracellular enzyme activities (10 g, stored up to 5 days at 4 °C). The field cores were analyzed only for soil properties and gas production rates, and served to provide context for interpreting the experimental manipulations.

Environmental analyses

At both the 6- and 18-month sampling events, soil redox potential and pH were measured using a Hanna Combo pH and ORP probe (QA Supplies, Norfolk, VA, USA). Soil moisture (%) was determined gravimetrically (100 ± 5 °C for 72 h), and OM (%) was measured as the mass loss on ignition following combustion at 500 °C for 4 h. Total carbon and nitrogen contents were determined using a Perkin Elmer CHNS/O Analyzer (Waltham, MA, USA) following grinding and acidification of samples using 10 % hydrochloric acid.

In addition, at the 18-month sampling, porewater was extracted and analyzed for dissolved nutrient concentrations. Briefly, water was collected from 50-ml soil samples by centrifugation (3,000×*g* for 15 min), filtered using a 0.45 μm pore-size mixed cellulose ester syringe filter, and stored at −20 °C until it could be analyzed for: (i) ammonium (NH_4^+) using the indophenol colorimetric assay of Grasshoff et al. (1983), (ii) dissolved organic carbon (DOC) using a Shimazdu TOC analyzer (Columbia, MD, USA), (iii) total dissolved nitrogen (TDN) and phosphorus (TDP) using a Skalar Sans Plus System (Buford, GA, USA).

Molecular analyses

Whole-community DNA was extracted from 0.5-g subsamples of soil using the MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA, USA) and stored at −20 °C. DNA purity and concentration were analyzed using a Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). All DNA extracts and PCR products were verified using agarose gel (1.5 %) electrophoresis and ethidium bromide staining.

Microbial gene abundance via qPCR

Quantitative polymerase chain reaction (qPCR) assays were performed to assess the genetic potential of the microbial communities (Smith and Osborn 2009). Assays were performed using SsoAdvanced SYBR Green qPCR Supermix (BioRad, Hercules, CA, USA) and a BioRad CFX 96 Real-Time System; data were analyzed using Bio-Rad CFX Manager Version 2.1. Results were reported as the $\log_{(10)}$ of the number of gene copies per gram of OM after averaging three technical replicates per sample and comparing to appropriate standard curves.

To estimate total bacterial abundance, the primers Eub338 and Eub517 were used to target the *16S rRNA* gene (Fierer et al. 2005). Genomic DNA from *Escherichia coli* (Strain 11775, ATCC, Manassas, VA, USA) was used to establish the standard curve (average efficiency = 101 %, $r^2 = 0.99$). Reactions (20 μl) were performed with 1.2 ng DNA template and 0.1 μM concentrations of each primer; thermal cycling conditions were: 95 °C for 4 min, and 40 cycles of 30 s at 95 °C, 30 s at 55.5 °C, and 60 s at 72 °C. The abundance of archaea was estimated using the primers Arch 967F and Arch-1060R (Karlsson et al. 2012), again targeting the *16S rRNA* gene. Standard curves (average efficiency = 94 %,

$r^2 = 0.99$) used genomic DNA from *Methanococcus voltae* (Strain A3, ATCC). Reactions (20 µl) used 2 ng DNA template and 0.3 µM primers; thermal cycling conditions were: 95 °C for 5 min, and 40 cycles of 20 s at 95 °C, 20 s at 59 °C, and 30 s at 72 °C. Finally, methanogen abundance was estimated using the mlas and mcrA-rev primers to target the methyl coenzyme-M reductase encoding *mcrA* functional gene (Steinberg and Regan 2009). As with archaea, *Methanococcus voltae* genomic DNA was used for the standard curve (average efficiency = 92 %, $r^2 = 0.99$). Reactions (20 µl) had 2 ng DNA template and 0.56 µM mlas and 0.70 µM mcrA-rev primer concentrations; thermal cycling conditions were: 95 °C for 5 min, and 50 cycles of 20 s at 95 °C, 20 s at 59 °C, and 45 s at 72 °C.

Community structure via T-RFLP

Microbial community structure was analyzed using terminal restriction fragment length polymorphism (T-RFLP) targeting the *16S rRNA* gene of bacteria and archaea and the functional gene *mcrA* for methanogens. All PCR reactions (50 µl) were performed with 10 mM Tris HCl (pH 8.3), 50 mM KCl, 200 µM of each dNTP, 20 µg BSA, and 2.5 units of AmpliTaq DNA polymerase (reagents obtained from Applied Biosystems, Foster City, CA, USA). Bacteria PCRs including the domain-specific primers 27F (labeled with FAM) and 1492R at a concentration of 0.2 µM each (Lane 1991), 3.0 µM $MgCl_2$, and 1.2 ng DNA. Thermal cycling conditions were: 95 °C for 3 min, 30 cycles of 45 s at 95 °C, 60 s at 57 °C, 120 s at 72 °C, followed by 72 °C for 7 min (PTC-100 Thermal Controller, MJ Research, Waltham, MA, USA). Archaea PCRs included the primers 21F (labeled with FAM) and 958R (Cytryn et al. 2000), each at 0.2 µM, as well as 1.5 µM $MgCl_2$, and 4 ng DNA. Thermal cycling conditions were: 94 °C for 3 min, 34 cycles of 60 s at 95 °C, 60 s at 55 °C, 60 s at 72 °C, followed by 72 °C for 7 min. The methanogen *mcrA* gene was targeted with MLf (labeled with FAM) and MLr (Smith et al. 2007) in a reaction with 60 nM of each primer, 2 µM $MgCl_2$, and 4–8 ng DNA. Touchdown PCR thermal cycling conditions were: 95 °C for 3 min, six cycles of 45 s at 95 °C, 60 s at 56 °C (-0.5 °C $cycle^{-1}$), 60 s at 72 °C, followed by 31 cycles of 45 s at 95 °C, 60 s at 53 °C, 60 s at 72 °C and final extension at 72 °C for 7 min.

PCR products were purified using the MinElute 96 UF PCR purification kit (Qiagen, Valencia, CA, USA) prior to restriction enzyme digest (associated materials from New England Biolabs, Ipswich, MA, USA). Digests were conducted in 1× Buffer #4 with 30 ng BSA, using either 10 units of Hha1 (*16S rRNA*) or 20 units of RsaI (*mcrA*). After digestion (16 h at 37 °C, 20 min at 65 °C), amplicons were purified using the MinElute kit, recovered in molecular-grade water, and detected using capillary electrophoresis with a MegaBACE 1000 DNA Analysis System (Amersham Biosciences, Buckinghamshire, UK). An aliquot of 50–120 ng of purified, digested PCR product was combined with 0.3 µl of MapMarker 400 ROX ladder (Bioventures, Murfreesboro, TN, USA) plus 4.75 µl injection buffer (0.1 % Tween-20). Samples were injected at 3 kV for 100 s, and electrophoresed using genotyping filter set 1 for 100 min at 10 kV. T-RFLP fragments between 70 and 400 base pairs (bp) were analyzed using Fragment Profiler software (Version 1.2; Amersham Biosciences) using a 1 bp size differential and a 15 relative fluorescent unit peak height threshold. Samples were standardized by calculating peak area as a percent of the total sample fluorescence; peaks accounting for <1 % of total sample fluorescence were removed prior to analysis.

Extracellular enzyme activity (EEA)

Soil slurries were prepared fresh each day of analysis by sonicating 1.0 g soil in 100 ml sterile deionized water (15 W for 2 min; Misonix Sonicator 3000, Farmingdale, NY, USA). The slurries were kept on a shaker table (150 rpm) during use to prevent settling of the soil particles. The activities of five hydrolytic enzymes were measured using fluorometric assays following modified protocols from Stursova et al. (2006) and German et al. (2011) with reagents obtained from Sigma-Aldrich Co. Ltd. (Table 1). Three technical replicates of each sample were assayed at each of ten substrate concentrations, as were three negative (no sample) controls. For the methylumbelliferone (MUB) assays, MES buffer (0.1 M, pH 6.1) was used, and quench curves were established for each sample using a range from 0 to 9 nmol MUB. For the amino-4-methylcoumarin hydrochloride (AMC) assay, a Trisma buffer was used (50 mM, pH 7.8) and quench curves ranged from 0 to 7.5 nmol AMC.

Table 1 Summary of enzymes assays used in this study, their natural substrates and products, as well as artificial substrates and concentration ranges used for determination of enzyme kinetics

Enzyme	Abbreviation	Target molecule → product	Artificial substrate (Sigma-Aldrich #)	Enzyme commission#	Assay conc. (μM)
β-1,4-glucosidase	BG	Cellulose → glucose	4-MUB-β-D-glucopyranoside (M3633)	3.2.1.21	2–800
1,4-β-cellobiosidase	CBH	Cellulose → disaccharide	4-MUB-β-D-cellobioside (M6018)	3.2.1.91	2–800
β-D-xylosidase	BX	Hemicellulose → xylose	4-MUB-β-D-xylopyranoside (M7008)	3.2.1.37	2–800
Leucyl aminopeptidase	LAP	Polypeptides → leucine	L-leucine-7-AMC (L2145)	3.4.11.1	1–600
Alkaline phosphatase	AP	Phospho-monoesters → phosphate	4-MUB-phosphate (M8883)	3.1.3.1	1–600
Phenol oxidase	POX	Lignin → oxidized lignin	3,4-dihydroxy-L-phenylalanine (D9628)	1.10.3.2	6500

The phenol oxidase assay was colorimetric and measured reaction velocity at only one substrate concentration

MUB methylumbelliferone, *AMC* amido-4-methylcoumarin hydrochloride

Plates were prepared by adding soil slurry (50 μl) first, followed by substrate, and then buffer sufficient to achieve a final volume of 200 μl per well. Plates were pre-incubated at 30 °C for either 1 h (for CBH, LAP, and AP) or 4 h (for BG and BX), and then read for an additional 6 h using a Synergy 2 plate reader (Biotek, Winooski, VT, USA) programmed for 360 nm excitation and 460 nm emission wavelengths and an incubation temperature of 30 °C. Activity was calculated for each sample after fitting a regression of the fluorescent reading versus MUB or AMC concentration for the corresponding quench curve. Rates were determined as the change in MUB or AMC generated in each sample during the 6 h incubation in the plate reader (each plate was read a minimum of twelve times). Technical replicates were averaged, and maximum reaction velocity (V_{max}) and half-saturation constant (K_m) values were calculated on Sigma Plot Version 10 (San Jose, CA, USA) using the Michaelis–Menten hyperbola function in the regression wizard.

Phenol oxidase (POX) activity was measured colorimetrically (Sinsabaugh et al. 2003). Three technical replicates containing soil slurry (50 μl), 50 mM sodium bicarbonate buffer (pH 6.1), and l-DOPA (6.5 mM) were performed for each sample, as were triplicate no-sample and no l-DOPA controls. The plates were incubated in the dark at 30 °C for 30 min and then read on the Synergy 2 at 460 nm wavelength for 6 h at 30 °C.

Anaerobic CO_2 and CH_4 Production

Production of CO_2 and CH_4 was measured using an anaerobic slurry assay (Neubauer et al. 2005). Briefly, homogenized soil samples (7.0 ± 0.2 g) were combined with 7 ml of filtered (glass microfiber filter GF/C; Whatman Piscataway, NJ, USA), deoxygenated porewater in a 125-ml serum bottle under anaerobic conditions using an N_2-filled glove bag. Two technical replicates were prepared for each sample, and slurries were pre-incubated overnight (~16 h at room temperature, 23 °C). The next morning, the headspace in each bottle was thoroughly flushed with N_2 to initiate a two-day experimental incubation. Gas samples (5 ml) were obtained from the headspace at 0, 8, 22, 32, and 46 h by shaking the slurry briefly, injecting 5 ml of N_2, and immediately withdrawing 5 ml of gas. Measuring headspace gas concentrations will underestimate potential production rates to the extent that gases accumulate in the slurry liquid rather than in the headspace, although this will not affect the relative comparison between our treatments since experimental conditions (pH, salinity, volumes of liquid and headspace) were similar in all bottles. Concentrations of CO_2 were measured on a LI-COR LI-7000 infrared

gas analyzer (Lincoln, NE, USA), and CH_4 was measured on a Shimadzu GC-14A gas chromatograph with flame ionization detector. All samples showed a linear increase in gas concentration over time, and production rates (nmol CO_2 or CH_4 produced per g of OM per hour) were calculated using linear regression. Median correlation coefficients were 0.97 for CO_2 and 0.99 for CH_4. Analytical precision was ±0.87 % for CO_2 and CH_4 (mean coefficient of variation for replicate injections of CO_2 and CH_4 standards).

Statistical analyses

By design, our treatments differed in their OM content. To account for this in data analysis, we normalized all microbial abundance, enzyme activity, and gas production data per gram of OM; this allowed us to focus on the effect of OM *type* without the confounding effect of *amount*. Shapiro–Wilks tests confirmed the soil properties, gas production rates, and microbial abundance data were normally distributed within each population making them appropriate for analysis using parametric techniques. Soil properties and gas production rates for field cores and control samples were compared using a Student's *t* test (n = 5 per group, df = 8). Effects of treatment (control, plant litter, or compost) on environmental variables, microbial abundance, EEA, and gas fluxes were analyzed using one-way analysis of variance (ANOVA; n = 5 per group, df = 14) with Tukey's HSD for post hoc comparisons. Analyses were performed using the JMP statistical software (Version JMP Pro 9.0.2, Cary, NC, USA; Sall 2005) with a 0.05 significance level.

T-RFLP results were analyzed using principal coordinates analysis (PCoA) applied to the Bray–Curtis index of similarity derived from normalized fluorescence data; the first two axes from each analysis were plotted to visualize relative similarity in community structure across samples. Treatment effects were analyzed using a non-parametric multivariate ANOVA (NP-MANOVA), again applied to the Bray-Curtis similarity index. All community analyses were conducted using the PAST Version 2.16 statistical package (Hammer 2001).

For the 18-month data (July 2012), correlation analysis was performed to examine the relationships among the environmental, microbial, enzyme, and gas production data. (SPSS Statistics Version 20, Armonk, NY, USA). Multivariate normality was confirmed using Doornik and Hansen omnibus test in PAST prior to selecting Pearson's coefficient.

Results

Controls versus field samples

After 6 months, the control samples did not differ significantly from field cores for any of the soil properties (pH, redox, OM, soil moisture, C:N; all $|t| < 2.0$ with $p > 0.05$). In contrast, for the 18-month sampling, *t*-tests revealed significant differences for all parameters except pH (for pH: t = 0.8 and p = 0.45; for all others, $|t| > 2.5$, all $p < 0.05$). Though the magnitude of the differences were small, redox (mV, mean ± SE; control: −128 ± 13, field: −83 ± 9), soil moisture (%; control: 52 ± 1, field: 60 ± 1), and OM (%; control: 7.8 ± 0.2, field: 9.7 ± 0.3) were all lower in the control samples; C:N was slightly higher (control: 9.6 ± 0.2, field: 8.4 ± 0.1). Gas flux rates (nmol g OM^{-1} h^{-1}) were also measured for the 18-month sampling event, and no significant differences were observed for CO_2 (control: 329.8 ± 50.4, field: 369.8 ± 120.0), CH_4 (control: 5.1 ± 0.8, field: 40.9 ± 39.9), or total C gas production (control: 335.0 ± 50.9, field: 410.8 ± 157.5; all $t < 1.0$, $p > 0.30$).

Effects of organic matter manipulation

Environmental analyses

Addition of plant litter and compost increased soil OM relative to the controls; these differences persisted throughout the study (Fig. 1a; Table 2). After 6 months, OM content in the plant litter (14.5 %) and compost treatments (13.7 %) was similar to the levels at the start of the study (averaged across both treatments: 14.0 ± 1.4 %). However, after 18 months, average OM for these treatments decreased (litter: 10.0 %, compost: 11.9 %), but was still significantly higher than the unamended control (7.8 %).

The compost and litter amendments also affected soil C:N, which was always lower in the controls (averaged by date: 9.5 ± 0.1) relative to the experimental treatments (Fig. 1b). At the 6-month sampling, average C:N was higher in the plant litter treatment (11.8) than in the compost treatment (10.6), reflecting

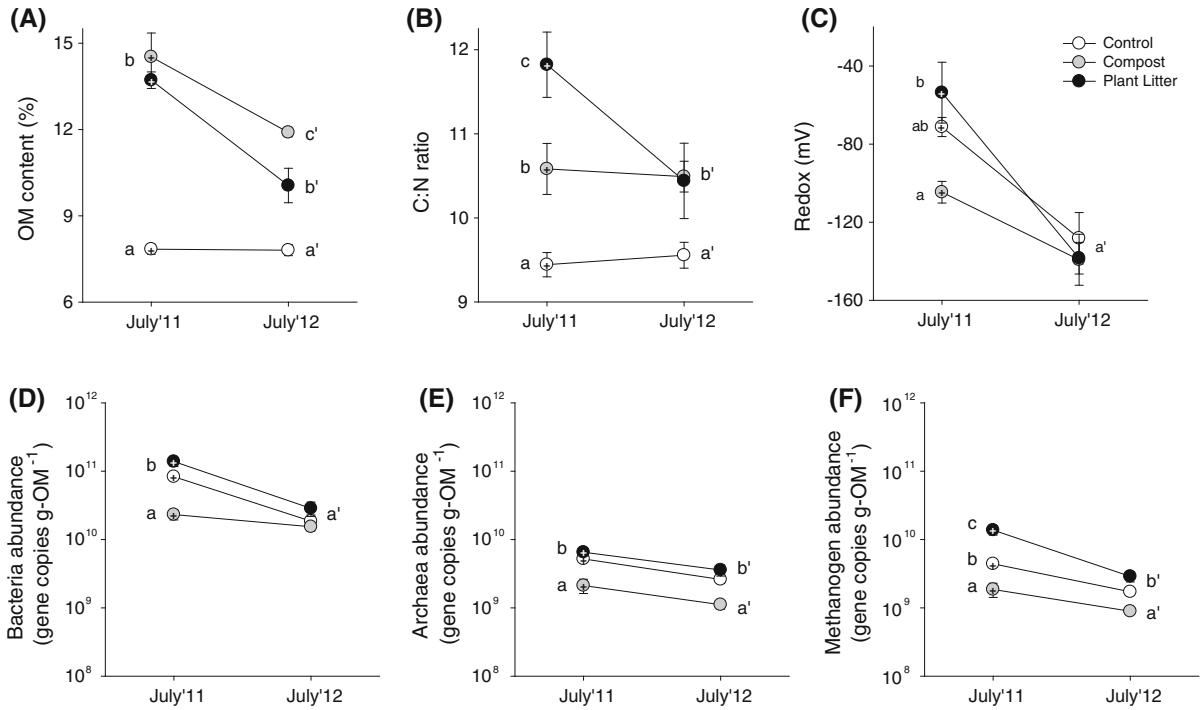

Fig. 1 Treatment effects on environmental parameters (**a**, **b**, **c**) and microbial abundance (**d**, **e**, **f**) following 6 (July 2011) and 18 (July 2012) months of in situ field incubation. Treatment effects were evaluated using one-way ANOVA within each sampling event; data are presented as mean ± SE, n = 5 per group. For all the graphs, *lowercase letters* denote statistically significant subgroups within the 6-month data set; letters with *prime* were used for the 18-month data. Treatments are color-coded and the symbols for the 6 month data are distinguished with a (*plus*) on the *symbol*

a substantial decrease from the start of the study (Litter: 24.4 ± 2.4, Compost: 13.9 ± 0.2). These differences disappeared after 18 months of incubation (combined average for both treatments: 10.5 ± 0.2).

For all treatments at all times, redox potential was negative (Fig. 1c). After 6 months of incubation, there were modest differences between the plant litter (−54 mV) and compost treatments (−105 mV), but neither was significantly different from the control (−82 mV). After an additional year of incubation, there were no significant differences between any treatments (combined average across treatments: −135 ± 6.4), though values were generally more negative than at 6-months.

Soil moisture was consistently lower in the compost-amended soils (%; 6-months: 47.4 ± 0.5, 18-month: 50 ± 1.2) relative to the plant litter (6-months: 56 ± 1.1, 18-month: 52.1 ± 0.9) and control soils (6-months: 55.0 ± 2.1, 18-month: 57.1 ± 2.0) (Table 2). For pH, there were significant treatment effects at the 6-month sampling event only; pH was higher in the compost (6.1 ± 0.1) compared to the control (5.7 ± 0.1) or plant litter amendment (5.6 ± 0.1). There were no significance differences in porewater chemistry for any of the parameters (mg L^{-1}, mean ± S.E.; DOC: 5.9 ± 0.9, TDN: 0.50 ± 0.08, TDP: 0.06 ± 0.01, NH_4^+: 0.18 ± 0.04).

Microbial abundance and community structure

For all three groups, abundance was lowest in the compost-amended soils, where it changed little over time [gene copies g OM^{-1}; averaged across both sampling events for bacteria: 17.9×10^9, archaea: 1.4×10^9, methanogens: 1.2×10^9 (Fig. 1 d–f)]. Bacterial abundance was ∼fivefold higher in the control and litter-added soils for the 6-month sampling, and not significantly different at the 18-month sampling. For both times, archaea abundance in the control and litter-added soils was similar and ∼ threefold higher than in the compost. For methanogens, significant differences were detected across all treatments for the 6-month sampling (gene copies g OM^{-1}; control: 4.3×10^9, litter: 12.9×10^9, compost:

Table 2 Statistical results evaluating treatment effects

Parameter	July 2011 (6 months)		July 2012 (18 months)	
	F	*p*	F	*p*
Environmental variables				
Soil				
pH	9.1	0.004*	2.5	0.12
Redox	6.8	0.01*	0.3	0.76
Moisture	11.8	0.001*	11.8	0.001*
OM	50.5	<0.001*	30.2	<0.001*
C:N	16.1	<0.001*	6.2	0.01*
Porewater				
DOC	–	–	0.9	0.43
NH_4^+	–	–	2.1	0.17
TDN	–	–	0.1	0.91
TDP	–	–	0.9	0.43
Microbial community				
Abundance[a]				
Bacteria	30.5	<0.001*	1.8	0.20
Archaea	12.7	0.01*	14.4	<0.001*
Methanogens	21.5	<0.001*	19.7	<0.001*
Structure				
Bacteria	6.3	<0.001*	3.2	<0.001*
Archaea	8.2	<0.001*	7.1	<0.001*
Methanogens	3.2	<0.001*	2.9	0.006*
Enzyme activity				
V				
POX	–	–	84.5	<0.001*
V_{max}				
BG	–	–	5.7	0.02*
CBH	–	–	2.6	0.12
BX	–	–	45.6	< 0.001*
LAP	–	–	16.6	< 0.001*
AP	–	–	3.8	0.05*
K_m				
BG	–	–	0.4	0.66*
CBH	–	–	7.0	0.001*
BX	–	–	1.2	0.33
LAP	–	–	11.4	0.002
AP			1.5	0.26
Gas production				
CO_2	–	–	6.1	0.01*
CH_4	–	–	74.9	< 0.001*
Total	–	–	11.9	0.001*
Fraction CH_4	–	–	76.4	< 0.001*

Analysis of microbial community structure was performed using NP-MANOVA; all other parameters were analyzed using ANOVA

– No data available

* Statistically significant with $\alpha = 0.05$

[a] Measures were $\log_{(10)}$ transformed prior to analysis

1.6×10^9). Abundance was slightly lower for all three treatments at the 18-month sampling, and the control and litter-added soils were no longer different (gene copies g OM^{-1}; control and litter: 2.2×10^9, compost: 0.9×10^9).

Treatment effects on microbial community structure were visualized using PCoA (Fig. 2a–c) and statistical significance was evaluated using NP-MANOVA (Table 2). For all three groups and both sampling events, community structure in the plant litter treatment was significantly different from that in the control and the compost addition. Generally, the compost treatment did not significantly alter microbial community structure relative to the control; the only exception is for the archaea community at the 18-month sampling.

Extracellular enzyme analysis

Treatment effects varied depending on the substrate tested and the kinetic parameter of interest (Table 2; Figs. 3 and 4; see Online Resource 1 for examples of the saturation curves from which these data were obtained). In general, the addition of plant litter corresponded to an increased V_{max} relative to the control, which was statistically significant for BG (200 % higher), BX (550 %), and LAP (50 %). Conversely, the addition of compost suppressed V_{max} relative to the control, though the trend was only statistically significant for AP ($\sim$25 % decrease). For K_m, fewer treatment effects were observed, and there were never any differences in K_m between the control and compost soils. In the presence of plant litter, K_m was significantly lower for CBH (by 75 % vs. control) and LAP (by 15 %). The activity of POX was reduced by 10 % (relative to controls) in the litter-amended soils and by roughly 40 % when compost was added.

Anaerobic CO_2 and CH_4 production

Potential rates of CH_4 and/or CO_2 production in anaerobic slurries increased in response to the OM additions (Table 2; Fig. 5). The rate of CH_4 production did not change significantly for compost but increased $\sim$tenfold for plant litter. Rates of CO_2 production in the compost and plant litter soils were 40–50 % higher than rates in the controls, with no significant differences in CO_2 production between the

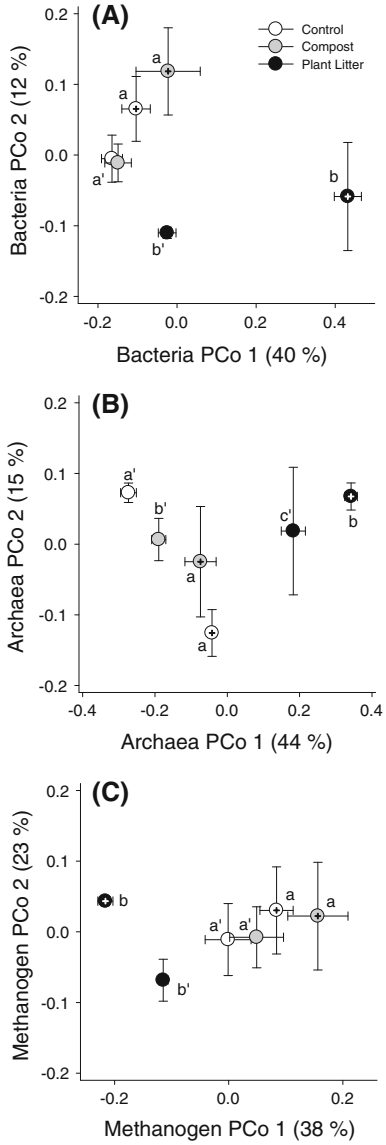

Fig. 2 Treatment effects on microbial community structure following 6 (July 2011) and 18 (July 2012) months of in situ field incubation. Patterns in microbial community structure were visualized using PCoA and statistical significance was evaluated by NP-MANOVA. Data are presented as mean ± SE, n = 5 per group, and the percent of variance explained by each axis is provided. Statistically significant subgroups are designated using *lowercase letters* for the 6-month data set, and letters with *prime* were used for the 18-month data. Treatments are color-coded and the 6 month data are distinguished with a (*plus*) on the *symbol*

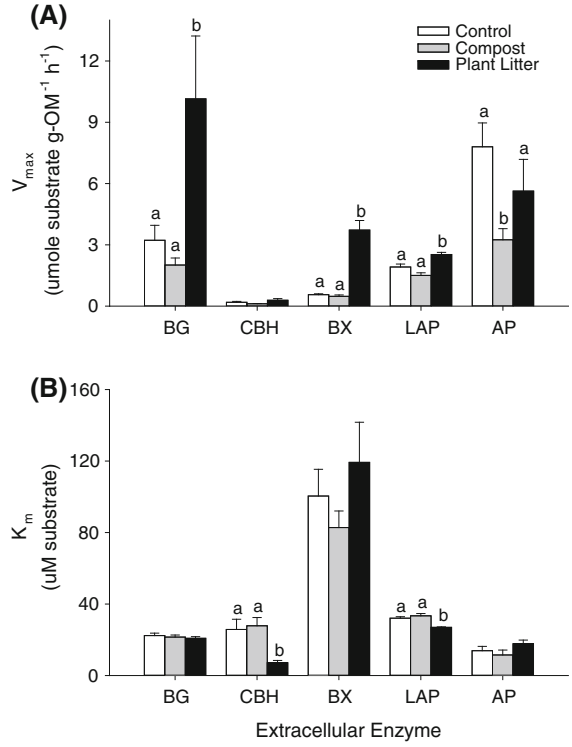

Fig. 3 Effects of treatment on enzyme kinetics, evaluated after 18 months of in situ field incubation (mean ± 1 SE, n = 5 each; sampled July 2012): **a** maximum reaction velocity (V_{max}) and **b** half-saturation constant (K_m). *Lowercase letters* denote significant differences as determined via one-way ANOVA and Tukey's HSD

two. Relative to the controls, total C gas production (CO_2 + CH_4) increased by 40 % in the compost treatment and by 70 % in the plant litter treatment (Fig. 5c). CH_4 accounted for ~2 % of the total C gas production in the control and compost treatments, but that fraction increased to ~15 % in the treatment with the addition of plant litter (Fig. 5d).

Correlation analysis

When C gas production rates were correlated with the environmental variables, only the relationship between soil C:N and CO_2 ($r = 0.61$, $p = 0.01$) was significant (other results not presented; all $r < 0.50$, $p > 0.08$). Similarly, microbial abundance did not show a strong relationship to gas production, except for the modest positive correlation of methanogen abundance and CH_4 rates (Table 3). To examine how microbial community structure and gas production were linked, correlation analysis was performed using the scores from each PCoA. In each case, the first axis describing bacterial, archaeal, and methanogen community structure was strongly correlated with CH_4 production (Table 3), and no significant correlations

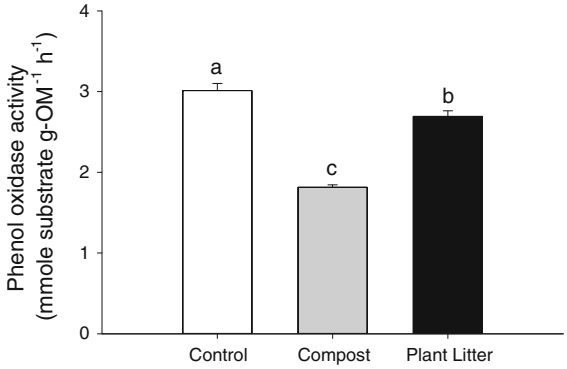

Fig. 4 Phenol oxidase (POX) activity as affected by treatment, evaluated after 18 months of in situ field incubation (mean ± 1 SE, n = 5 each; sampled July 2012). *Lowercase letters* denote significant differences as determined via one-way ANOVA and Tukey's HSD

were obtained for any of the second axes (not presented; all $r < 0.39$ and $p > 0.15$). For EEA, CH_4 production was significantly positively correlated with V_{max} for BG, CBH, BX and LAP, and negatively correlated with K_m for CBH and LAP (Table 3). The only significant correlation with CO_2 production was for K_m of BG ($r = -0.60$, $p = 0.02$).

Several aspects of the microbial community were correlated with one another, including a significant relationship between the abundance of all three microbial groups (Table 3). Similarly, bacterial, archaeal, and methanogen community composition were correlated when each PCoA 1 was considered. Significant correlations were found between microbial community composition and EEA for V_{max} for BG, BX and LAP, and with K_m for CBH and LAP. In general, correlations were highest between EEA and bacterial or archaeal community composition, and were significant less often for methanogens.

Discussion

The loss of natural wetland ecosystems is often mitigated by construction or restoration of wetlands elsewhere in the watershed (EPA 2008). As a means of improving soil quality and promoting plant productivity, OM amendments are regularly included in these mitigation projects (Davis 1995; Mitsch and Gosselink 2000). Our study complements other research that has examined how this practice affects soil characteristics, redox gradients, vegetation, and nutrient cycling (O'Brien and Zedler 2006; Bruland et al. 2009; Sutton-Grier et al. 2009), and demonstrates that amendments can alter rates of C mineralization and induce shifts in microbial community structure and function. Furthermore, because our manipulation produced long-term changes in soil OM and C:N with relatively limited effects on other soil parameters (e.g., redox, pH, and soil moisture), we were able to isolate how OM characteristics can affect C biogeochemistry

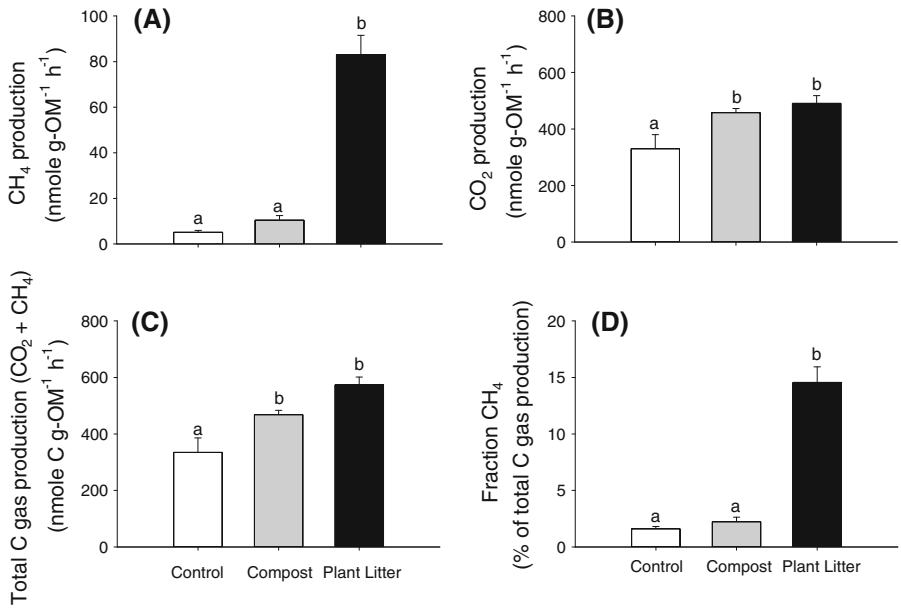

Fig. 5 Treatment effects on the production of CH_4 (**a**), CO_2 (**b**) and total C gas (**c**), as well as the fraction of total C gas that is CH_4 (**d**), evaluated after 18 months of in situ field incubation measured in anaerobic slurries (mean ± 1 SE, n = 5 each; sampled July 2012). *Lowercase letters* denote significant differences as determined via one-way ANOVA and Tukey's HSD

Table 3 Pearson's correlations coefficients (r, above and bolded) and *p*-values (below) associated with the comparison of gas production rates, microbial community attributes, and enzymatic variables at the July 2012 (18-month) sampling

Category	Parameter	Gas		Abundance			Community composition[a]		
		CH_4	CO_2	Bacteria	Archaea	Meth	Bacteria	Archaea	Meth
Gas	CH_4	–	**0.42**	**0.25**	**0.40**	**0.55***	**−0.86****	**0.93****	**−0.72****
	CO_2	0.11	–	**0.17**	**−0.06**	**0.02**	**−0.34**	**0.50**	**−0.21**
Abundance	Bacteria	0.37	0.54	–	**0.57***	**0.68****	**−0.55***	**0.41**	**−0.26**
	Archaea	0.14	0.84	0.03*	–	**0.95****	**−0.46**	**0.41**	**−0.44**
	Methanogens	0.03*	0.94	0.01**	<0.01**	–	**−0.63****	**0.59***	**−0.53***
Composition[a]	Bacteria	<0.01**	0.21	0.03*	0.09	0.01**	–	**−0.86****	**0.67****
	Archaea	<0.01**	0.06	0.13	0.13	0.02*	<0.01**	–	**−0.63****
	Meth.	<0.01**	0.46	0.34	0.10	0.04*	0.01**	0.01**	–
Enzymes	POX[b]	0.53	0.17	0.57	<0.01**	0.01**	0.42	0.82	0.17
	BG V_{max}	0.01**	0.19	0.31	0.17	0.07	0.02*	0.01**	0.17
	CBH V_{max}	0.04*	0.79	0.94	0.08	0.04*	0.41	0.12	0.08
	BX V_{max}	<0.01**	0.14	0.09	0.03*	<0.01**	<0.01**	<0.01**	0.01**
	LAP V_{max}	<0.01**	0.42	0.22	<0.01**	<0.01**	<0.01**	0.01**	0.11
	AP V_{max}	0.86	0.17	0.38	0.12	0.18	0.50	0.70	0.70
	BG K_m	0.42	0.02*	0.67	0.69	0.81	0.76	0.34	0.78
	CBH K_m	0.01**	0.46	0.18	0.15	0.05*	<0.01**	<0.01**	0.05*
	BX K_m	0.20	0.77	0.66	0.16	0.18	0.31	0.44	0.25
	LAP K_m	0.01**	0.27	0.15	0.01**	0.01**	0.02*	<0.01**	0.05*
	AP K_m	0.18	0.31	0.58	0.08	0.05*	0.51	0.12	0.97

Table 3 continued

Category (cont'd)	Parameter (cont'd)	Enzyme activity										
		POX[b]	V_{max}					K_m				
			BG	CBH	BX	LAP	AP	BG	CBH	BX	LAP	AP
Gas	CH_4	**0.18**	**0.66****	**0.54***	**0.89****	**0.71****	**0.05**	**−0.23**	**−0.71****	**0.35**	**−0.71****	**0.36**
	CO_2	**−0.37**	**0.36**	**0.07**	**0.40**	**0.23**	**−0.38**	**−0.60***	**−0.21**	**−0.08**	**−0.31**	**0.28**
Abundance	Bacteria	**0.16**	**0.28**	**0.02**	**0.45**	**0.34**	**−0.24**	**−0.12**	**−0.36**	**0.12**	**−0.39**	**0.16**
	Archaea	**0.75****	**0.37**	**0.47**	**0.57***	**0.69****	**0.42**	**−0.11**	**−0.39**	**0.38**	**−0.65****	**0.47**
	Methanogens	**0.67****	**0.48**	**0.53***	**0.69****	**0.74****	**0.37**	**−0.07**	**−0.52***	**0.37**	**−0.67****	**0.52***
Composition[a]	Bacteria	**−0.22**	**−0.58***	**−0.23**	**−0.82****	**−0.68****	**0.19**	**0.08**	**0.81****	**−0.28**	**0.61***	**−0.18**
	Archaea	**0.06**	**0.64****	**0.41**	**0.85****	**0.66****	**−0.11**	**−0.27**	**−0.69****	**0.22**	**−0.73****	**0.42**
	Meth.	**−0.38**	**−0.37**	**−0.46**	**−0.66****	**−0.43**	**−0.11**	**0.08**	**0.52***	**−0.31**	**0.52***	**−0.01**
Enzymes	POX[b]	–	**0.19**	**0.38**	**0.24**	**0.53***	**0.64****	**0.06**	**−0.20**	**0.36**	**−0.48**	**0.20**
	BG V_{max}	0.49	–	**0.37**	**0.78****	**0.80****	**0.12**	**−0.05**	**−0.56***	**0.31**	**−0.52***	**0.62****
	CBH V_{max}	0.16	0.17	–	**0.58***	**0.49**	**0.76****	**0.03**	**−0.24**	**0.60***	**−0.43**	**0.47**
	BX V_{max}	0.38	<0.01**	0.02*	–	**0.81****	**0.10**	**−0.08**	**−0.69****	**0.55***	**−0.69****	**0.44**
	LAP V_{max}	0.04*	<0.01**	0.07	<0.01**	–	**0.34**	**−0.14**	**−0.61***	**0.50**	**−0.60***	**0.71****
	AP V_{max}	0.01**	0.68	<0.01**	0.72	0.21	–	**0.14**	**0.07**	**0.47**	**−0.12**	**0.44**
	BG K_m	0.84	0.86	0.92	0.78	0.62	0.62	–	**−0.14**	**0.03**	**0.41**	**−0.33**
	CBH K_m	0.47	0.03*	0.40	<0.01**	0.02*	0.79	0.63	–	**−0.35**	**0.38**	**−0.14**
	BX K_m	0.19	0.26	0.02*	0.03*	0.06	0.08	0.92	0.20	–	**−0.31**	**0.18**
	LAP K_m	0.07	0.05*	0.11	<0.01**	0.02*	0.67	0.13	0.16	0.27	–	**−0.32**
	AP K_m	0.47	0.01**	0.07	0.10	<0.01**	0.10	0.23	0.61	0.52	0.25	–

* Statistically significant with $0.01 < *p \leq 0.05$; ** $p \leq 0.01$

[a] Correlation reported for PCoA 1; no significant results obtained for PCoA 2 (all $p \geq 0.10$)

[b] POX reaction velocity only recorded at one substrate concentration, as described in the methods

and identify key microbial drivers and feedbacks to the multi-stage process of decomposition. The similarities between measurements on control soils and intact field cores suggest that the results remain applicable to unaltered wetlands soils.

The amendments used in this study differ considerably in their biochemical composition. Compost, although derived from plant materials, undergoes a humification process that yields chemically-complex OM with few residual plant polymers and increased microbial necromass (Tiquia et al. 1996; Tuomela et al. 2000). Compost may be similar to native soil OM, which is also considered to have a significant portion of C of microbial origin (Simpson et al. 2007; Liang and Balser 2010; Throckmorton et al. 2012). In general, microbial necromass contains a larger fraction of proteins and lipids than does plant litter, and has only a small fraction of the carbohydrates and lignins dominant in plant litter (Nelson and Baldock 2005; Simpson et al. 2007; Throckmorton et al. 2012). Thus, while nutrient availability may have varied between the control and compost-amended soils, the chemical composition of the OM was probably more similar between these treatments and distinct from that in the litter-amended soil (that is, microbially-dominated vs. plant-dominated).

Treatment effects and carbon gas production

Overall, we found that both the plant litter and compost-added treatments exhibited higher potential rates of anaerobic C gas production relative to unamended soils on a per gram-OM basis (Fig. 5), which indicates that a greater fraction of the OM was mineralized compared to the control soil. One explanation for this result is that the OM in the plant litter and compost amendments was more labile than the existing soil OM. Additional factors at play include the potential for enhanced decomposition of native material through "priming" (Blagodatskaya and Kuzyakov 2008; Nottingham et al. 2009) or the possibility that some of the native soil OM was physically inaccessible (e.g., via sorption onto mineral surfaces), which could limit decomposition regardless of inherent lability (Kalbitz et al. 2000).

We did not identify any significant relationships between either soil environmental conditions or porewater chemistry and any of the following: microbial community composition, abundance, EEA, or C gas production, excepting a small positive correlation between C:N and CO_2. Given the narrow range of C:N for our treatment soils (Fig. 1b), it is likely that C:N is not the driver of decomposition rates per se but instead a co-variant associated with finer-scale OM characteristics such as the degree of humification or OM lability as discussed above. The general lack of correlation between environmental variables and either the soil microbial community or biogeochemical response metrics suggests environmental conditions were not major divers of the observed treatment effects. It further supports our assertion that differences in C gas production were the result of microbial responses to OM type and not an unintended consequence of treatment on abiotic soil characteristics.

Role of microbial communities

Extracellular enzyme activity

Microorganisms can detect substrates in their environment and regulate enzyme production accordingly to balance resource needs with metabolic costs (Bhat and Bhat 1997; Shackle et al. 2000; Allison and Vitousek 2005; Allison et al. 2011; Shi 2011). In this study, we found considerable evidence that OM type (i.e., polymer availability) can influence EEA. For example, in the plant litter treatment, enzymes that target compounds abundant in plant litter (e.g., cellulose and hemicellulose) were elevated. This response was observed for V_{max} of all of the C and N hydrolytic enzymes we measured, and was statistically significant for BG, BX, and LAP (Fig. 3; Table 2). Microbial adjustments of EEA are also evident in the compost treatment, where the reduced V_{max} of AP was likely a response to decreased P limitation. The compost we used contained 0.2 % P, and microorganisms generally produce fewer acquisition enzymes for nutrients that are readily available (Sinsabaugh and Moorhead 1994; Allison and Vitousek 2005). Similarly, POX decreased approximately 50 % following compost addition, likely due to lower lignin content in compost (Sinsabaugh 2010).

Our results suggest that this variation in EEA across OM types may be due, at least in part, to changes in microbial community structure. Specifically, the decrease in K_m associated with CBH and LAP in the plant litter treatment indicates the synthesis of isoenzymes with higher substrate affinity, which reflects more efficient allocation of resources (Marx et al.

2005; Stone et al. 2012). While multiple isoenzymes are known to occur within an individual organism (Esser et al. 2013), shifts in isoenzymes are also consistent with changes in microbial community composition (Farrell et al. 1994; Martinez et al. 1996; Tabatabai et al. 2002). The significant correlations between K_m of CBH and LAP with bacteria PCoA 1 and archaea PCoA 1 (Table 3) further support our conceptual model that community structure can influence enzyme activity (Fig. 6). We anticipate that most of the EEA in our soils is of bacterial origin, as bacterial abundance was ~tenfold greater than that of archaea (average bacteria:archaea *16S rRNA* gene ratio at the 18 month sampling, with a range from 4 to 21). Although enzyme parameters were correlated with archaea in our study (Table 3), and archaea have been demonstrated to produce extracellular enzymes in marine sediments (Lloyd et al. 2013), we suggest that these groups were not significant producers of enzymes in our system. This assertion is based on the fact that most of our archaea were likely methanogens (average archaea *16S rRNA*:methanogen *mcrA* ratio was 1.3, with a range from 1.0 to 1.7), and methanogens exclusively use fermentation products and CO_2 as their carbon source (Thauer et al. 2008; Reddy and De Laune 2008). Thus, it is unlikely they would expend resources to produce enzymes for carbon polymer breakdown (e.g., BG, CBH, BX, and POX) to liberate products they cannot directly utilize. Instead, we propose that the correlations we observed between EEA and archaea/methanogens are indirect based on methanogen consumption of fermentation products affecting upstream pathways of organic carbon breakdown (Fig. 6).

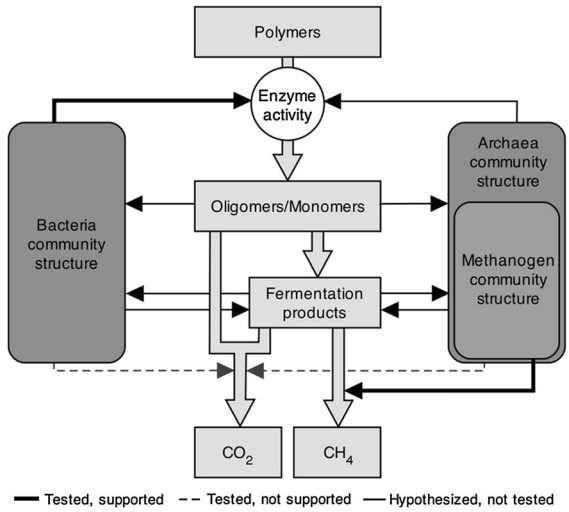

Fig. 6 Conceptual model diagramming the hypothesized role of microbial community structure and extracellular enzyme activity in wetland organic matter decomposition. Microbially mediated flows of carbon are represented as *thick arrows* beginning with polymers and concluding with the terminal decomposition end products CO_2 and CH_4 (after Megonigal et al. 2004). Interactions between microbial structure and carbon pools/flows are designated as supported, not supported, or not tested in the current study

Microbial community structure

We demonstrated that the plant litter amendment supported a distinct microbial community compared to the control and compost-added soils (Fig. 2a–c), and propose these differences developed in response to C substrate availability as mediated by EEA (Fig. 6). The initial mechanism for this OM effect is selection for a distinct set of heterotrophs capable of directly metabolizing the unique oligomers and monomers generated from EEA on plant litter. Given the current knowledge on wetland soil microbiology (Reddy and De Laune 2008; Wüst et al. 2009), we anticipate a large fraction of these organisms are fermentative bacteria. Fermentation generates acetate and other simple organic acids that support methanogens, the main archaea in our system. Thus we hypothesize the plant litter addition *directly* affected bacterial community structure (similar to Nemergut et al. 2010), and resulted in greater availability and altered composition of fermentation end products (e.g., acetate vs. propionate, Uz and Ogram 2006). Then, because many methanogen genera can use only a specific subset of fermentation products (Garcia et al. 2000), this altered substrate availability was the *indirect* mechanism for the observed change in community structure of methanogens (and archaea). These changes in community structure have the potential to impact C mineralization rates and the balance of CO_2 and CH_4 production.

Microbial regulation of C gas production

If polymer breakdown is the rate-limiting step in decomposition, there should be a positive correlation between EEA V_{max} and C mineralization (e.g., Schimel and Weintraub 2003). In this study, no such relationships were observed for the CO_2 production rates (Table 3). This may be partly due to the

particular suite of enzymes we considered. Although commonly tested in soils, BG, BX, and CBH are fairly selective for plant polymers, and thus may not be as responsive to the availability of microbial necromass or humified material, which potentially dominated our control and compost treatments. Nonetheless, our results are consistent with the work of Freeman et al. (1997, 1998), who similarly found that BG activity did not correlate with CO_2 production in wetland soils. The production of CO_2 was also unrelated to microbial community structure, similar to the work of Bell et al. (2005) and Fromin et al. (2012). This may be because CO_2 is generated by a plethora of microbial species with diverse metabolic strategies, creating considerable functional redundancy in natural communities (Botton et al. 2006; Griffiths et al. 2000).

In contrast to CO_2, we did observe strong relationships between CH_4 production, microbial community composition, and EEA, which we hypothesize are mediated through OM and bacterial community effects on the abundance, composition, and activity of methanogens (Fig. 6). Because methanogenesis is a fairly well-conserved function, performed by a monophyletic group of organisms (Garcia et al. 2000) that can utilize a limited range of organic substrates, there is relatively low functional redundancy associated with CH_4 production and the contribution of individual species to overall ecosystem function should be more important (Allison and Martiny 2008; McGuire and Treseder 2010). We were able to identify two terminal restriction fragments (T-RF) in our data associated with the genus *Methanosarcinales* (after Smith et al. 2007) and found their relative abundance was positively correlated with CH_4 production (Spearman correlation; T-RF 95 bp: $r = 0.62$, $p = 0.01$; T-RF 179 bp: $r = 0.56, p = 0.02$). These results suggest that specific taxa of methanogens may be strong drivers of CH_4 production and are consistent with several other recent studies (Beckmann et al. 2011; Angel et al. 2012; Parkes et al. 2012). Additional research into the ecological and physiological attributes of these community members could further enhance our understanding of ecosystem-scale CH_4 dynamics.

Conclusions

Our results have been used in conjunction with current knowledge on wetland decomposition to develop a conceptual model that incorporates microbial community structure and EEA to expand our understanding of CO_2 and CH_4 production rates (Fig. 6). Models such as this may be particularly helpful in understanding methanogenesis, since rates of CH_4 production were strongly correlated with microbial community structure and multiple enzyme kinetic parameters. Relationships of enzyme activity and microbial community composition with CO_2 production were considerably more tenuous. This may be a consequence of the numerous microorganisms, substrates, and metabolic pathways associated with anaerobic CO_2 production (see Megonigal et al. 2004).

This work has direct implications for wetland restoration as plant litter and compost produced disparate changes in C gas production. Both OM sources increased total rates of anaerobic C mineralization relative to unamended soils, but only the plant litter additions increased rates of CH_4 production (by roughly an order of magnitude). Similar results have also been reported for rice paddy soils (Singh et al. 2009; Ruirui et al. 2011), suggesting that the incorporation of highly decomposed OM amendments such as compost may help with wetland restoration (Stauffer and Brooks 1997; Sutton-Grier et al. 2009) while minimizing production of the greenhouse gas CH_4.

Acknowledgments This research was funded by the VCU Rice Center for Environmental Life Sciences Student Research Award to E.M. Morrissey. Thanks to Joseph Morina, Jaimie Gillespie, Chansotheary Dang, Joseph Battistelli, Aaron Porter, Rana Mehr, and Olivia De Meo for laboratory and field help. Sincere thanks to the anonymous reviewers whose feedback greatly enhanced this manuscript. This paper is contribution #38 from the VCU Rice Center for Environmental Life Sciences and contribution #1677 from the University of South Carolina's Belle W. Baruch Institute for Marine and Coastal Sciences.

References

Allison SD, Martiny JB (2008) Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci USA 105:11512–11519

Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37:937–944

Allison SD, Weintraub MN, Gartner TB, Waldrop MP (2011) Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. In: Shukla D, Varma A (eds) Soil enzymology. Springer, New York, pp 229–244

Angel R, Claus P, Conrad R (2012) Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J 6:847–862

Arnosti C, Holmer M (2003) Carbon cycling in a continental margin sediment: contrasts between organic matter characteristics and remineralization rates and pathways. Estuar Coast Shelf Sci 58:197–208

Arnosti C, Jørgensen B (2006) Organic carbon degradation in arctic marine sediments, Svalbard: a comparison of initial and terminal steps. Geomicrobiol J 23:551–563

Baltar F, Aristegui J, Sintes E, van Aken H, Gasol J, Herndl G (2009) Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub)tropical Atlantic. Environ Microbiol 11:1998–2014

Beckmann S, Lueders T, Krüger M, von Netzer F, Engelen B, Cypionka H (2011) Acetogens and acetoclastic *Methanosarcinales* govern methane formation in abandoned coal mine. Appl Environ Microbiol 77:3749–3756

Bell T, Newman JA, Silverman BW, Turner SL, Lilley AK (2005) The contribution of species richness and composition to bacterial services. Nature 436:1157–1160

Bhat MK, Bhat S (1997) Cellulose degrading enzymes and their potential industrial applications. Biotechnol Adv 15:583–620

Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45:115–131

Botton S, van Heusden M, Parsons JR, Smidt H, van Straalen N (2006) Resilience of microbial systems towards disturbances. Crit Rev Microbiol 32:101–112

Bruland GL, Richardson CJ, Daniels WL (2009) Microbial and geochemical responses to organic matter amendments in a created wetland. Wetlands 29:1153–1165

Cleveland C, Nemergut D, Schmidt S, Townsend A (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82:229–240

Conrad B (2009) The global methane cycle: recent advances in understanding the microbial processes involved. Environ Microbiol Rep 1:285–292

Costa AL, Paixão SM, Caçador I, Carolino M (2007) CLPP and EEA profiles of microbial communities in salt marsh sediments. J Soil Sediment 7:418–425

Cytryn E, Minz D, Oremland RS, Cohen Y (2000) Distribution and diversity of archaea corresponding to the limnological cycle of a hypersaline stratified lake (Solar Lake, Sinai, Egypt). Appl Environ Microbiol 66:3269–3276

Davis L (1995) Handbook of constructed wetlands volume 1: a guide to creating wetlands for agricultural wastewater, domestic wastewater, coal mine drainage, stormwater in the mid-Atlantic. Gordon Press Publishers, Washington, DC

Environmental Protection Agency (2008) Compensatory mitigation for losses of aquatic resources. Fed Reg 73(70): 19594–19705

Esser D, Kouril T, Talfournier F, Polkowska J, Schrader T, Bräsen C, Siebers B (2013) Unraveling the function of paralogs of the aldehyde dehydrogenase super family from *Sulfolobus solfataricus*. Extremophiles 17:205–216

Farrell RE, Gupta VVSR, Germida JJ (1994) Effects of cultivation on the activity and the kinetics of arylsulfatase in Saskatchewan soils. Soil Biol Biochem 26:1033–1040

Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl Environ Microbiol 71:4117–4120

Freeman C, Liska G, Ostle NJ, Lock MA, Hughes S, Reynolds B, Hudson J (1997) Enzymes and biogeochemical cycling in wetlands during a simulated drought. Biogeochemistry 39:177–187

Freeman C, Nevison GB, Hughes S, Reynolds B, Hudson J (1998) Enzymic involvement in the biogeochemical responses of a Welsh peatland to a rainfall enhancement manipulation. Biol Fertil Soils 27:173–178

Freeman C, Ostle N, Kang H (2001) An enzymic 'latch' on a global carbon store. Nature 409:149

Fromin N, Porte B, Lensi R, Hamelin J, Domenach A, Buatois B, Roggy J (2012) Spatial variability of the functional stability of microbial respiration process: a microcosm study using tropical forest soil. J Soil Sediment 12:1030–1039

Galand PE, Fritze H, Yrjala K (2003) Microsite-dependent changes in methanogenic populations in a boreal oligotrophic fen. Environ Microbiol 5:1133–1143

Gallo M, Amonette R, Lauber C, Sinsabaugh RL, Zak DR (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. Microb Ecol 48:218–229

Garcia JL, Petal BKC, Ollivier B (2000) Taxonomic, phylogenetic, and ecological diversity of methanogenic archaea. Anaerobe 6:205–226

German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397

Goberna M, Garcia C, Insam H, Hernández MT, Verdú M (2012) Burning fire-prone Mediterranean shrublands: immediate changes in soil microbial community structure and ecosystem functions. Microb Ecol 64:242–255

Grasshoff K, Ehrhardt M, Kremeling K (1983) Methods of seawater analysis. Chemie, Weinheim

Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S, Ekelund F, Sørensen SJ, Bååth E, Bloem J, de Ruiter PC, Dolfing J, Nicolardot B (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. Oikos 90:279–294

Hammer Ø (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:1–9

Kaiser C, Koranda M, Kitzler B, Fuchslueger L, Schnecker J, Schweiger P, Rasche F, Zechmeister-Boltenstern S, Sessitsch A, Richter A (2010) Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytol 187:843–858

Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci 165:277–304

Karlsson AE, Johansson T, Bengtson P (2012) Archaeal abundance in relation to root and fungal exudation rates. FEMS Microbiol Ecol 80:305–311

Kayranli B, Scholz M, Mustafa A, Hedmark A (2010) Carbon storage and fluxes within freshwater wetlands: a critical review. Wetlands 20:111–124

Kourtev PS, Ehrenfeld JG, Häggblom M (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biol Biochem 35:895–905

Lane DI (1991) 16S/23S sequencing. In: Stackcbrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–176

Lazar CS, Parkes RJ, Cragg BA, L'haridon S, Toffin L (2012) Methanogenic activity and diversity in the centre of the Amsterdam Mud Volcano, Eastern Mediterranean Sea. FEMS Microbiol Ecol 81:243–254

Li Y, Wang L, Zhang W, Wang H, Fu X, Le Y (2011) The variability of soil microbial community composition of different types of tidal wetland in Chongming Dongtan and its effect on soil microbial respiration. Ecol Eng 37:1276–1282

Liang C, Balser TC (2010) Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. Nat Rev Microbiol 9:75

Liu DY, Ding WX, Jia ZJ, Cai ZC (2011) Relation between methanogenic archaea and methane production potential in selected natural wetland ecosystems across china. Biogeosciences 8:329–338

Lloyd KG, Schreiber L, Peterson DG, Kjeldsen KU, Lever MA, Steen AD, Stepanauskas R, Richter M, Kleindienst S, Lenk S, Schramm A, Jørgensen BB (2013) Predominant archaea in marine sediments degrade detrital proteins. Nature 496:215–218

Martinez J, Smith DC, Steward GF, Azam F (1996) Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in sea. Aquat Microb Ecol 10:223–230

Marx MC, Kandeler E, Wood M, Wermbter N, Javis SC (2005) Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biol Biochem 37:35–48

McGuire KL, Treseder KK (2010) Microbial communities and their relevance for ecosystem models: decomposition as a case study. Soil Biol Biochem 42:529–535

Megonigal JP, Hines ME, Visscher PT (2004) Anaerobic metabolism: linkages to trace gases and aerobic metabolism. In: Schlesinger WH (ed) Biogeochemistry. Elsevier, Pergamon, Oxford, pp 317–424

Mitra S, Wassman R, Vlek PLG (2005) An appraisal of global wetland area and its organic carbon stock. Curr Sci 88:25–35

Mitsch WJ, Gosselink JG (2000) Wetlands, 3rd edn. Wiley, New York, pp 687–724

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil function. Eur J Soil Sci 54:655–670

Nelson PN, Baldock JA (2005) Estimating the molecular composition of a diverse range of natural organic materials from solid-state ^{13}C NMR and elemental analyses. Biogeochemistry 72:1–34

Nemergut DR, Cleveland CC, Wieder WR, Washenberger CL, Townsend AR (2010) Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biol Biochem 42:2153–2160

Neubauer SC, Givler K, Valentine SK, Megonigal JP (2005) Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. Ecology 86:3334–3344

Nottingham AT, Griffiths H, Chamberlain PM, Stott AW (2009) Soil priming by sugar and leaf-litter substrates: a link to microbial groups. Appl Soil Ecol 42:183–190

O'Brien EL, Zedler JB (2006) Accelerating the restoration of vegetation in a southern California salt marsh. Wetl Ecol Manag 35:453–461

Parkes RJ, Brock F, Banning N, Hornibrook E, Roussel EG, Weightman A, Fry JC (2012) Changes in methanogenic substrate utilization and communities with depth in a saltmarsh, creek sediment in southern England. Estuar Coast Shelf Sci 96:170–178

Reddy R, DeLaune R (2008) Biogeochemistry of wetlands: science and applications. CRC, Taylor and Francis, Boca Raton

Ruirui C, Xiangui L, Yiming W, Junli H (2011) Mitigating methane emissions from irrigated paddy fields by application of aerobically composed livestock manures in eastern China. Soil Use Manag 27:103–109

Sall J (2005) JMP start statistics: a guide to statistics and data analysis using JMP and JMP IN software. SAS Institute, Cary

Schimel J (1995) Ecosystem consequences of microbial diversity and community structure. In: Kroner C (ed) Arctic and alpine biodiversity: patterns, causes and ecosystem consequences. Springer, Berlin, pp 239–254

Schimel JP, Weintraub MN (2003) The implication of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563

Segers R (1998) Methane production and methane consumption: a review of processes underlying wetland methane fluxes. Biogeochemistry 41:23–51

Shackle VJ, Freeman C, Reynolds B (2000) Carbon supply and the regulation of enzyme activity in constructed wetlands. Soil Biol Biochem 32:1935–1940

Shi W (2011) Agriculture and ecological significance of soil enzymes: soil carbon sequestration and nutrient cycling. In: Shukla G, Varma A (eds) Soil enzymology. Springer, New York, pp 43–60

Simpson AJ, Simpson MJ, Smith E, Kelleher BP (2007) Microbially derived inputs to soil organic matter: are current estimates too low? Environ Sci Technol 41:8070–8076

Singh SK, Bjaradwaj V, Thakur TC, Pachauri SP, Singh PP, Mishra AK (2009) Influence of crop establishment methods on methane emission from rice fields. Curr Sci 97:84–89

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42:391–404

Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biol Biochem 26:1305–1311

Sinsabaugh RL, Antibus RK, Linkins AE (1991) An enzymatic approach to the analysis of microbial activity during plant litter decomposition. Agric Ecosyst Environ 34:43–54

Sinsabaugh RL, Saiya CK, Long T, Osgood MP, Neher DA (2003) Soil microbial activity in a Liquidambar plantation unresponsive to CO_2-driven increases in primary production. Appl Soil Ecol 24:263–271

Smith CJ, Osborn AM (2009) Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS Microbial Ecol 67:6–20

Smith JM, Castro H, Ogram A (2007) Structure and function of methanogens along a short-term restoration chronosequence in the Florida everglades. Appl Environ Microbiol 73:4135–4141

Stauffer AL, Brooks RP (1997) Plant and soil responses to salvaged marsh surface and organic matter amendments at a created wetland in central Pennsylvania. Wetlands 17:90–105

Steinberg LM, Regan JM (2009) *mcrA*-targeted real-time quantitative PCR method to examine methanogen communities. Appl Environ Microb 75:4435–4442

Stone MM, Weiss MS, Goodale CL, Adams M, Fernandez IJ, German DP, Allison SD (2012) Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. Glob Chang Biol 18:1173–1184

Stursova M, Crenshaw CL, Sinsabaugh RL (2006) Microbial responses to long-term N deposition in a semiarid grassland. Microb Ecol 51:90–98

Sutton-Grier AE, Ho M, Richardson CJ (2009) Organic amendments improve soil conditions and denitrification in a restored riparian wetland. Wetlands 29:343–352

Tabatabai MA, García-Manzanedo AM, Acosta-Martínez V (2002) Substrate specificity of arylamidase in soils. Soil Biol Biochem 34:103–110

Thauer RK, Kaster A, Seedorf H, Buckel W, Hedderich R (2008) Methanogenic archaea: ecologically relevant differences in energy conservation. Nat Rev Microbiol 6:579–591

Throckmorton HM, Bird JA, Dane L, Firestone MK, Horwath WR (2012) The source of microbial C has little impact on soil organic matter stabilization in forest ecosystems. Ecol Lett 15:1257–1265

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. Bioresour Technol 65:201–206

Tuomela M, Vikman M, Hatakka A, Itävaara M (2000) Biodegradation of lignin in a compost environment: a review. Bioresour Technol 72:169–183

Uz I, Ogram AV (2006) Cellulolytic and fermentative guilds in eutrophic soils of the Florida Everglades. FEMS Microbiol Ecol 57:396–408

Wüst PK, Horn MA, Drake HL (2009) Trophic links between fermenters and methanogens in a moderately acidic fen soil. Environ Microbiol 11:1395–1409

Zedler JB, Kercher S (2005) Wetland resources: status, trends, ecosystem services, and restorability. Annu Rev Environ Resour 30:39–74