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Trade-offs between soil-based functions in wetlands restored with soil amendments of differing lability

Katherine A. Ballantine,[1,5] Johannes Lehmann,[2] Rebecca L. Schneider,[3] and Peter M. Groffman[4]

[1]*Department of Environmental Studies, Mount Holyoke College, South Hadley, Massachusetts 01075 USA*
[2]*Department of Crop and Soil Science, Cornell University, Ithaca, New York 14853 USA*
[3]*Department of Natural Resources, Cornell University, Ithaca, New York 14853 USA*
[4]*Cary Institute of Ecosystem Studies, Millbrook, New York 12545 USA*

Abstract. Soil amendments have been proposed as a means to speed the development of plant and soil processes that contribute to water quality, habitat, and biodiversity functions in restored wetlands. However, because natural wetlands often act as significant methane sources, it remains unknown if amendments will also stimulate emissions of this greenhouse gas from restored wetlands. In this study, we investigated the potential trade-offs of incorporating soil amendments into wetland restoration methodology. We used controlled field-scale manipulations in four recently restored depressional freshwater wetlands in western New York, USA to investigate the impact that soils amended with organic materials have on water-quality functions and methane production in the first three years of development. Results showed that amendments, topsoil in particular, were effective for stimulating the development of a suite of biological (microbial biomass increased by 106% and respiration by 26%) and physicochemical (cation exchange capacity increased by 10%) soil properties indicative of water-quality functions. Furthermore, increases in microbial biomass and activity lasted for a significantly longer period of time (years instead of days) than studies examining less recalcitrant amendments. However, amended plots also had 20% times higher potential net methane production than control plots three years after restoration. Wetlands restoration projects are implemented to achieve a variety of goals, commonly including habitat provision, biodiversity, and water-quality functions, but also carbon sequestration, flood abatement, cultural heritage and livelihood preservation, recreation, education, and others. Projects should strive to achieve their specific goals while also evaluating the potential tradeoffs between wetland functions.

Key words: biochar; cation exchange capacity; ecosystem function; methane; microbial biomass; microbial respiration; soil amendment; wetland restoration; wetland soil.

Introduction

Wetlands perform numerous important ecological functions, including water purification, aquifer recharge, long-term carbon storage, flood abatement, and habitat provision (Costanza et al. 1997). Unfortunately, more than half of the earth's wetlands have been degraded or destroyed due to agriculture and development (Dahl 1990). Recognition of the value of lost functions led to wetland protection under Section 404 of the U.S. Clean Water Act (CWA) in 1972. The mandate of the CWA requires that mitigation efforts prioritize functional replacement rather than areal or structural replacement of lost wetlands (EPA 1990). However, there has been little effort to assess ecological functions in the planning, implementation, or monitoring stages of the mitigation process (Zedler 1996, Hoeltje and Cole 2007).

Functional success in wetland restoration is usually determined by dominant vegetation type, percent herbaceous cover, and wetland size (Mitsch and Wilson 1996, Hoeltje and Cole 2007), but evidence suggests that these parameters are poor predictors of ecological functions (Cole and Shafer 2002). In fact, numerous reports reveal that restored wetlands are often not structurally or functionally equivalent to those they are meant to replace (Hanson et al. 1994, Stolt et al. 2000, NRC 2001, Campbell et al. 2002). For example, Ballantine and Schneider (2009) examined 35 restored wetlands in western New York and found that while the vegetative parameters commonly used to determine project success returned to levels of their natural counterparts relatively rapidly, soil properties indicative of overall wetland functioning lagged behind by decades to centuries. The slow development of soil properties has serious implications for the ability of restored wetlands to meet functional expectations within an acceptable timeframe.

Soils are fundamental to ecosystem structure and function, and are therefore recognized as one of the key state factors, which along with climate, organisms, topography, time, and humans, determine the state of

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[5] E-mail: kballant@mtholyoke.edu

an ecosystem (Amundson and Jenny 1997). Because soils are important drivers of ecosystem function, soil properties are often considered indicative of functional development (Hossler and Bouchard 2010). For example, in identifying indicators of structural and functional equivalence in a coastal marsh wetland restoration project, Craft et al. (2003) found that soil organic carbon is an ideal indicator of ecological attributes. Soil organic carbon and other soil variables are often used as such because they are relatively predictable, easy to measure, inexpensive, and have been shown in a variety of ecosystems to serve as excellent surrogates for overall function.

Wetland functions are predominantly dependent on extensive interactions between water and wetland soils. Therefore, the condition of the soil is one of the most critical components in restoration of wetlands. Of particular importance to overall wetland structure and function are soil microbial processes (Groffman et al. 1996), yet they are rarely examined when assessing ecosystem development in restored wetlands (Ahn and Peralta 2009). A number of microbial processes contribute to the water-quality maintenance value of wetlands, as microbial populations can immobilize significant quantities of a variety of nutrients and be important degraders of organic pollutants (Johnston 1991). Another proxy for the potential of a wetland to improve water quality is the soil's ability to hold cations (Brady and Weil 2002, Austin 2006). Soil with large amounts of negatively charged surface area binds positively charged pollutants, such as certain pesticides and especially metals, thereby reducing the quantities being transferred into adjacent surface and groundwater (Cox et al. 1998, Bajeer 2012, Cabrera et al. 2012).

While the importance of wetland functions such as water-quality improvement is widely recognized, concerns have also been raised regarding greenhouse gas emissions from wetlands (Thiere et al. 2011, Morse et al. 2012*a*, *b*, Burgin et al. 2013). Although wetlands may act as greenhouse gas sinks when carbon dioxide (CO_2) is removed from the atmosphere and stored in the soil carbon (C) pool (Bridgham et al. 2006), wetlands may also serve as significant sources of methane (Whiting and Chanton 2001, Burgin et al. 2013). Rice paddies and many types of natural wetlands are known to emit high amounts of methane, but converting aerobic agricultural soil to wetlands does not necessarily increase methane emissions. A number of studies have shown that restored wetlands do not significantly increase greenhouse gas emissions (Mander et al. 2005, Gleason et al. 2009, Morse et al. 2012*a*), and in some cases may even reduce emission of greenhouse gases (Smith et al. 2011). Methane emissions are largely controlled by water level and frequency of inundation, living vegetation, and soil C (Updegraff et al. 1995, Stadmark and Leonardson 2005). Soil C is the substrate for methanogenesis, so the typically low-C soils of newly restored wetlands may have reduced potential for methane emissions compared to the C-rich soils of their natural counterparts. Furthermore, site hydrology is dependent on the restoration design, and can sometimes be manipulated. Therefore, restored wetlands may have hydrologic and soil conditions that limit methane emissions for many years.

The addition of soil amendments as a part of restoration methodology has been suggested as a strategy to hasten soil development and stimulate associated wetland functions. Amendments such as compost, straw, and topsoil have been shown to improve soil by increasing C and nitrogen (N) pools (Ballantine et al. 2012). They have also been shown to increase soil moisture and phosphorus sorption, stimulate nutrient cycling and microbial community development, and decrease bulk density in both coastal and inland restored and created wetlands (Duncan and Groffman 1994, Bruland and Richardson 2004, Burchell et al. 2007, Jacinthe and Lal 2007). Specific recommendations for incorporating soil amendments into wetland restoration plans are rare, however, and of the research that has been published, recommendations are conflicting. Some studies recommend the use of amendments, while others report no beneficial effects, indicating that the time and money invested into incorporating amendments are not worthwhile (Stauffer and Brooks 1997, Bailey et al. 2007, Sutton-Grier et al. 2009). Furthermore, while amendments may stimulate water-quality functions, it is unknown whether they also stimulate methane production. Therefore, the potential trade-off between functions due to amendment additions is unknown.

The primary objective of this study was to investigate the potential tradeoffs of incorporating soil amendments into restoration methodology. These amendments ranged along a gradient of lability, allowing us to examine the effect of mineralizability on wetland function. Specifically, we hypothesized that several years after restoration, (1) microbial biomass, respiration, and methane production would be highest where the most labile organic materials were added, (2) soil chemical and physical improvements with respect to nutrient retention would be highest where the least labile organic materials were added, and (3) the greatest ecosystem function trade-offs would be observed with organic amendments with the highest lability.

Methods

Site description

The experiment was conducted in four newly restored wetlands, each within 120 km of Ithaca, New York, USA. Each wetland was restored in July 2007 on retired agricultural fields by removing topsoil and using that soil to build a flood-control berm. Although they were all similar in topography, size, and history, they differed in soil type and hydrology (Table 1). Sites 1 and 2 were restored on the property of Jim Carter by Marshland Excavating and were permitted by the Seneca County

TABLE 1. Site characteristics of the four restored wetlands examined in this study.

Site	Location	Landscape position	Soil type	Soil saturation	Area (ha)
1	42°55′39″ N, 76°51′31″ W	depression	Canandaigua: very deep, poorly drained, fine-silty, nonacid, mesic Mollic Endoaquepts	consistent	1.2
2	42°55′37″ N, 76°51′22″ W	depression	Alden: deep, poorly drained, fine-loamy, nonacid, mesic Mollic Endoaquepts	consistent	0.8
3	42°23′11″ N, 76°18′17″ W	depression	Canandaigua: very deep, poorly drained, fine-silty, nonacid, mesic Mollic Endoaquepts	intermittent	0.8
4	43°10′11″ N, 75°56′04″ W	depression	Middlebury: very deep, moderately well drained, coarse-loamy, mesic Fluvaquentic Eutrudepts	intermittent	2.4

Note: From Ballantine et al. (2012).

Soil and Water Conservation District as a part of the USDA Natural Resources Conservation Service Wetland Reserve Program. Site 3 was restored on the property of the Cornell University Biological Field Station, also as a part of the USDA Natural Resources Conservation Service Wetland Reserve Program. Site 4 was restored by the Upper Susquehanna Coalition as a mitigation wetland and is located in the Goetchius Wetland Preserve, now property of the Finger Lakes Land Trust. The restored wetlands were all palustrine emergent depressional wetlands (Cowardin et al. 1979).

Experimental design

Immediately after restoration, before flooding occurred, at each of the four sites, we established 25 2×2 m experimental plots to measure soil parameters (five replicates of each of five treatments). Each plot was separated from its nearest neighbors by 2 m. To ensure minimal elevation variation between plots, the bottom topography was leveled with bulldozers during restoration.

The treatments (in order of decreasing lability: straw, topsoil, a 50:50 mix of straw and biochar, and biochar) were assigned to plots in a randomized block design. Carbon content was equalized across all treatments, with 8 kg of organic C added to each plot. This represented an increase of 66% to over 350% above the amount of pretreatment C levels, depending on the site. All plots, including the control plots, were rototilled to 0.1 m depth. The straw treatment was composed of dry stalks of organically grown *Triticum aestivum* ssp. *spelta* obtained from Oescher Farm in Newfield, New York. The biochar was made from a mixture of hardwoods by fast pyrolysis at 450°C with a retention time of less than five seconds (Dynamotive, Vancouver, British Columbia, Canada). The topsoil amendment of each site was taken from homogenized topsoil of that same site (Table 2).

Before treatments were applied, 0.1 m deep soil cores of both topsoil and subsoil were taken at each site using a chrome molybdenum corer (19 mm diameter; AMS, American Falls, Idaho, USA) pushed gently into the soil. Eight randomly distributed cores of topsoil were collected before restoration at each site, and eight more randomly distributed cores were taken again of the subsoil post-excavation. One core per treatment plot was taken in July of 2008 and 2010, Year 1 and Year 3 after the wetlands were restored. All cores were stored at 4°C in the dark until analysis, following homogenization and rock removal. In order to isolate the effects of soil functions, plants were removed from plots.

Each site was surveyed and the water level was measured with a series of 12 0.6 m deep PVC wells distributed evenly throughout each site. Elevation of the water table was measured in wells once monthly during the growing season Year 1 and Year 3. Water table depths relative to the soil surface were averaged to create a single overall index of soil flood condition across each site.

Laboratory analysis

Soil cores collected in July of Year 1 and Year 3 were analyzed for microbial biomass and respiration, cation exchange capacity (CEC), pH, soil C, and soil moisture. Microbial biomass and respiration were measured within three days of sampling using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976). Microbial cells in the soil samples were killed and lysed by fumigation for 20 h with chloroform. The fumigated samples were then inoculated with a small amount of fresh soil containing microbes that used the lysed cells as substrate to grow. The flush of CO_2 released by the actively growing cells during a 10-d incubation is considered to be directly proportional to the amount of C in the microbial biomass of the original soils. Microbial biomass was determined using a proportionality constant (0.45) for calculating biomass C from the CO_2 flush (Jenkinson and Powlson 1976). A Shimadzu GC-14 gas chromatograph with a thermal conductivity detector (Shimadzu, Kyoto, Japan) was used to measure CO_2.

Microbial respiration was quantified as the amount of CO_2 evolved over a 10-d incubation in a nonfumigated sample. Potential net methane emissions were measured

TABLE 2. Site topsoil and amendment chemical properties based on 2007 pre-restoration conditions; units are mg/kg except for C and N (g/kg) and OM (%).

Treatment	C	N	P	K	Mg	Ca	Fe	Al	Mn	Zn	Cu	pH	NO_3	OM
Straw	441.7	4.4												
Biochar	614.7	6.6	34.4	6028	274	2346	70.4	0.40	48.0	3.42		7.18	0.00	50.96
Topsoil														
Site 1	45.9		4.18	55.2	413	3658	3.54	8.00	6.92	0.21	0.72	6.68	0.00	9.19
Site 2	198.6		3.20	31.0	485	7067	495	140	17.7	7.90	1.90	5.21	27.0	39.72
Site 3	39.3		2.84	30.6	689	5699	6.40	15.2	19.2	0.45	1.70	7.11	1.20	7.86
Site 4	25.8		1.34	49.2	101	664	37.1	161	39.2	1.20	0.30	5.38	0.00	5.16

Notes: Soils were sampled to 0.1 m depth. P, K, Mg, Ca, Fe, Al, Mn, Zn, Cu, and NO_3 were extracted using the Morgan method (Morgan 1941). Soil organic matter (OM) was determined by loss on ignition (Black 1965). Adapted from Ballantine et al. (2012).

from these same samples on a Shimadzu GC-8 gas chromatograph with a flame ionization detector. Moisture (%) of each sample was measured to calculate the values per gram dry soil from the field-moist samples that were analyzed. This was done by drying each sample at 105°C for 24 h.

Potential CEC (CEC) was measured by replacing the exchangeable cations in the soil with ammonium ions from ammonium acetate at pH 7.0. After washing with isopropyl alcohol to remove excess ammonium acetate, the adsorbed ammonium ions were displaced by sodium chloride. The ammonium ions in the final extract were determined by an automated Nesslerization procedure on a Technicon AutoAnalyzer (Technicon, Tarrytown, New York, USA). The initial ammonium acetate extract was analyzed for individual exchangeable cations via an inductively coupled plasma spectrometer (ICP-AES, Spectro CIROS, Kleve, Germany). Exchangeable bases (EB) were calculated from the sum of calcium, magnesium, potassium, and sodium ions. All extractions were performed using a mechanical vacuum extractor (Sumner and Miller 1996).

Soil pH was measured in 10 mL distilled water using 5 g soil after occasionally stirring soil for one hour. Soil C was analyzed using an Elementar Vario elemental analyzer (Elemantar Analysensysteme GmbH, Hanau, Germany) coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK) by the Stable Isotope Facility, University of California, Davis, California, USA.

Statistical analysis

A mixed-model MANOVA (treatment, site, year, treatment by site, treatment by year, site by year, treatment by site by year as fixed effects; plot ID as random effect) was performed to assess significant effects across all soil variables measured in this study (statistical package R; R Core Research Team 2004). Next, univariate mixed-model ANOVAs were performed using the same model design as the MANOVA to assess significant effects for individual response variables (JMP version 9, SAS Institute, Cary, North Carolina, USA). In cases where significant fixed effects were detected, pairwise comparisons among groups were made with Tukey's test of honestly significant difference (HSD). All variables were tested for normality and homoscedascity and were transformed to meet these criteria where necessary.

RESULTS

The addition of soil amendments (straw, topsoil, a 50:50 mix of straw and biochar [mix], and biochar) in restored wetlands was effective for stimulating the development of a suite of biological and physicochemical soil properties indicative of water-quality functions. The addition of topsoil resulted in the highest increases in microbial variables associated with water-quality functions. Furthermore, microbial biomass C and respiration increased over time in topsoil plots. Three years after restoration, however, CH_4 production was significantly higher in amended plots than control plots. The mixed-model MANOVA identified significant effects of treatment, site, year, site by year, treatment by year, and treatment by site across all response variables (Wilks' lambda $P = <0.0001$, <0.0001, <0.0001, <0.0001, 0.0289, 0.0003, respectively). Site differences are reported, but only discussed where relevant to the study's overall objectives and hypotheses.

Pre-amendment and hydrologic site characteristics

Physical and chemical properties of the subsoil differed among the newly restored sites (Table 3). In particular, subsoil C differed among sites ($P = 0.0251$), and was highest in Site 2, followed by Site 1, Site 3, and finally, by Site 4. Initial pH also differed significantly among sites ($P \leq 0.0001$). Site 4 had significantly more acidic soils than all other sites, while the soil of Site 2 was close to neutral, and Sites 1 and 3 were significantly more basic than the other sites. Soil N did not differ significantly among sites.

Water level also differed among sites. Sites 1 and 2 were consistently inundated for much of the growing seasons of Year 1 and Year 3, with water levels dropping below the soil surface in August of Year 1 in Site 1, and August of Year 1 and Year 3 in Site 2. In contrast, Site 4 was drier, with intermittent inundation throughout the growing season. Site 3 was not submerged in Year 1, but flooded for much of Year 3 (Fig. 1).

TABLE 3. Site subsoil chemical properties based on 2007 pre-restoration conditions; units are mg/kg except for C and N (g/kg) and OM (%).

Treatment	C	N	P	K	Mg	Ca	Fe	Al	Mn	Zn	Cu	pH	NO_3	OM
Site 1	21.3	1.1	0.80	38.7	1077	14427	29.8	35.9	62.4	0.18	19.2	7.90	0.00	1.17
Site 2	30.2	1.2	0.96	24.8	820	6491	70.1	43.9	27.6	1.64	1.75	6.98	0.00	3.09
Site 3	16.6	0.6	0.96	31.6	1074	13182	3.78	51.8	30.4	0.17	16.2	7.88	1.10	1.18
Site 4	06.2	1.1	0.66	23.4	47.9	370	30.6	120.4	17.3	0.43	0.42	5.13	0.00	1.62

Notes: Soils were sampled to 0.1 m depth. P, K, Mg, Ca, Fe, Al, Mn, Zn, Cu, and NO_3 were extracted using the Morgan method (Morgan 1941). Soil organic matter (OM) was determined by loss on ignition (Black 1965). Adapted from Ballantine et al. (2012).

Biological soil variables

The addition of soil amendments significantly increased microbial biomass (Fig. 2). The mixed-model ANOVA found a significant effect of treatment, site, and year (P = 0.0025, 0.0077, 0.0414, respectively). Topsoil plots showed the highest increases, followed by straw, mix, biochar, and finally, control plots. Site 1 had the highest microbial biomass, followed by Site 3, and was significantly greater than Sites 4 and 2. Microbial biomass increased from Year 1 to Year 3, though this trend was clearly driven by an increase in topsoil plots.

The mixed-model ANOVA found that respiration was significantly influenced by treatment ($P \leq 0.0001$). Plots amended with straw (straw and mix) had the highest rates of respiration, followed by topsoil, biochar, and control plots (Fig. 3). Although Year was not significant in the overall model, post-hoc analysis revealed that respiration increased from Year 1 to Year 3 in topsoil plots only.

Physicochemical soil variables

The mixed-model ANOVA of CEC found a significant effect of treatment, site, and site by year (P = 0.0314, <0.0001, <0.0001, respectively). Topsoil plots had the highest CEC, followed by mix, control, biochar, and straw plots (Fig. 4). Cation exchange capacity decreased from Year 1 to Year 3 in Sites 1 and 4, and increased in Sites 2 and 3.

The mixed-model ANOVA of EB found a significant effect of site, year, and site by year ($P \leq 0.0001$, <0.0001, 0.0111, respectively). Post-hoc analyses revealed that EB decreased in all sites from Year 1 to Year 3, though the change was not significant in Site 1 (Fig. 5). Exchangeable bases also decreased across treatments, though the change was only significant in biochar plots.

Exchangeable bases were positively correlated with pH within and across all treatments, and these trends were driven almost entirely by differences in pH among sites ($P \leq 0.0001$ for all). Treatment, site, year, and site by year had significant effects on pH, according to the mixed-model ANOVA (P = 0.0215, <0.0001, <0.0001, <0.0001, respectively). Post-hoc analyses revealed no differences among treatments, but showed that Sites 2 and 3 had higher pH than Site 1, and all three sites had higher pH than Site 4. The pH at Sites 1 and 2 was constant over time, whereas the pH of Sites 3 and 4 decreased from Year 1 to Year 3 (Fig. 5).

Soil C increased with the use of amendments. The mixed-model ANOVA found a significant effect of treatment and site ($P \leq 0.0001$ for both). Biochar and mix plots had the highest increases, followed by topsoil, straw, and finally, control plots (Fig. 6). Sites 2 and 1 had significantly higher soil C than site 3, and all sites had significantly higher levels than Site 4.

The mixed-model ANOVA of soil moisture found a significant effect of treatment and site (P = 0.0012, <0.0001, respectively). Topsoil and straw plots had the highest soil moisture, followed by mix, biochar, and finally, control plots. Soil moisture was highest in Site 1, followed by Sites 2, 4, and finally, 3.

CH_4 production

Three years after restoration, CH_4 production was significantly higher in amended plots than control plots

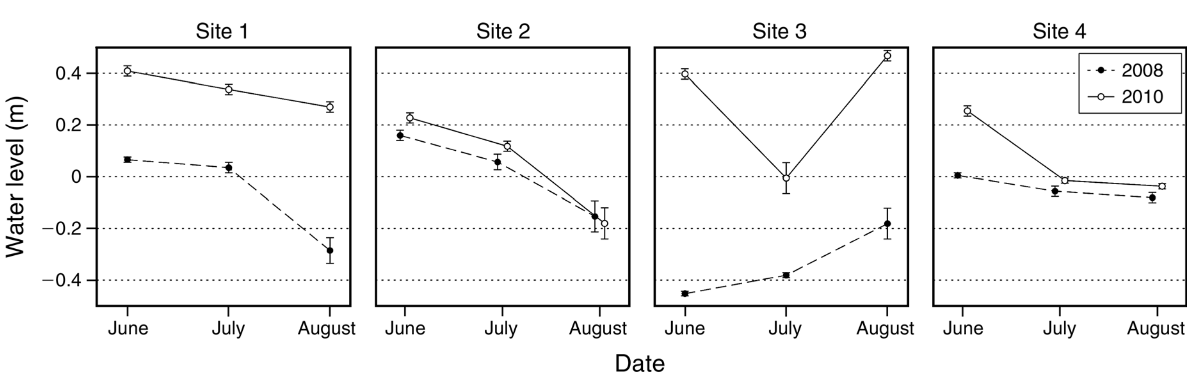

FIG. 1. Water level (m) above or below soil surface (zero level) as an average of 12 well measurements across each site on each date (means ± standard error). From Ballantine et al. (2012).

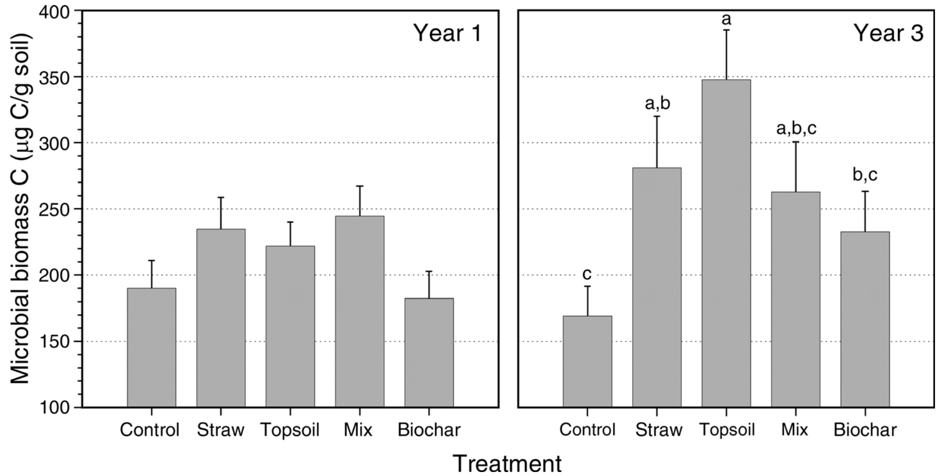

FIG. 2. Microbial biomass by treatment and year, averaged across all sites (means + standard error). A post-hoc test for Year 1 showed no significant ($P < 0.05$) differences. Letters above the bars indicate significant differences identified by a post-hoc test for Year 3. Sites not linked by a common letter are significantly different.

(Fig. 7). Potential net methane production had significant effects of treatment, site, treatment by year, and site by year ($P \leq 0.0001$, <0.0001, 0.0008, <0.0001, respectively). Soil moisture was positively correlated with CH_4 production within and across all treatments ($P \leq 0.0001$ for all).

DISCUSSION

Soils are a key factor influencing ecosystem function, and are often used as indicator variables for assessing the overall state of an ecosystem (Amundson and Jenny 1997). This is particularly relevant when evaluating restored ecosystems, where monitoring is required and highly indicative, yet predictable, easy to measure, inexpensive indicators are desirable. This study revealed that the use of soil amendments as a part of wetland restoration methodology stimulated the development of soil properties. Topsoil amendments were particularly effective at increasing microbial biomass and CEC. However, within three years of restoration, CH_4 production was higher from amended soils. Topsoil amendments yielded the greatest increases in soil variables related to water quality and had similar CH_4 production as other amendments. Three years after restoration, however, levels of the soil properties measured were far below those of comparable natural wetlands, and the long-term tradeoff between functions of differently amended soils in restored wetlands remains an important area of inquiry.

Amendments stimulate soil-based water-quality functions

Microbial biomass and respiration increased over time with the use of soil amendments, particularly in topsoil plots. That levels did not increase in plots containing straw may indicate that the most readily

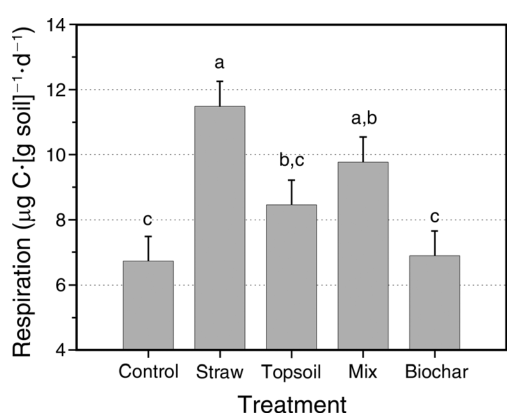

FIG. 3. Respiration by treatment, averaged across all sites and years (means + standard error). Letters above the bars summarize the results of post-hoc comparisons among treatments. Sites not linked by a common letter are significantly ($P < 0.05$) different.

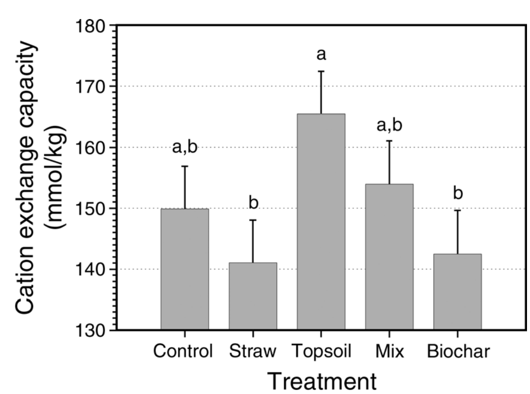

FIG. 4. Cation exchange capacity by treatment, averaged across all sites and years (means + standard error). Letters above the bars summarize the results of post-hoc comparisons among treatments. Sites not linked by a common letter are significantly ($P < 0.05$) different.

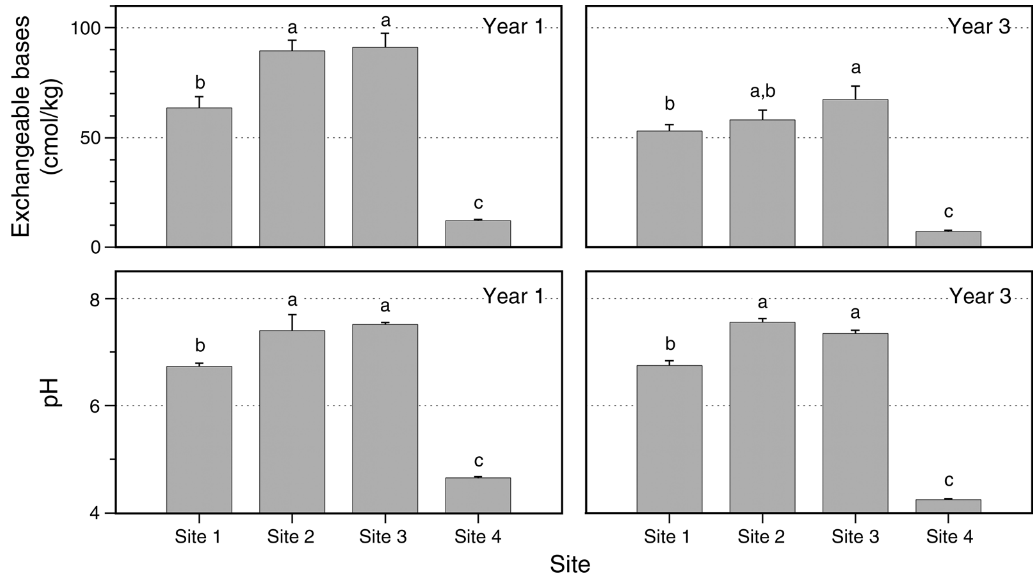

FIG. 5. Exchangeable bases and pH by site and year, averaged across all treatments (means + standard error). Letters above the bars summarize the results of post-hoc comparisons among sites. Within each year, sites not linked by a common letter are significantly ($P < 0.05$) different.

available C was metabolized quickly, leaving only more recalcitrant forms of organic matter that are less available for microbial use over the long term. The increase over time of microbial biomass and respiration in topsoil plots may indicate that conditions suitable for microbial growth improved as the soil developed. In particular, topsoil amendments may also add an active microbial community, effectively acting as a microbial inoculant (Ballantine et al. 2012). The increase in microbial biomass and respiration in topsoil plots was not related exclusively to soil C because this variable did not increase from Year 1 to Year 3.

Due to the increases in microbial biomass and respiration in topsoil plots, by Year 3, there were no significant differences in these biological indicators among topsoil- and straw-containing plots. The higher microbial biomass and respiration of topsoil, straw, and mix plots suggest that these amendments have greater potential to jumpstart biological degradation of organic compounds and improve the wetland's ability to immobilize nutrients and buffer or absorb pollutant inputs from the surrounding landscape.

Surprisingly, microbial biomass and respiration in biochar plots had not risen above control plots even three years after restoration. We originally hypothesized that the porous structure and nutrient-binding capability of biochar would foster active microbial communities over time. The lack of increase in microbial indicator variables in biochar plots suggests that the biochar used here does not jumpstart water-quality functions in the first three years as anticipated. Some studies of biochar amendments in agricultural systems have reported,

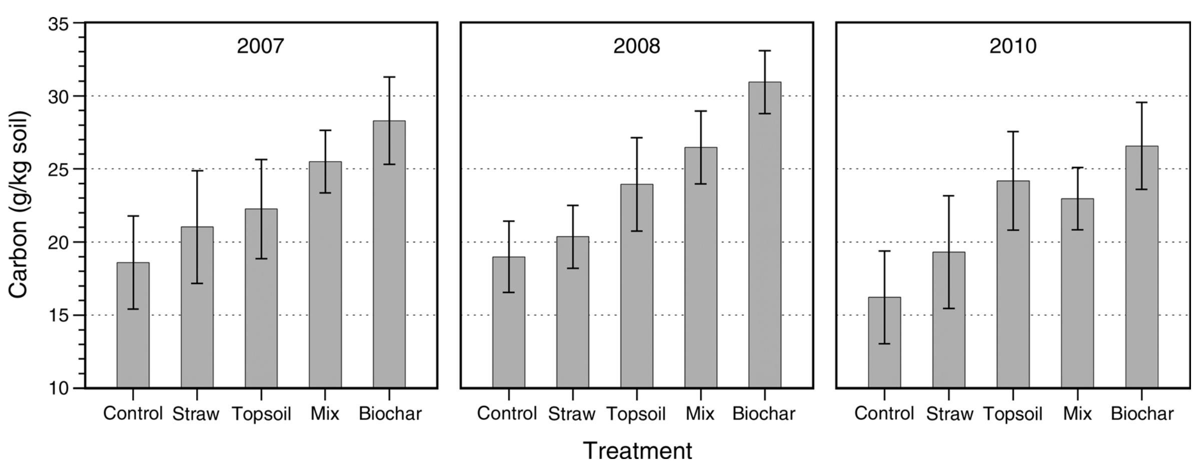

FIG. 6. Soil carbon by treatment and year averaged across all sites (means ± standard error). From Ballantine et al. (2012).

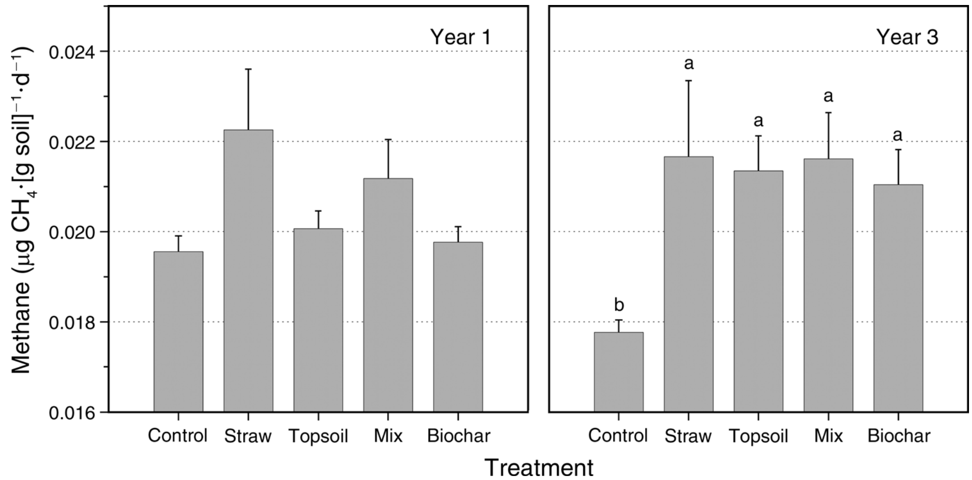

FIG. 7. Potential net methane production by site and year, averaged across all treatments (means + standard error). A post-hoc test for Year 1 showed no significant ($P < 0.05$) differences. Letters above the bars indicate significant differences identified by a post-hoc test for Year 3. Sites not linked by a common letter are significantly different.

however, that the beneficial effects of biochar additions were not significant in the first years following addition (Major et al. 2010). This delay may be even longer in wetlands due to the anoxic nature of submerged soils.

While amendments increased microbial parameters, these levels were far below those reported for comparable natural wetlands. Restored wetland soils often have relatively low levels of C and microbial activity compared to natural wetlands (Craft et al. 1988, Ballantine and Schneider 2009). For example, Duncan and Groffman (1994) found that constructed marshes in Massachusetts, USA, which were amended with 0.3 m of organic substrate from a nearby pond-dredging project, had microbial biomass C ranging from 768 to 1088 μg C/g. Even the low end of this range is an order of magnitude greater than the highest values measured in our study. These results indicate that amendment with substrate can increase microbial properties of wetland soils, and suggest that the amount of substrate added in our study (66% to over 350% increase over the amount of pretreatment C levels) was not sufficient to see substantial effects.

Some studies have found it difficult to estimate the long-term efficacy of soil amendments based on short-term observations. For example, compost amendments have been found to stimulate microbial biomass, but only for the first days to weeks after addition (Saison et al. 2006). In our study, the magnitude of increased microbial biomass in amended soils was smaller than other published amendment studies, but remained significantly greater even three years after restoration. Thus, while our amendments had a relatively small effect on microbial biomass, they remained active for a longer period. The sustained productivity in our plots could be due to the nature of the amendments we chose, which may be more recalcitrant than the compost amendments examined in the other studies.

Unlike the biological properties of microbial biomass and respiration, the physicochemical property of CEC is highly reflective of a substrate's structure. Cation exchange capacity differed among treatments, with topsoil plots having the highest levels. The CEC of topsoil plots was likely higher than that of plots containing straw, because topsoil contains more humus and heavy fraction soil organic matter (SOM), which have higher CEC than the light fraction SOM present in fresh organic matter such as straw (Bendfeldt et al. 2001). As the straw continues to decompose over time, we expect to see CEC increase in straw plots. We expect the CEC of mix plots to rise even faster, as both straw decomposition and biochar oxidation will contribute to an increase in negatively charged sites on the soil surfaces.

All of the CEC levels observed in this study were much lower than those of comparable natural wetlands. For example, Ballantine and Schneider (2009) reported CEC levels of natural wetlands in central New York more than five times greater than the mean of our topsoil plots. That same study reported CEC values similar to ours for wetlands of approximately the same age (3–5 years). Based on the results of the Ballantine and Schneider (2009) study, we expect that CEC will be very slow to develop in our restored wetlands. Our results show, however, that topsoil additions increase CEC, and it is possible that larger amounts would more quickly establish CEC levels found in natural reference wetlands.

In contrast to the other variables, differences in exchangeable bases (EB) were explained primarily by site differences in pH. Soil pH is known to be important for EB because as pH increases and the soil becomes less acidic, the number of negative charges on the colloids increases, thereby increasing EB. Likewise, as pH drops, EB decreases (Downie et al. 2010). Exchangeable bases did not differ among treatments, but decreased from Year 1 to Year 3 across all sites. The most striking instance was in Site 4, where both pH and EB were significantly lower than other sites in Year 1, and decreased still further in Year 3. If the pH of Site 4 continues to decrease, we expect EB will also continue to decrease. This would result in more highly acidic soil with few of the nutrients essential for supporting microbial communities and plant growth. Subsequently, the ability of this soil to bind and process pollutants and improve water quality would be limited.

Amendments stimulate methane production

While amendments, particularly topsoil, increased some soil variables indicative of water-quality functions, they also increased methane production. Contrary to expectations, however, CH_4 production did not differ with amendment type.

The higher CH_4 production in wetlands three years after restoration may have been due in part to differences in soil moisture. Moisture status in control plots was consistently drier than the other treatments in both years. The higher oxygen status of control plot soils over time likely led to insufficiently reduced conditions for methanogenesis, and may have allowed for more methane depletion by methanotrophs, thereby lowering overall potential methane production.

In addition to oxygen status, methane emissions are influenced by temperature, pH, plant productivity, and C availability. Unlike temperature, pH, and plant productivity (Ballantine et al. 2012), soil C differed significantly among treatments. Control plots had the lowest C both years, and appeared to drop slightly from Year 1 and Year 3, though not significantly so (Ballantine et al. 2012). The decrease in soil C may have also contributed to the decreased CH_4 production in control plots from Year 1 to Year 3. This supports other studies reporting reduced methane emissions in low C soils. For example, Altor and Mitsch (2008) found lower methane emissions from newly restored wetland soils than older restored wetlands, and attributed the difference to lower SOM in the newly restored sites. The low microbial biomass and respiration in control plots may also have limited methane production (Segers 1998). Whiting and Chanton (2001), for example, found that the quantity of methane contained belowground and emitted to the atmosphere was closely associated with the activity of the microbial community. It is possible that as C accumulates in restored wetlands over time, methane emissions may increase. This process is likely to be very slow in the examined sites, however, as C has been shown to accumulate slowly in similar restored wetlands (Ballantine and Schneider 2009). There is also likely an interaction between soil SOM and soil moisture, as SOM increases the water-holding capacity of the soil.

Plants are also known to influence methane emissions in multiple ways. In addition to providing carbon for microbial activity, aerenchyma within the tissues of some plants serve as conduits for direct travel of gases to and from plant roots (Wang et al. 1996). Methane can enter the atmosphere through this transport system. Likewise, oxygen enters the root zone through these same channels, potentially enhancing methane consumption by methanotrophs (Cao et al. 1998, Askaer et al. 2011). To isolate the effects of amendments on soil functions, we removed vegetation from the plots. We therefore cannot derive any conclusions about plant effects on methane emissions.

Weighing the benefits vs. the risks

Our results revealed that while the use of soil amendments in wetland restoration may hasten the development of water-quality functions, it may also stimulate methane production. Other studies investigating the potential tradeoffs between greenhouse gas emissions and desirable functions of wetlands suggest that over time, C sequestration and the other benefits of wetland restoration will outweigh the risks. For example, studies from the Timberlake Restoration Project in the North Carolina coastal plain found that restoration of wetland hydrology led to significant nitrate retention and removal (Ardón et al. 2010), but did not increase greenhouse gas emissions (Morse et al. 2012*a*). In comparing C sequestration and methane emissions from natural wetlands, Whiting and Chanton (2001) concluded that, integrated over a 500-year time horizon, natural wetlands will be sinks for greenhouse gas warming potential and therefore will attenuate the greenhouse warming of the atmosphere. Furthermore, several studies have found that methane emissions can remain transient or low in periodically inundated restored wetlands (Altor and Mitsch 2008, Jerman et al. 2009). Other studies reveal that constructed wetlands receiving high but naturally occurring nitrate inputs, such as those built to provide water-quality functions, constrain the production of methane (Stadmark and Leonardson 2005).

It is also important to consider that restoring the hydrology of historically drained agricultural fields, a common method for restoring wetlands, typically has the goal of restoring the functions of wetlands that were historically lost to agriculture and development, as mandated under the Clean Water Act (EPA 1990). In addition to the undesirable potential emission of methane, many of these functions are desirable, among them habitat provision, flood abatement, water-quality functions, and long-term C sequestration.

Conclusions and Recommendations

Wetlands restoration projects have goals ranging from habitat provision and biodiversity to carbon sequestration and water-quality functions to recreation and education. By aiming to restore the functions of natural wetlands, should we consider the less desirable functions a necessary corollary to restoring desirable functions (Burgin et al. 2013)? Or instead, should we strive to develop methods (such as soil amendment and hydraulic manipulation) that promote certain functions while minimizing others? We recommend that these decisions be made on a case-by-case basis depending on the goals of each particular project, with the overall goal of maintaining a positive balance of desirable functions.

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