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Stimulating Nitrate Removal Processes of Restored Wetlands

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ABSTRACT: The environmental and health effects caused by nitrate contamination of aquatic systems are a serious problem throughout the world. A strategy proposed to address nitrate pollution is the restoration of wetlands. However, although natural wetlands often remove nitrate via high rates of denitrification, wetlands restored for water quality functions often fall below expectations. This may be in part because key drivers for denitrification, in particular soil carbon, are slow to develop in restored wetlands. We added organic soil amendments that range along a gradient of carbon lability to four newly restored wetlands in western New York to investigate the effect of carbon additions on denitrification and other processes of the nitrogen cycle. Soil carbon increased by 12.67−63.30% with the use of soil amendments ($p \leq 0.0001$). Soil nitrate, the carbon to nitrogen ratio, and microbial biomass nitrogen were the most significant predictors of denitrification potential. Denitrification potential, potential net nitrogen nitrification and mineralization, and soil nitrate and ammonium, were highest in topsoil-amended plots, with increases in denitrification potential of 161.27% over control plots. While amendment with topsoil more than doubled several key nitrogen cycling processes, more research is required to determine what type and level of amendment application are most effective for stimulating removal of exogenous nitrate and meeting functional goals within an acceptable time frame.

INTRODUCTION

Water pollution caused by excessive inputs of nutrients (eutrophication) is a serious problem throughout the world and has been consistently ranked as a top cause of degradation in U.S. waters.[1] Nitrate (NO_3^-) contamination of coastal aquatic systems is a particular concern. Anthropogenic activities have doubled the cycling of reactive N on Earth, leading to air and soil pollution, human health concerns, and increased delivery of NO_3^-, the most common and mobile form of reactive N, to receiving waters.[2] A strategy proposed for addressing eutrophication of aquatic ecosystems and human health problems is the restoration and creation of wetlands.[3,4] Researchers have estimated that movement through such wetlands and riparian zones can reduce NO_3^- content of upland runoff from 30 to 85% annually.[5,6]

Wetlands reduce NO_3^- primarily through plant uptake, microbial immobilization, and dissimilatory respiration processes.[7,8] Storage in plant or microbial biomass can result in N-enrichment of the wetland and these pools can be saturated as excessive inputs result in decreased removal potential of NO_3^- over time.[9] In contrast, NO_3^- can be completely removed from the wetland system by reduction of NO_3^- to the N gases nitric oxide (NO), nitrous oxide (N_2O), or harmless diatomic nitrogen (N_2).[2] This process is known as denitrification, and is traditionally seen as the most important removal mechanism of NO_3^- from ecosystems, although other dissimilatory processes such as the reduction of nitrate to ammonium (DNRA), anaerobic ammonium oxidation (anammox), and

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Table 1. Site Characteristics of the Four Restored Wetlands Examined in This Study, From Ballantine et al. (2012)

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-laomy, mesic Fluvaquentic Eutrudepts	intermittent	2.4

chemoautotrophic denitrification through iron or sulfur oxidation have also been shown to be important.[10]

Denitrification is primarily carried out by facultative anaerobic bacteria, most of which are heterotrophic and use carbon (C) as a source of energy.[11] In wetland soils, denitrification rates are controlled by oxygen status and the presence of sufficient amounts of NO_3^- and organic carbon (C_{org}).[12,13] Organic C provides an energy source for denitrifying bacteria.[5,6,14] Because the established vegetation and topsoil are often removed as part of the restoration methodology, restored wetlands often have relatively low levels of soil C and microbial activity relative to natural wetlands.[7,8,15−17] Despite the importance of soil C in providing the substrate for NO_3^- removal functions, soil conditions are often the least considered aspect of wetland restoration.[9,18]

The addition of C via soil amendments has been suggested as a way to hasten soil development and associated water quality functions of restored wetlands.[2,19] Amendments such as compost, straw, and topsoil have been shown to increase C and N pools. They also have 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 wetlands.[10,20−25] Specific recommendations for incorporating soil amendments into wetland restoration plans are rare, however, and there has been little analysis of amendments in sites specifically designed for NO_3^- removal. 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 is not worthwhile.[11,21,25,26] Much confusion remains regarding whether the cost of amendments is worthwhile, and if so, how much and what type of amendment to incorporate into the soil for maximum NO_3^- removal.

We hypothesize that the unique properties of different amendments are particularly influential to NO_3^- removal. Specifically, we expect that N dynamics are influenced by the effects of amendments on the pool of labile C (C_L), NO_3^- levels, and the carbon to nitrogen ratio (C:N) of the soil. In this study, we added organic soil amendments that range along a gradient of carbon lability to four newly restored wetlands in western New York to investigate the effect of carbon additions on denitrification and other processes of the nitrogen cycle and to address the following questions: (1) Are differences in hydrology and background soil conditions among sites more powerful indicators of denitrification potential than treatment differences? (2) Do differences in the C_L of wetland soil influence denitrification potential and N cycling in restored wetlands? (3) Can the C_L of newly restored wetland soil can be influenced through addition of soil amendments?

MATERIALS AND METHODS

Site Description. The experiment was conducted in four newly restored wetlands, each within 120 km of Ithaca, New York. 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).[12,13,19] Sites 1 and 2 were restored on the property of Jim Carter by Marshland Excavating and were permitted by the Seneca County Soil & 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.[27−30]

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

The treatments (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% over the amount of pretreatment C levels, depending on the site. All plots, including the control plots, were roto-tilled to 0.1 m depth. The straw treatment was composed of dry stalks of organically grown *Triticum aestivum* subsp. *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, Canada). The topsoil amendment of each site was taken from homogenized topsoil of that same site.

Before treatments were applied, 0.1 m deep soil cores were taken of both topsoil and subsoil were taken at each site using a chrome molybdenum corer (19 mm diameter) pushed gently

Table 2. Site Soil and Amendment Chemical Properties Based on 2007 Pre-Restoration Conditions[a]

treatment	C (g/kg)	N (g/kg)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Fe (mg/kg)	Al (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	pH	NO_3 (mg/kg)
straw	441.7	4.4											
biochar	614.7	6.6	34.40	6028.00	274.00	2346.00	70.40	0.40	48.00	3.42		7.18	0.00
topsoil (site 1)	45.9		4.18	55.20	413.34	3658.40	3.54	8.00	6.92	0.21	0.72	6.68	0.00
topsoil (site 2)	198.6		3.20	31.00	485.40	7067.00	495.20	140.30	17.70	7.90	1.90	5.21	27.02
topsoil (site 3)	39.3		2.84	30.60	689.04	5699.40	6.40	15.24	19.20	0.45	1.70	7.11	1.20
topsoil (site 4)	25.8		1.34	49.20	101.08	664.00	37.12	161.94	39.18	1.20	0.30	5.38	0.00
subsoil (site 1)	21.3	1.1	0.80	38.67	1077.57	14427.67	29.80	35.93	62.43	0.18	19.20	7.90	0.00
subsoil (site 2)	30.2	1.2	0.96	24.80	820.46	6491.20	70.14	43.94	27.60	1.64	1.75	6.98	0.00
subsoil (site 3)	16.6	0.6	0.96	31.60	1074.92	13182.60	3.78	51.82	30.42	0.17	16.16	7.88	1.10
subsoil (site 4)	06.2	1.1	0.66	23.40	47.88	370.20	30.56	120.42	17.28	0.43	0.42	5.13	0.00

[a]Soils were sampled to 0.1 m depth. P, K, Mg, Ca, Fe, Al, Mn, Zn, Cu, and NO3 extracted using the Morgan method (Morgan 1941). From Ballantine et al. (2012).

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 postexcavation. 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. To isolate the effects of amendments on soil functions, we removed vegetation from the plots. This enabled us to differentiate the effect of soil amendments on the response variables. For example, we would not be able to confidently say that increases in denitrification potential were due to the addition of amendments if the plant biomass or composition differed across plots. A separate study ultimately revealed that amendments did not influence plant biomass or diversity and there was minimal plant growth (Ballantine et al. 2012).

Each site was surveyed and the water level was measured with a series 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 denitrification potential, potential net N mineralization and nitrification, and levels of microbial biomass N, soil NO_3^- and ammonium (NH_4^+). Properties that influence denitrification were also analyzed, including C_L, soil C, pH, soil moisture, and site hydrology. Soil cores were stored at 4 °C after sampling and analyzed within 3 days at field moisture following homogenization and rock removal.

Denitrification potential was measured using the denitrification enzyme activity assay described by Smith and Tiedje.[2,31−33] This procedure is based on the ability of acetylene (C_2H_2) to inhibit the reduction of N_2O to N_2. Therefore, in the presence of C_2H_2, N_2O becomes the terminal product of denitrification.[33,34] Soils in the lab were combined with a mixture of (1) acetylene (10 kPa) to prevent N_2O from being reduced to N_2, (2) glucose (40 mg kg^{-1}) and KNO_3 (100 mg N kg^{-1}) to provide excess substrate for existing enzymes, and (3) chloramphenicol (10 mg kg^{-1}) to block production of new enzymes during incubation. The soil slurries were made anaerobic by evacuation and flushing with N_2 gas and placed on an orbital shaker. Gas samples were taken at 30 and 90 min, stored in evacuated glass tubes, and analyzed for N_2O by electron capture gas chromatography.

The pool of labile C, microbial biomass N, potential net N mineralization and nitrification, and levels of soil NO_3^- and NH_4^+ were measured using the chloroform fumigation-incubation method.[35] 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 to introduce microorganisms that metabolize the lysed microbial cells in the original sample. The carbon dioxide (CO_2) flush and extractable mineral nitrogen (NH_4^+ and NO_3^-) released by the actively growing cells during a 10-day incubation are directly proportional to the amounts of carbon and nitrogen in the microbial biomass of the original soils.

Ten-day incubations of nonfumigated samples provided estimates of the pool of C_L and potential net mineralization and nitrification rates. The pool of C_L in the soil was quantified as the amount of CO_2 evolved over the 10-day incubation of the nonfumigated sample.[36] Potential net N mineralization was quantified as the accumulation of mineral N (NH_4^+ and NO_3^-) and potential net N nitrification was quantified as the accumulation of NO_3^- during this incubation. Nitrate and NH_4^+ were quantified colorometrically using a Lachat Quikchem 8100 flow injection analyzer. CO_2 production was measured by thermal conductivity gas chromatography. Soil moisture was quantified by drying at 105 °C for 24 h.

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 Ltd., Cheshire, UK) by the Stable Isotope Facility, University of California, Davis, CA.

Statistical Analysis. A mixed-model MANOVA (fixed effects = treatment, site, year, treatment×site, treatment×year, site×year, treatment×site×year; random effect = plot ID) was performed to assess significant effects across all soil variables measured in this study (Statistical package R). Next, univariate

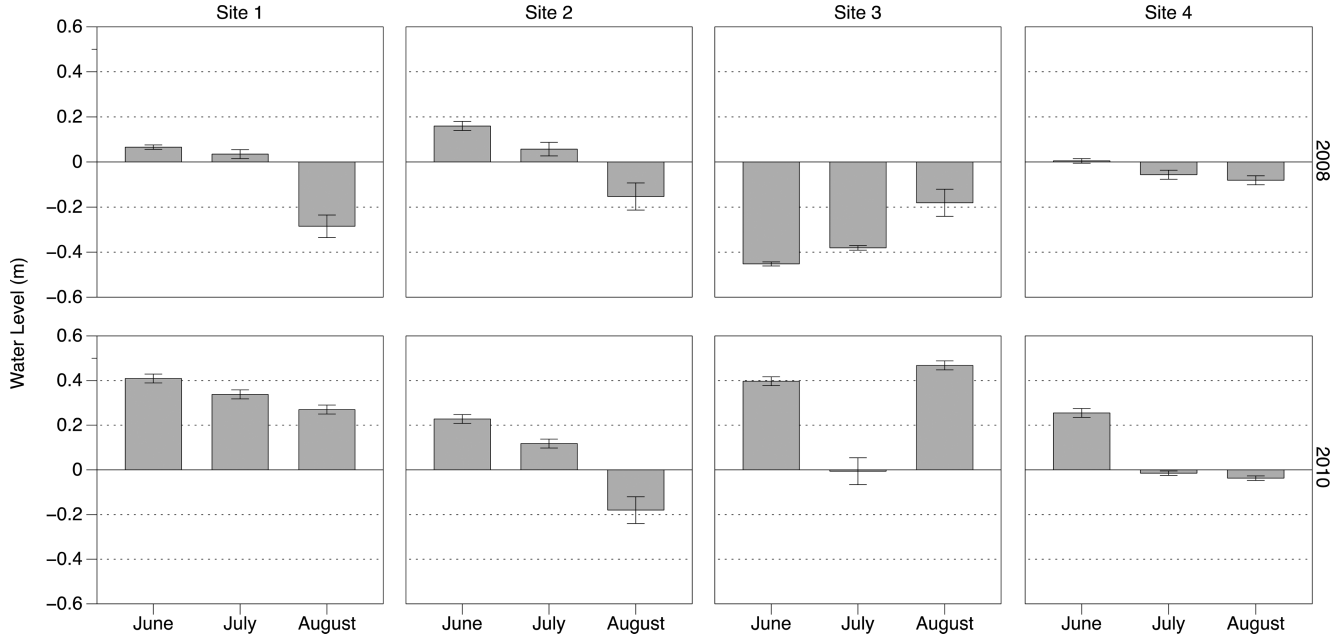

Figure 1. Water level (m) above or below soil surface (zero level) as an average of 12 well measurements across each site on each date (mean + standard error). From Ballantine et al. (2012).

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, Inc.). Because there were no consistent differences in any of the response variables across time, year was also included as a random variable in the ANOVA model. In cases where significant fixed effects were detected, pairwise comparisons among groups were made with Tukey's test of Honestly Significant Difference (HSD). In addition, bivariate regression analyses were performed to compare specific response variables that have been reported in the literature to influence N cycling. All variables were tested for normality and homoscedascity and were transformed to meet these criteria where necessary.

RESULTS

Preamendment and Hydrologic Site Characteristics. Physical and chemical properties of the subsoil differed among the newly restored sites (Table 2). In particular, pretreatment soil C differed among sites ($p = 0.0251$), and was highest in Site 2 (30.2 g/kg), followed by Site 1 (21.3 g/kg), Site 3 (16.6 g/kg) and, finally by Site 4 (06.2 g/kg). Initial pH also differed significantly among sites ($p \leq 0.0001$). Site 4 had significantly more acidic soils than all other sites (5.13), while the soil of Site 2 was close to neutral (6.98), and Sites 1 and 3 were significantly more basic than the other sites (7.9 and 7.88, respectively). 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 intermittently inundated throughout the growing season. Site 3 was not submerged in Year 1, but was flooded for much of Year 3 (Figure 1).

Physical and Chemical Soil Variables. The addition of different soil amendments to the four restored wetlands significantly influenced processes and products of the N cycle, as well as soil variables known to influence NO_3^- removal via denitrification. The mixed model MANOVA of all response variables identified significant effects of treatment, site, year, treatment×year, site×year, and treatment×site across all response variables (Wilks' Lambda $p \leq 0.0001$, <0.0001, <0.0001, 0.0279, <0.0001, <0.0001, respectively).

The mixed model ANOVA of C_L, our primary intended treatment effect, found a significant effect of treatment and site ($p < 0.0001$, 0.0410, respectively). Plots amended with straw (Straw and Mix) had higher C_L, while Topsoil plots had higher C_L than Biochar and Control plots, though not significantly so (Figure 2). Site 1 had significantly higher C_L than all the other sites.

The addition of soil amendments also significantly increased soil C relative to controls, with biochar and topsoil amendments showing the highest increases (Figure 3A). The mixed model ANOVA of soil C found a significant effect of treatment and site ($p \leq 0.0001$ for both). Soil C was significantly higher in

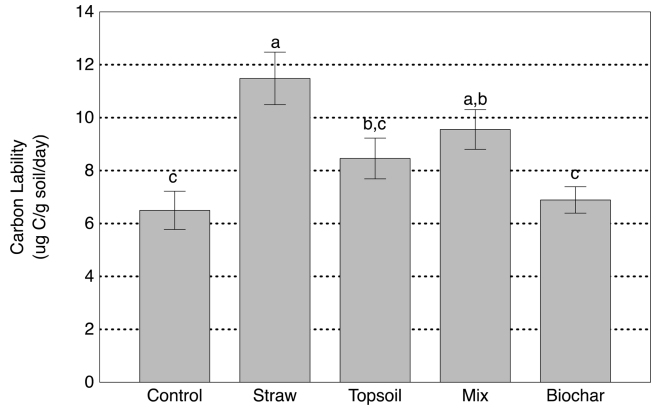

Figure 2. Pool of labile carbon by treatment averaged across all sites and years (mean + standard error). Letters above the bars summarize the results of post hoc comparisons among treatments. Treatments not linked by a common letter are significantly different.

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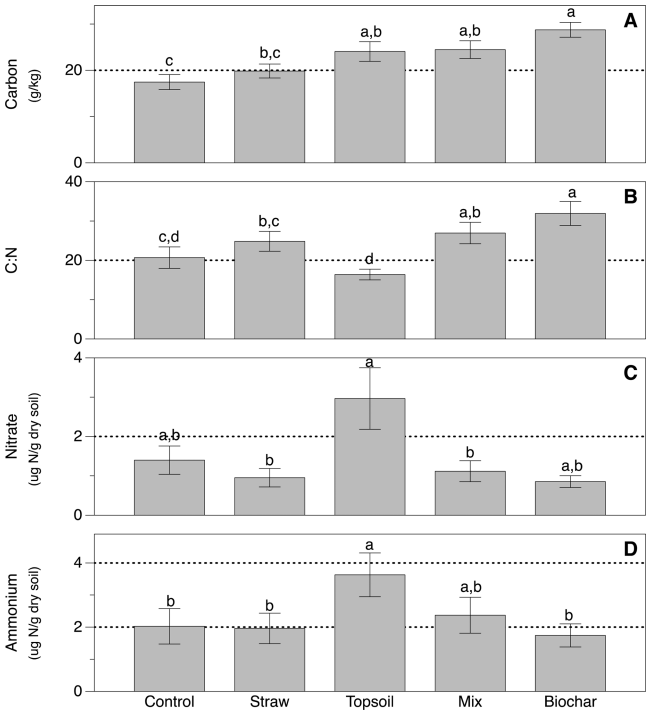

Figure 3. Concentrations of soil carbon, C:N, nitrate, and ammonium by treatment across all sites and years (mean + standard error of raw data). Letters above the bars summarize the results of post hoc comparisons among treatments for each variable. Treatments not linked by a common letter are significantly different. Note: post hoc comparisons of C:N, nitrate, and ammonium were performed on transformed data.

Sites 1 and 2, than in Site 3, which was significantly higher than Site 4.

Like the C variables, other soil properties known to influence N cycling differed significantly among treatments and sites. The mixed model ANOVA of C:N found a significant effect of treatment and site ($p < 0.0001$ for both). Biochar and Mix plots had the highest C:N, followed by Straw, Control, and Topsoil plots. Site 2 had significantly higher C:N than all other sites, followed by Sites 1 and 3, which were significantly higher than Site 4 (Figure 3B).

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 significantly higher in Site 1 than all other sites, followed by Site 2, Site 4, and finally Site 3, which had significantly lower soil moisture than Site 2. Treatment and site also had significant effects on pH, according to the mixed model ANOVA ($p = 0.0215$, <0.0001, respectively). Posthoc analyses revealed no differences among treatments, but showed that Sites 2 and 3 had significantly higher pH than Site 1, and all three sites had significantly higher pH than Site 4.

Soil N pools were also significantly influenced by treatment and site differences. The mixed model ANOVA of NH_4^+ found a significant effect of treatment and ($p = 0.0073$, <0.0001, respectively). Levels were significantly higher in Topsoil plots than in Biochar, Straw, and Control plots. Ammonium was significantly greater in Sites A and D than Site B, all of which were greater than Site 3 (Figure 3D). For soil NO_3^-, there was a significant effect of treatment and site ($p = 0.0013$, <0.0001, respectively). Soil NO_3^- in Topsoil plots was significantly higher than in Mix and Straw plots, but not significantly different from Biochar and Control plots (Figure 3C). Sites D and C had significantly higher levels than Sites B and A.

Processes and Products of Nitrogen Cycling. Denitrification potential was significantly influenced by the amendment added ($p \leq 0.0001$), with the highest rates in plots that had been amended with Topsoil, followed by plots that had been amended with Biochar (Figure 4A). Specifically, Topsoil

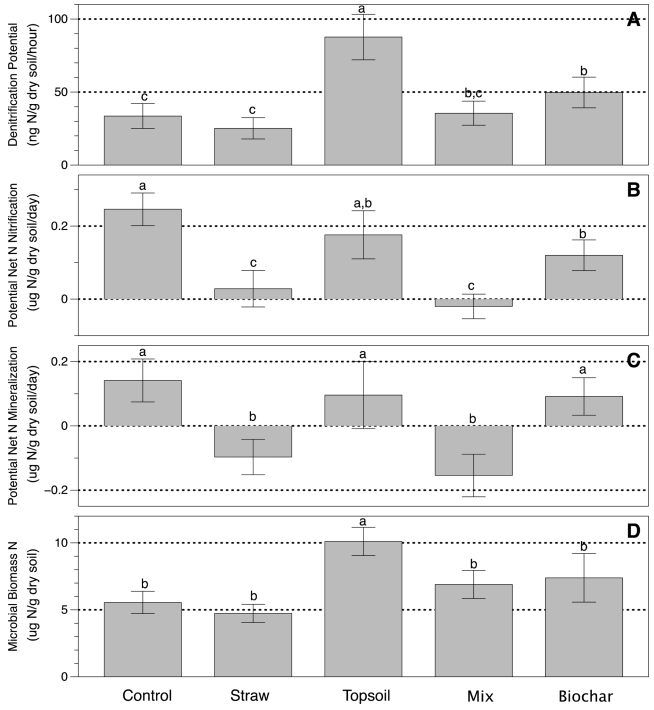

Figure 4. Denitrification potential, potential net N nitrification, potential net N mineralization, microbial biomass N by treatment across all sites and years (mean + standard error of raw data). Letters above the bars summarize the results of post hoc comparisons among treatments and were performed on transformed data. Treatments not linked by a common letter are significantly different.

plots had significantly greater denitrification potential than all other plots, while Biochar plots had significantly greater denitrification potential than Straw and Control plots. In addition to a treatment effect, the mixed model ANOVA of denitrification potential found a significant effect of site ($p = 0.0147$). Specifically, Sites 4 and 3 had significantly greater denitrification potential than Site 2, but not Site 1.

Potential net N nitrification and mineralization showed similar patterns across treatments and sites. Both processes were significantly influenced by the amendment added ($p \leq 0.0001$ for both). Potential net nitrification rates were higher in Control and Topsoil plots than in Straw and Mix plots, and potential net N mineralization was higher in Control, Topsoil, and Biochar plots than Straw and Mix plots (Figure 4B and C). Site effects were also found to be significant in mixed model ANOVAs of potential net nitrification and N mineralization ($p \leq 0.0001$, 0.0052, respectively). Specifically, Sites 1 and 3 had higher nitrification rates than Sites 2 and 4.

The addition of topsoil amendments increased microbial biomass N over all other treatments ($p \leq 0.0001$). A mixed model ANOVA also found a significant effect of site ($p \leq 0.0001$), in which Site 4 had significantly higher levels than all the other sites, followed by Site 1, Site 3, and, finally, Site 2,

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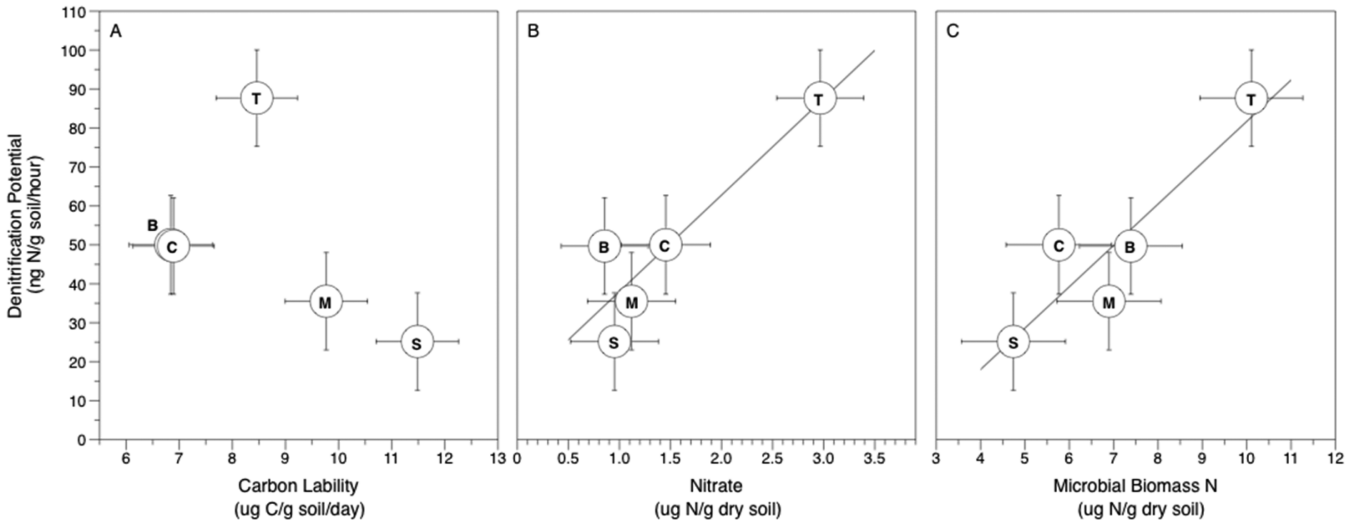

Figure 5. Treatment means of denitrification potential versus treatment means of the labile carbon pool (A), soil nitrate (B), and microbial biomass N. Point labels indicate treatments (*C* = Control, *S* = Straw, *T* = Topsoil, M = Mix *B* = Biochar). Error bars represent standard error. Fit lines in plots B and C correspond with significant correlations.

which had significantly lower microbial biomass N than Site 1 (Figure 4D).

Bivariate regression analyses identified significant positive correlations between mean denitrification potential for each treatment and the corresponding treatment means of soil NO_3^- (r^2 = 0.825; p = 0.032; Figure 5B) and microbial biomass N (r^2 = 0.822; p = 0.034; Figure 5C), but there was no correlation with treatment means of C_L (r^2 = 0.189; p = 0.464; Figure 5A).

DISCUSSION

Processes and products of the N cycle essential for NO_3^- removal from surface and groundwater were significantly influenced by the addition of soil amendments to restored wetlands. In particular, our data confirms that soil NO_3^-, C:N, and microbial biomass N can be significantly influenced by the addition of soil amendments, and, in turn, these characteristics influence N cycling processes.

Site Factors Controlling Denitrification Potential and Nitrogen Cycling. Denitrification potential differed among sites, with the intermittently flooded Sites 3 and 4 having significantly higher rates than the consistently flooded Site 2. This finding is consistent with previous work suggesting that alternating periods of saturation and drying create optimal conditions for denitrification, with aerobic periods recharging pools of labile C and NO_3^-.[37]

Higher denitrification potential in Sites 3 and 4 may also have been driven by the relatively high microbial biomass N and levels of NO_3^- in these sites. Although soil C was lowest in Site 4, microbial biomass N was significantly higher in Site 4 than the other sites. This trend appears to have been driven by relatively high native microbial biomass N in the subsoil and topsoil of Site 4 compared to other sites. Soil NO_3^- was also significantly higher in Sites 3 and 4. The majority of NO_3^- in wetlands is supplied by incoming water and nitrification of mineralized NH_4^+. It is unlikely that the amounts of NO_3^- entering Sites 1 and 2 were significantly different than the amounts in the ground and surface water entering Sites 3 and 4. However, NO_3^- supplies could be greater in these sites due to more intermittent periods of drying, during which nitrification could occur.

It does not appear that soil pH was a factor driving denitrification potential in our sites and surprisingly, the site with the lowest pH had the highest denitrification potential. Denitrification is commonly positively correlated with pH, so it is notable that the opposite was true in this study. However, similar results have been observed in low pH (≤4.5) soils of tropical rainforests,[14] created nontidal freshwater wetlands,[38] and mixed hardwood and heath wetlands,[39] revealing that denitrifying bacteria can be active in strongly acidic soils.[40]

Amendment effects on Denitrification Potential and associated Nitrogen Cycling processes. While some studies have reported that denitrification in restored wetlands is limited by C availability,[15,41−43] in these wetlands soil NO_3^-, C:N, and microbial biomass N were stronger predictors of denitrification potential than C_L. A positive influence of soil NO_3^- on denitrification potential is well-established,[39,44−51] and microbial biomass N and C:N have also been shown to be useful indices of N richness in wetland soils and important predictors of N cycling.[52,53]

Topsoil plots had the highest rates of denitrification potential, potential net N nitrification and mineralization, the lowest C:N and the highest levels of NH_4^+, NO_3^-, and microbial biomass N. The relatively high denitrification potential in Topsoil plots is likely explained by an optimal C:N ratio for producing high labile C and N for the processes of the N cycle. The treatments with the highest C_L (Mix and Straw) had the highest C:N and the lowest soil nitrate. It is likely that the high C:N stimulated immobilization and reduced net mineralization and nitrification of soil N, creating N limitation of denitrification and reducing the influence of C_L on denitrification potential. Whereas the Topsoil amendment provided a mix of labile C and N that stimulated the highest rates of denitrification, the high C:N of Straw and Biochar plots likely did not provide enough N to stimulate denitrification potential and internal N cycling. Given the high C_L in plots containing straw, N cycling may be stimulated by sufficient inputs of exogenous N. As high inputs of exogenous N are found in many restored wetlands, future research will compare straw and topsoil amendments under different NO_3^- loading scenarios.

Rates of potential net N nitrification and mineralization across all treatments were in the normal range of similar restored wetlands, but significantly lower than values reported for comparable natural wetlands, suggesting that they have less well developed capacities for nutrient cycling and sustained plant production.[15,53] Negative nitrification values are not uncommon, and indicate a net immobilization of NO_3^-. Negative mineralization values indicate that while the sites are not a source of NO_3^- to the surrounding environment, supply of N to plants is low.

Soil Amendments and Labile Carbon Pools. C_L was significantly influenced by the addition of amendments, with Straw and Mix plots having the highest C_L. The pool of labile C is affected by production from organic matter and the addition of amendments as well as by consumption by microbes. The differences in this study were likely driven primarily by the unique structural and chemical composition of the amendments. Highly labile C sources are usually quickly decomposed into CO_2 and stable soil organic matter. The speed and extent of decomposition is determined by the structure and chemical composition of the substrate as well as by environmental conditions such as temperature, which did not differ significantly among plots. Differences in soil quality influence the abundance, composition, and activity of decomposers, which in turn also determine rates of decomposition.[54]

Straw is composed exclusively of plant material and thus contains a greater proportion of decomposable nonhumic substances (e.g., carbohydrates, proteins, and fats) and phenolic substances (e.g., lignins and tannins) than other amendments. Because straw is intermediately labile, it produces a steady supply of labile C. Topsoil is composed of a complex of plant, microbial, and animal products in various stages of decomposition as well as an inorganic mineral component (e.g., sand and clay). Like straw, topsoil contains nonhumic and phenolic substances. However, topsoil also contains highly decomposed and complex mixtures of humic substances.[55] Humic substances are composed of high-molecular weight aromatic structures formed by dynamic alterations of resistant tannins and lignins by abiotic and biotic reactions and are highly recalcitrant. The higher proportion of C bound up in humic compounds was likely responsible for the lower C_L of these plots.

The pool of labile C in Straw plots was almost twice that of the Biochar plots. Amending subsoil with biochar did not increase C_L relative to controls, likely due to the structure of biochar, which is dominated by a core of aromatic C rings.[56] These C rings are highly stable thereby making biochar far more resistant to microbial decomposition than unmodified organic matter such as straw. In terrestrial ecosystems, biochar has been shown to have a very long residence time in the soil (e.g., hundreds to thousands of years).[57] Due to the slow decomposition rate of biochar, it has the potential for long-term C sequestration in the soil. Because of this, it has been suggested that the application of biochar to soil could be a significant long-term sink for atmospheric CO_2, making it a possible strategy to mitigate climate change.[58,59]

Costs Versus Benefits of Soil Amendments in Restored Wetlands. While natural wetlands are known to have highly effective water quality functions, wetlands restored to replace lost ecosystem functions often go unevaluated or fall below expectations. Previous examination of restored wetlands have revealed that key drivers for denitrification, such as the amount of C_{org} in the soil, are slow to develop to the levels of their natural counterparts.[17] This research shows that C_L and levels of C_{org} of restored wetland soil can be increased with the use of soil amendments. Denitrification potential among amendments was correlated with soil NO_3^-, C:N, and microbial biomass N, but not with C_L as hypothesized. Rates of denitrification potential were more than three times as high in Topsoil plots than in unamended Control plots. The potential benefits of straw and biochar treatments for increasing denitrification potential were less clear. Neither straw nor biochar inhibited N cycling, but more research is needed to determine if there are benefits of these amendments (e.g., long-term carbon sequestration, denitrification of exogenous nitrate) that justify the additional cost and effort of application. In particular, future studies should compare topsoil and straw amendments under different N input scenarios to determine how C:N affects internal vs external N cycling. While topsoil additions provided the best mix of labile C and N and thus was the most balanced amendment promoting internal N cycling, straw's high C:N might stimulate high denitrification rates in the presence of exogenous NO_3^-.

Addition of any amendment, particularly topsoil, adds to the cost of a restoration project. Costs can be minimized, however, if the topsoil used is salvaged from the restoration site itself instead of transferred from elsewhere. Restoration projects that require soil removal instead of, for example, simple drain tile removal, can be bulldozed slightly deeper to make room for addition of topsoil that was separated in the initial phases of restoration. In this way, topsoil can be used as a soil amendment and the desired water level can still be managed. Before choosing to use a site's native soil as a topsoil source, however, care must be taken to make sure it is free of invasive species whose propagules and seeds may be contained in the seed bank.

In this study, we found that soil NO_3^-, C:N, and microbial biomass N were significant factors explaining differences in denitrification potential among sites. These findings reflect the potential influence of background soil conditions in stimulating water quality functions of restored wetlands, and emphasize the importance of considering soil conditions when selecting potential restoration sites. For example, the high variability in rates of N cycling processes of Topsoil plots reflects that the degree of benefit from topsoil additions will depend on the nature of the topsoil itself. Therefore, it is important to thoroughly test topsoil as a part of site selection and amendment planning.

Some site variables generally found to influence denitrification did not explain the observed site differences. While pH, soil C, soil moisture, and site hydrology did not appear to be indicators of denitrification potential in our sites, we continue to recommend that these variables be considered in site selection for restored wetlands with goals of water quality functions. Future research will reveal how differences in denitrification potential among treatments and sites change over time, and perhaps further illuminate mechanisms explaining the observed differences among sites.

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Notes

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■ REFERENCES

(1) Conley, D. J.; Paerl, H. W.; Howarth, R. W.; Boesch, D. F.; Seitzinger, S. P.; Havens, K. E.; Lancelot, C.; Likens, G. E. Ecology. Controlling eutrophication: Nitrogen and phosphorus. *Science* **2009**, *323*, 1014−1015.

(2) Seitzinger, S.; Harrison, J. A.; Böhlke, J. K.; Bouwman, A. F.; Lowrance, R.; Peterson, B.; Tobias, C.; Van Drecht, G. Denitrification across landscapes and waterscapes: A synthesis. *Ecol. Appl.* **2006**, *16*, 2064−2090.

(3) Zedler, J. B.; Kercher, S. Wetland resources: Status, trends, ecosystem services, and restorability. *Annu. Rev. Environ. Resour.* **2005**, *30*, 39−74.

(4) Zedler, J. B. Wetlands at your service: Reducing impacts of agriculture at the watershed scale. *Front. Ecol. Environ.* **2003**, *1*, 65−72.

(5) Seitzinger, S. P.; Styles, R. V.; Boyer, E. W.; Alexander, R. B.; Billen, G.; Howarth, R. W.; Mayer, B.; Breemen, N. Nitrogen retention in rivers: Model development and application to watersheds in the northeastern U.S.A. In *The Nitrogen Cycle at REgional to Global Scales*; Springer Netherlands: Dordrecht, 2002; pp 199−237.

(6) Sutton, A. J.; Fisher, T. R.; Gustafson, A. B. Effects of restored stream buffers on water quality in non-tidal streams in the Choptank River Basin. *Water, Air, Soil Pollut.* **2010**, *208*, 101−118.

(7) Hey, D. L.; Kostel, J. A.; Crumpton, W. G.; Mitsch, W. J.; Scott, B. The roles and benefits of wetlands in managing reactive nitrogen. *J. Soil Water Conserv.* **2012**, *67*, 47A−53A.

(8) Burgin, A. J.; Yang, W. H.; Hamilton, S. K.; Silver, W. L. Beyond carbon and nitrogen: How the microbial energy economy couples elemental cycles in diverse ecosystems. *Front. Ecol. Environ.* **2011**, *9*, 44−52.

(9) Willems, H.; Rotelli, M.; Berry, D.; Smith, E.; Renaue, R., Jr; Mostaghimi, S. Nitrate removal in riparian wetland soils: Effects of flow rate, temperature, nitrate concentration and soil depth. *Water Res.* **1997**, *31*, 841−849.

(10) Burgin, A. J.; Hamilton, S. K. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Front. Ecol. Environ.* **2007**, *5*, 89−96.

(11) Groffman, P. Denitrification in freshwater wetlands. *Curr. Topics Wetland Biogeochem.* **1994**, *1*, 15−35.

(12) Ahn, C.; Peralta, R. M. Ecological Engineering. *Ecol. Eng.* **2012**, *49*, 130−136.

(13) Seitzinger, S. P. Linkages between organic-matter mineralization and denitrification in eight riparian wetlands. *Biogeochemistry* **1994**, *25*, 19−39.

(14) Tiedje, J.; Sexstone, A.; Myrold, D.; Robinson, J. Denitrification: Ecological niches, competition and survival. *Antonie van Leeuwenhoek* **1982**, *48*, 569−583.

(15) Duncan, C. P.; Groffman, P. M. Comparing microbial parameters in natural and constructed wetlands. *J. Environ. Qual.* **1994**, *23*, 298−305.

(16) Craft, C.; Megonigal, P.; Broome, S.; Stevenson, J.; Freese, R.; Cornell, J.; Zheng, L.; Sacco, J. The pace of ecosystem development of constructed *Spartina alterniflora* marshes. *Ecological Applications* **2003**, *13*, 1417−1432.

(17) Ballantine, K.; Schneider, R. Fifty-five years of soil development in restored freshwater depressional wetlands. *Ecol. Appl.* **2009**, *19*, 1467−1480.

(18) Bruland, G. L.; Hanchey, M. F.; Richardson, C. J. Effects of agriculture and wetland restoration on hydrology, soils, and water quality of a Carolina bay complex. *Wetlands Ecol. Manage.* **2003**, *11*, 141−156.

(19) Ballantine, K.; Schneider, R.; Groffman, P.; Lehmann, J. Soil properties and vegetative development in four restored freshwater depressional wetlands. *Soil Sci. Soc. Am. J.* **2012**, *In Press*, 1482.

(20) Bruland, G. L.; Richardson, C. J. Hydrologic gradients and topsoil additions affect soil properties of Virginia created wetlands. *Soil Sci. Soc. Am. J.* **2004**, *68*, 2069−2077.

(21) Bailey, D. E.; Perry, J. E.; Daniels, W. L. Vegetation dynamics in response to organic matter loading rates in a created freshwater wetland in southeastern Virginia. *Wetlands* **2007**, *27*, 936−950.

(22) Burchell, M. R.; Skaggs, R. W.; Lee, C. R.; Broome, S.; Chescheir, G. M.; Osborne, J. Substrate organic matter to improve nitrate removal in surface-flow constructed wetlands. *J. Environ. Qual.* **2007**, *36*, 194−207.

(23) Jacinthe, P.-A.; Lal, R. Carbon storage and minesoil properties in relation to topsoil applications techniques. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1788−1795.

(24) Bruland, G. L.; Richardson, C. J.; Daniels, W. L. Microbial and geochemical responses to organic matter amendments in a created wetland. *Wetlands* **2009**, *29*, 1153−1165.

(25) Sutton-Grier, A. E.; Ho, M.; Richardson, C. J. Organic amendments improve soil conditions and denitrification in a restored riparian wetland. *Wetlands* **2009**, *29*, 343−352.

(26) Stauffer, A. L.; Brooks, R. P. Plant and soil responses to salvaged marsh surface and organic matter amendments at a created wetland in central Pennsylvania. *Wetlands* **1997**, *17*, 90−105.

(27) Morse, J. L.; Ardón, M.; Bernhardt, E. S. Using environmental variables and soil processes to forecast denitrification potential and nitrous oxide fluxes in coastal plain wetlands across different land uses. *J. Geophys. Res.* **2012**, *117*, GO2323.

(28) Cowardin, L. M.; Carter, V.; Golet, F. C.; La Roe, E. T. *Classification of Wetlands and Deepwater Habitats of the United States*; U.S. Fish and Wildlife Service, U.S. Department of the Interior: Washington, DC, 1979; p 103.

(29) Song, K.; Kang, H.; Zhang, L.; Mitsch, W. J. Seasonal and spatial variations of denitrification and denitrifying bacterial community structure in created riverine wetlands. *Ecol. Eng.* **2012**, *38*, 130−134.

(30) Fisher, J.; Acreman, M. C. Wetland nutrient removal: A review of the evidence. *Hydrol. Earth Syst. Sci.* **2004**, *8*, 673−685.

(31) Smith, M. S.; Tiedje, J. M. Phases of denitrification following oxygen depletion in soil. *Soil Biol. Biochem.* **1979**, *11*, 261−267.

(32) Galloway, J. N.; Dentener, F. J.; Capone, D. G.; Boyer, E. W.; Howarth, R. W.; Seitzinger, S. P.; Asner, G. P.; Cleveland, C. C.; Green, P. A.; Holland, E. A. Nitrogen cycles: Past, present, and future. *Biogeochemistry* **2004**, *70*, 153−226.

(33) Mitsch, W. J.; Day, J. W.; Gilliam, J. W.; Groffman, P. M.; Hey, D. L.; Randall, G. W.; Wang, N. Reducing nitrogen loading to the Gulf of Mexico from the Mississippi River Basin: Strategies to counter a persistent ecological problem. *BioScience* **2001**, *51*, 373−388.

(34) Groffman, P. M.; Altabet, M. A.; Böhlke, J. K.; Butterbach-Bahl, K.; David, M. B.; Firestone, M. K.; Giblin, A. E.; Kana, T. M.; Nielsen, L. P.; Voytek, M. A. Methods for measuring denitrification: Diverse approaches to a difficult problem. *Ecol. Appl.* **2006**, *16*, 2091−2122.

(35) Jenkinson, D. S.; Powlson, D. S. Effects of biocidal treatments on metabolism in soil 0.1. Fumigation with chloroform. *Soil Biol. Biochem.* **1976**, *8*, 167−177.

(36) Zou, X. M.; Ruan, H. H.; Fu, Y.; Yang, X. D.; Sha, L. Q. Estimating soil labile organic carbon and potential turnover rates using a sequential fumigation-incubation procedure. *Soil Biol. Biochem.* **2005**, *37*, 1923−1928.

(37) Reddy, K.; Patrick, W. Effect of alternate aerobic and anaerobic conditions on redox potential, organic matter decomposition and nitrogen loss in a flooded soil. *Soil Biol. Biochem.* **1975**, *7*, 87−94.
(38) Peralta, R. M.; Ahn, C.; Voytek, M. A.; Kirshtein, J. D. Applied Soil Ecology. *Appl. Soil Ecol.* **2013**, *70*, 70−77.
(39) Seitzinger, S. P. Linkages between organic matter mineralization and denitrification in eight riparian wetlands. *Biogeochemistry* **1994**, *25*, 19−39.
(40) Schlesinger, W. H. *Biogeochemistry: An Analysis of Global Change*; Academic Press: San Diego, 1997.
(41) Hunter, R. G.; Faulkner, S. P. Denitrification potentials in restored and natural bottomland hardwood wetlands. *Soil Sci. Soc. Am. J.* **2001**, *65*, 1865−1872.
(42) Groffman, P. M.; Crawford, M. K. Denitrification potential in urban riparian zones. *J. Environ. Qual.* **2003**, *32*, 1144−1149.
(43) Gift, D. M.; Kaushal, S. S.; Mayer, P. M. Denitrification potential, root biomass, and organic matter in degraded and restored urban riparian zones. *Restor Ecol* **2010**, *18*, 113−120.
(44) Roley, S. S.; Tank, J. L.; Stephen, M. L.; Johnson, L. T.; Beaulieu, J. J.; Witter, J. D. Floodplain restoration enhances denitrification and reach-scale nitrogen removal in an agricultural stream. *Ecol. Appl.* **2012**, *22*, 281−297.
(45) Poe, A. C.; Pichler, M. F.; Thompson, S. P.; Paerl, H. W. Denitrification in a constructed wetland receiving agricultural runoff. *Wetlands* **2003**, *23*, 817−826.
(46) Smith, L. K.; Sartoris, J. J.; Thullen, J. S.; Andersen, D. C. Investigation of denitrification rates in an ammonia-dominated constructed wastewater-treatment wetland. *Wetlands* **2000**, *20*, 684−696.
(47) Merrill, A. G.; Zak, D. R. Factors controlling denitrification rates in upland and swamp forests. *Can. J. Forest Res.* **1992**, *22*, 1597−1604.
(48) Koerselman, W.; Caluwe, H.; Keiskamp, W. M. Denitrification and dinitrogen fixation in two quaking fens in the Vechtplassen area, The Netherlands. *Biogeochemistry* **1989**, *8*, 153−165.
(49) Westermann, P.; Ahring, B. Dynamics of methane production, sulfate reduction, and denitrification in a permanently waterlogged alder swamp. *Appl. Environ. Microbiol.* **1987**, *53*, 2554−2559.
(50) Scaroni, A. E.; Nyman, J. A.; Lindau, C. W. Comparison of denitrification characteristics among three habitat types of a large river floodplain: Atchafalaya River Basin, Louisiana. *Hydrobiologia* **2011**, *658*, 17−25.
(51) Piña-Ochoa, E.; Álvarez-Cobelas, M. Denitrification in aquatic environments: A Cross-system Analysis. *Biogeochemistry* **2006**, *81*, 111−130.
(52) Palta, M. M.; Ehrenfeld, J. G.; Groffman, P. M. Denitrification and potential nitrous oxide and carbon dioxide production in brownfield wetland soils. *J. Environ. Qual.* **2013**, *42*, 1507−1517.
(53) Groffman, P. M.; Hanson, G. C.; Kiviat, E.; Stevens, G. Variation in microbial biomass and activity in four different wetland types. *Soil Sci. Soc. Am. J.* **1996**, *60*, 622−629.
(54) Cornwell, W. K.; Cornelissen, J. H. C.; Amatangelo, K.; Dorrepaal, E.; Eviner, V. T.; Godoy, O.; Hobbie, S. E.; Hoorens, B.; Kurokawa, H.; Pérez-Harguindeguy, N.; et al. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol. Lett.* **2008**, *11*, 1065−1071.
(55) Brady, N. C.; Weil, R. R. *The Nature and Properties of Soils*,13 ed.; Prentice-Hall: Upper Saddle River, NJ, 2002.
(56) Lehmann, J.; Joseph, S. *Biochar for Environmental Management: Science and Technology*; Earthscan: London, 2009.
(57) Sohi, S. P.; Krull, E.; Lopez-Capel, E.; Boi, R. A review of biochar and its use and function in soil. *Adv. Agron.* **2010**, *105*, 47−82.
(58) Lehmann, J. A handful of carbon. *Nature* **2007**, *447*, 143−144.
(59) Jha, P.; Biswas, A.; Lakaria, B.; Subba Rao, A. Biochar in agriculture−prospects and related implications. *Curr. Sci.* **2010**, *99*, 1218−1225.