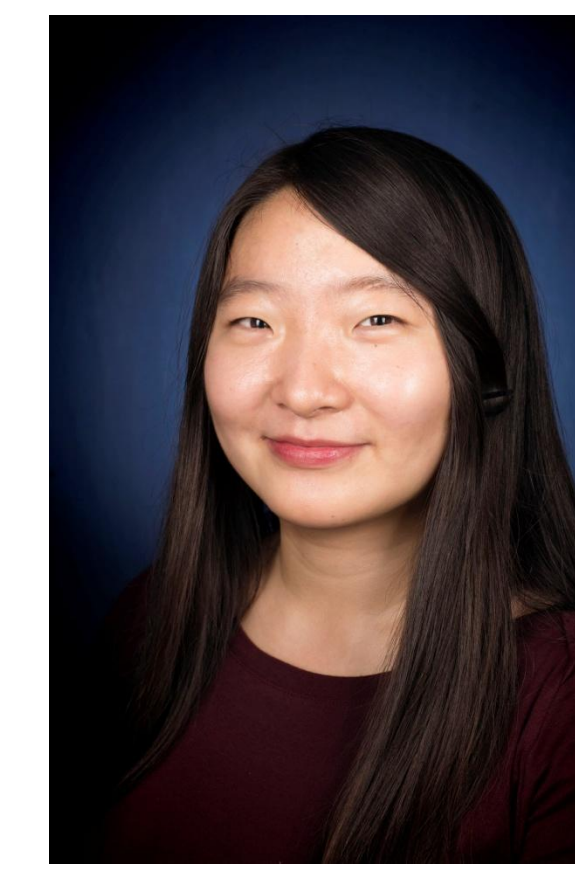




# Promoting Denitrification in Restored Wetlands with Amendments of Differing Carbon Lability

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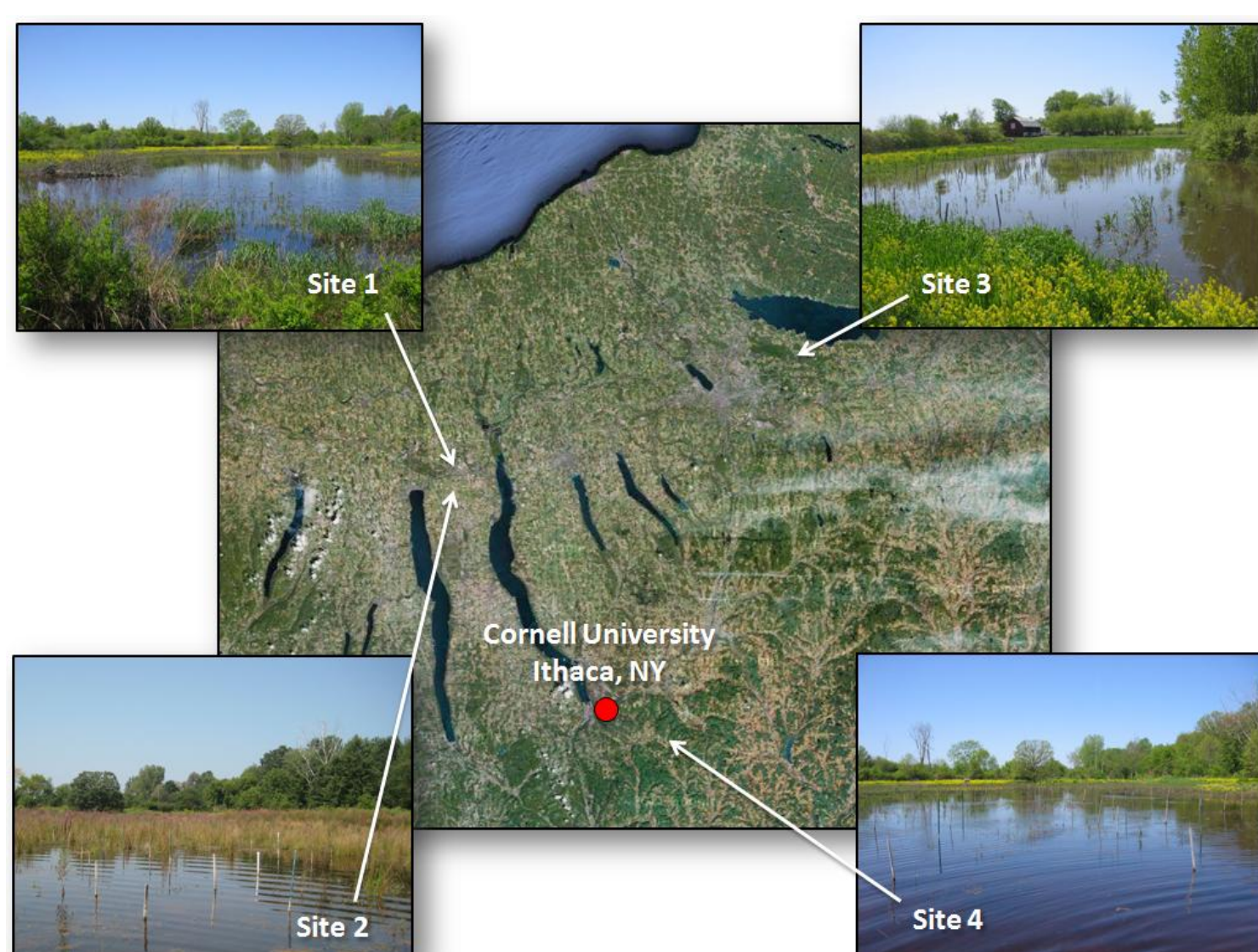
## Abstract

Wetlands contribute many ecosystem functions, but restored wetlands function at lower levels than the natural ones they replace. We evaluated the efficacy of using carbon amendments to promote denitrification potential in four restored wetlands. The amendments used during restoration were straw, topsoil, and biochar, which have differing levels of carbon lability and thus different rates of decomposition by soil microbes. Soil samples were collected six years after restoration and analyzed to quantify denitrification potential, organic carbon, respiration, microbial biomass, and pH. Denitrification potential was significantly higher in amended plots than in control plots, and it was significantly positively correlated with both soil organic carbon and microbial biomass nitrogen. This suggests that organic carbon availability in restored wetlands soils is vital for supporting the populations of microbes that carry out denitrification, and that the incorporation of carbon amendments can help provide this important requirement. However, denitrification potential in a natural reference wetland was at least 50 times higher than in the restored wetlands, highlighting the limitations of using restoration to compensate for the destruction of natural wetlands.

## Background

- High levels of nitrate in groundwater threaten human health and marine ecosystems.
- Nitrate can be transformed into atmospheric  $N_2$  through denitrification, a biogeochemical process.
- Wetlands are hotspots for denitrification due to their anaerobic soil and accumulated organic matter.
- Restoration of wetlands is meant to compensate for natural wetland area lost to development.
- However, restored wetlands take decades to achieve functional equivalency with natural wetlands<sup>1</sup>.
- In previous studies, denitrification rates increased in restored wetlands with addition of straw or topsoil<sup>2,3</sup>.
- Biochar may also be a promising amendment, having been shown to improve soil properties such as cation exchange capacity and soil surface area<sup>4</sup>.
- This study investigates the effect of straw, topsoil, and biochar amendments on denitrification potential and associated properties of restored wetland soils.

## Site Description



- The experimental sites were four restored freshwater depressional wetlands within 120 km of Ithaca, NY<sup>5</sup>.
- In each restored wetland, 2 m by 2 m plots for treatment replicates were set up in rows 2 m apart.
- A neighboring ecologically comparable natural wetland served as a reference site.

## Methods

- In each restored wetland, there were five replicate control plots, where no amendment was added, and five replicate plots each of straw, topsoil, and biochar treatment.
- Soil samples were collected and analyzed in May 2013, six years after restoration.
- Denitrification enzyme activity (DEA) assay<sup>6</sup> was used to quantify denitrification potential, i.e. how much denitrification occurs when all limiting factors are supplied.
- The chloroform fumigation-incubation method (CFIM)<sup>7</sup> was used to determine the amount of microbial biomass nitrogen as well as respiration, a proxy for pools of labile carbon present. Organic carbon content and pH were also measured.
- ANOVA analyses were done to identify significant differences, and linear regressions were performed to determine which properties correlate with denitrification potential.

## Results

- Denitrification potential was significantly higher in amended plots than in control plots.
- Organic carbon was higher in topsoil plots than in control plots and straw plots, and higher in biochar plots than in any other plot type.
- Microbial biomass nitrogen was higher in topsoil plots than in control and straw plots.
- There were no significant differences in respiration between treatments or sites.

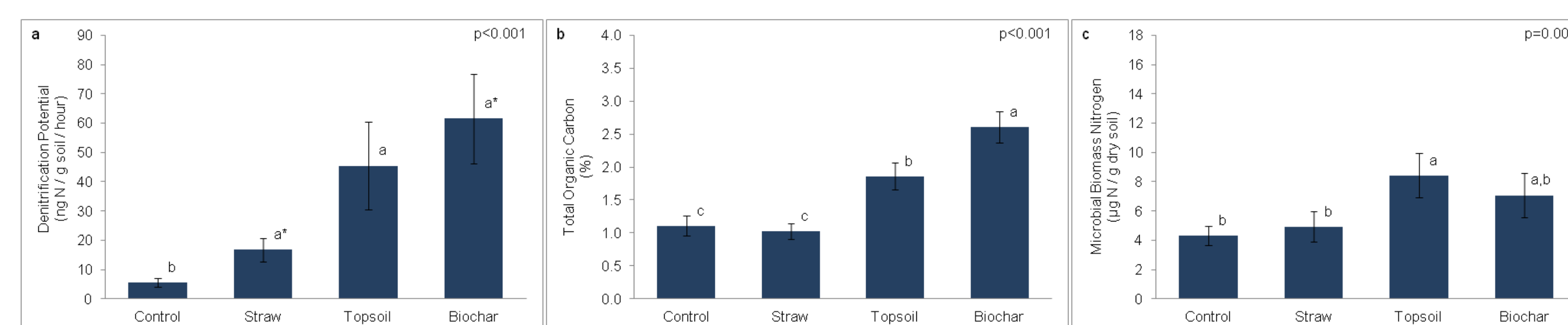


Figure 1. Differences by treatment for a) denitrification potential, b) organic carbon, and c) microbial biomass nitrogen. Tukey HSD post-hoc comparisons are summarized by the letters above each bar. Bars that do not share a letter are significantly different from each other, and asterisks indicate a marginally significant difference. Error bars show standard error.

- Organic carbon and microbial biomass nitrogen were both significantly positively correlated with denitrification potential.
- Organic carbon was also positively correlated with microbial biomass nitrogen.

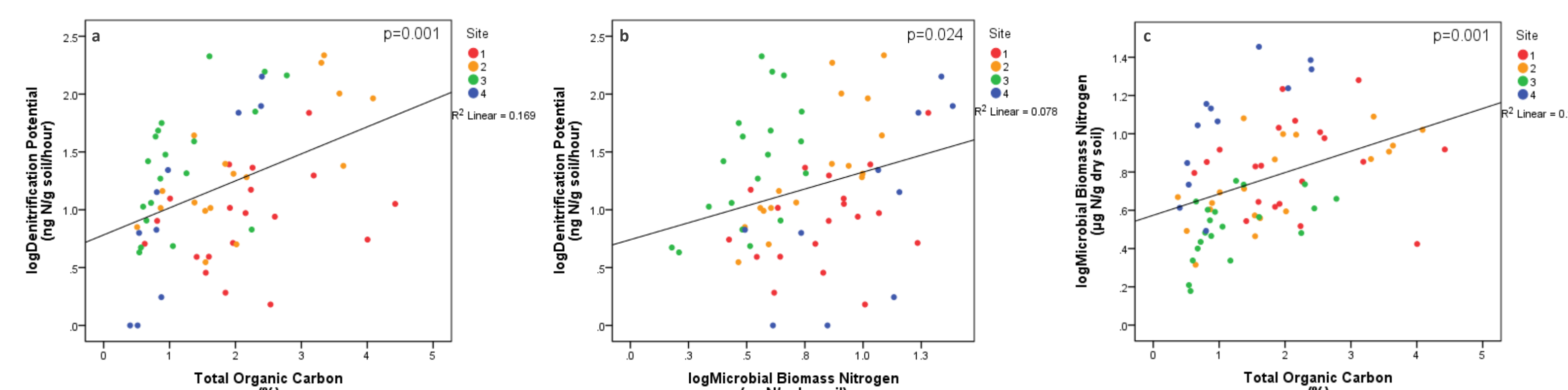


Figure 2. Scatterplots with linear fit lines showing significant positive correlations between a) organic carbon and denitrification potential, b) microbial biomass nitrogen and denitrification potential, and c) organic carbon and microbial biomass nitrogen.

- Site 4 had acidic soil with an average pH of 4.58.
- Denitrification potential was lower in Site 4 than in the other sites for control, straw, and topsoil, but Site 4 biochar plots had high denitrification potential.

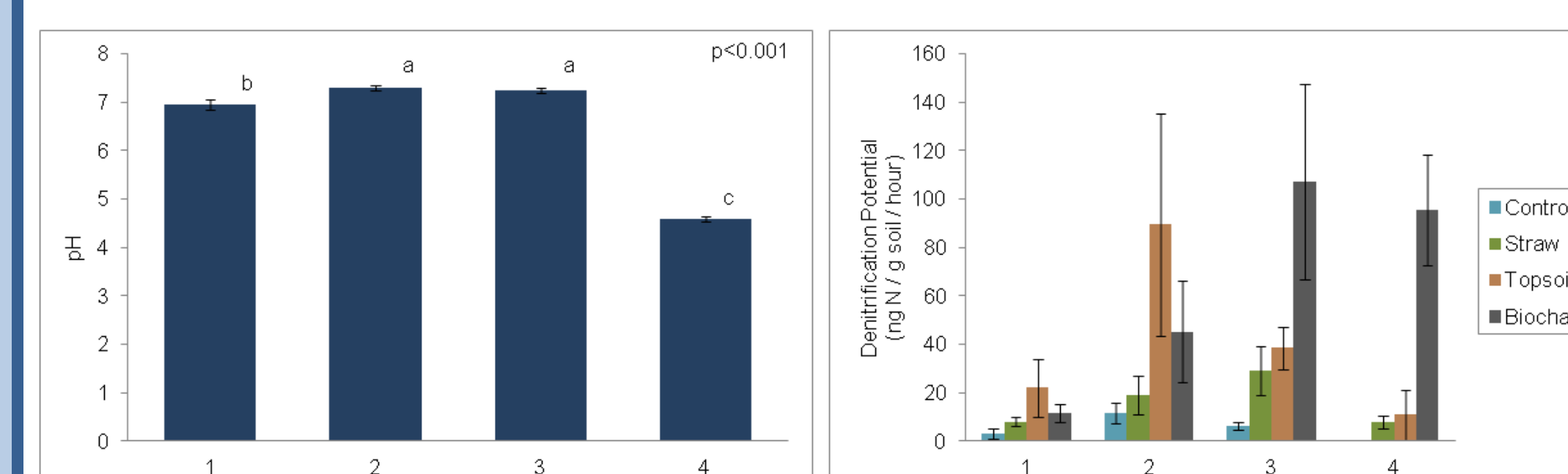


Figure 3. Differences in pH across sites are shown by Tukey HSD post-hoc comparisons. Bars that do not share a letter are significantly different from each other. Error bars show standard error.

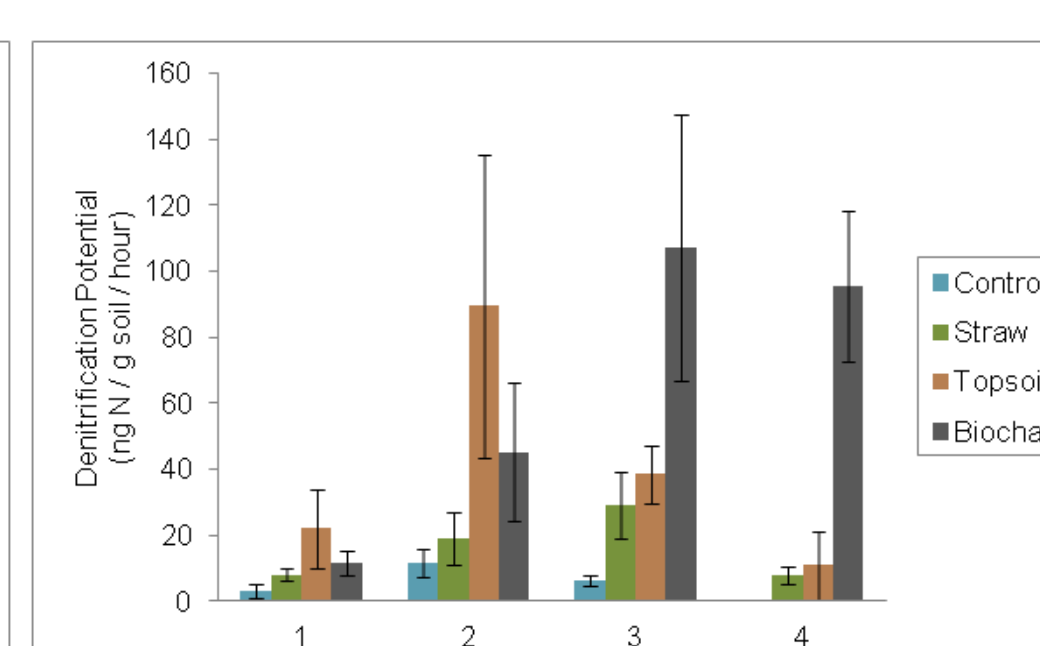


Figure 4. Denitrification potential for each treatment-by-site combination. Error bars show standard error.

- Denitrification potential in the natural reference wetland was over 600 times higher than in control plots and 55 times higher than in biochar plots.

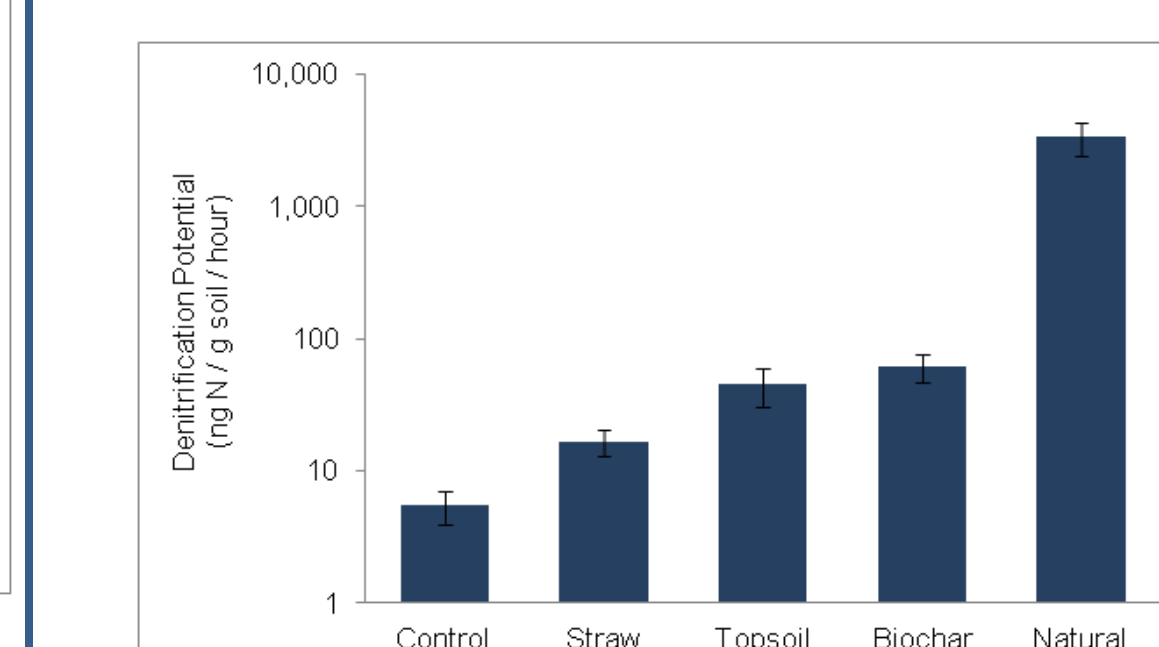


Figure 5. Differences in denitrification potential across treatments in comparison to the natural wetland. Data are in log scale for clarity. Error bars show standard error.

## Discussion

- The correlations between organic carbon, microbial biomass nitrogen, and denitrification potential suggest that organic carbon supports communities of microbes that perform denitrification.
- Labile pools of organic carbon can directly serve as a food source, but these are used up quickly. Meanwhile, less labile forms of carbon remain in the soil for a longer time and can provide other benefits.
- The carbon in biochar is in stable aromatic systems, and thus not available as an electron source for microbes. But, biochar can adsorb other nutrients<sup>8</sup>, which may make them more available to microbes.
- That high denitrification potential was maintained in biochar plots of the acidic restored wetland suggests that biochar may also provide pH buffering benefits.
- Continued monitoring may reveal that amended soils reach functional equivalency to natural levels sooner than restored wetland soils without amendments.

## Conclusions

- Carbon amendments significantly increased denitrification potential in restored wetland soils, so their use is a promising means of aiding wetland restoration.
- However, key soil properties were still much lower compared to the natural wetland, illustrating that the preservation of natural systems should be prioritized.
- Continued monitoring of how these amended soils develop over time will provide important insights into the long-term efficacy of carbon amendments in promoting ecosystem functions in restored wetlands.

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