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Wet Soil Redox Chemistry as Affected by Organic Matter and Nitrate

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ABSTRACT

Wet soil microcosms were established to determine effects of organic matter and nitrate additions on microbial respiration and redox potentials. Organic matter (1%) and nitrate (100 ppm and 200 ppm) treatments were applied in factorial combination. Soil pH, redox potential, and CO_2 emissions were measured. Data were analyzed by ANOVA for repeated measures and separately by sampling day. Addition of organic matter significantly ($P < 0.05$) and consistently increased CO_2 emissions and decreased redox potentials. On Day 42 nitrate significantly ($P < 0.05$) increased redox values. This study indicates a tendency for organic matter to decrease soil redox potential both in absolute terms and relative to the suboxic-anoxic boundary. Our findings portend that additions of organic matter may quickly and markedly decrease soil redox potentials and increase CO_2 emissions in wetlands, whereas additions of nitrate may have complex and sporadic effects on redox potentials.

Keywords: Oxidation; Reduction; Wetlands; Carbon Dioxide Emission

1. Introduction

Microbial respiration in the soil, accomplished by linking oxidation and reduction half-reactions, directly impacts the redox potential (Eh) of soil-water systems. Soil redox potential affects, and is affected by, many attributes of the ecosystem. In soil the primary electron donor for oxidation half-reactions is organic matter. In aerobic soil the prevailing electron acceptor for reduction half-reactions is molecular oxygen (O_2). Unlike higher organisms, microbial populations can use electron acceptors less susceptible to reduction than O_2, especially in environments where O_2 is absent. Where microbes and organic matter are abundant, the absence of O_2 (*i.e.*, an anaerobic condition) leads to the reduction of other substances, creating a low redox potential as expressed in units of millivolts or by the term pe implying the negative logarithm of concentration of electrons in search of an electron acceptor.

Electron acceptors are generally reduced in the following order as redox potentials decline: O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} [1]. Oxic soils (also termed normal, oxidized, and aerobic) are those soils with high redox potentials indicating that O_2 is present. Anoxic soils (also termed reduced, and water-logged) are soils in which O_2 has been reduced and is absent, as are sulfate $\left(SO_4^{2-}\right)$ and other less preferable electron acceptors. Suboxic soils (also termed wet but not water-logged) are those having intermediate redox potentials in which O_2 is ab-

sent but sulfate has not been completely reduced. A concept used to delineate anoxic and suboxic soils is the pe-pH boundary between oxidized and reduced sulfur, as described by Baas Becking *et al*. [2] and modified by Sposito [1] and Essington [3]. This boundary between suboxic and anoxic conditions is described by the equation: $pe = 9 - pH$.

Nitrate $\left(NO_3^-\right)$ is a particularly effective electron acceptor in the absence of O_2, and can presumably mitigate the reduction of sulfate and chemicals less preferable as electron acceptors, preventing very low redox potentials. Whitmire and Hamilton [4] demonstrate an increase in sulfate coinciding with denitrification, suggesting that the presence of nitrate for an electron acceptor may alleviate sulfate reduction or encourage sulfur oxidation. A decrease in wet soil redox potential has been linked to the loss of nitrate [5]. The phenomenon of denitrification occurs when nitrate, functioning as an electron acceptor, is reduced to gaseous nitrogen. The extent to which denitrification occurs depends upon many environmental factors, including the quality of the carbon in the system [6], the relation between organic N mineralization and the presence of inorganic electron acceptors [7], and competing fates of nitrate [8]. The effects of nitrate on soil redox potential are intrinsically complex. The presence of three soil factors, organic matter, O_2, and nitrate, could strongly influence microbial respiration and soil redox potential.

Wetlands, either natural or constructed, are used for nitrogen removal from water catchments. The effectiveness of wetlands for nitrate attenuation has been assessed in lab [9], mesocosm [10], and field studies [11], with differing results.

Also, wetlands are often constructed in locations where they did not formerly exist to compensate for the loss of wetlands elsewhere. As wetlands come into existence, one might well ask whether or not wetland functions have also come into existence, or has the process only created lands that are wet [12]. Answers to such questions hinge on the chemistry, biology, and hydrology of a site. Moreover, legally recognized diagnostic characteristics for wetlands in the United States [13] include the presence of hydric soils, *i.e.*, soils with anaerobic conditions. Yet some soils, natural or otherwise, can be inundated for long periods of time while maintaining high redox potentials, indicating a lack of anaerobic conditions. Soil redox potential influences many processes of practical importance, such as the degradation of pesticides [14]. Therefore understanding the nature of both constructed and natural wetlands requires an understanding of the factors associated with aerobic and anaerobic conditions as indicated by measurements of soil redox potential.

The primary objective of this study was to determine if, and to what extent, organic matter addition would increase respiration in wet soils therefore consuming O_2 and decreasing redox potentials. If redox potentials were reduced by organic matter, the secondary objective would be to determine whether or not nitrate would mitigate those effects, either in an absolute sense or in relation to the wet-waterlogged (suboxic-anoxic) boundary.

2. Materials and Methods

Experimental units were 18 wet soil microcosms consisting of 1-liter opaque bottles, each containing 1.0 kg of soil collected from a seasonally wet site of the Aransas National Wildlife Refuge in southern coastal Texas, USA. The soil was excavated from the upper 30 cm of a probable Mustang soil (Mixed, hyperthermic Typic Psammaquents) mapped as the Galveston-Mustang Association [15]. The soil contained 89.5% sand, 3.8% clay, 6.2% silt, 0.6% organic matter, 2.6 g kg^{-1} total nitrogen, and 31 mg kg^{-1} nitrate.

Experimental treatments were applied in a 2×3 factorial combination of 0 and 1% organic matter additions (0 and 10 g kg^{-1}); and 0, 100, and 200 ppm nitrate additions (0, 100, and 200 mg kg^{-1}), replicated three times. The organic matter added was dried Burmudagrass containing 1.33 mg kg^{-1} nitrogen. Nitrate was added as NaNO$_3$. The treatment combination that included neither organic matter nor nitrate constituted the control. Each microcosm was randomly assigned to a treatment. Soils were maintained in a wet condition with a few mm of water covering the surface, and incubated in a growth chamber held a 30˚C with 11 hours of simulated sunlight.

Electrode measurements of pH and redox potential were taken at 14-day intervals for a period of 112 days. Redox values were determined using a calomel electrode, then adjusted to standard Eh values relative to a hydrogen electrode by adding 245 mV to the calomel-electrode values [16]. Redox values relative to the sloping line separating suboxic and anoxic soils were calculated by determining the difference between the measured redox value and the threshold redox value corresponding to the soil pH, given that pe = 9 − pH, and that Eh expressed in millivolts = 59.16 pe.

Carbon dioxide emissions were determined by measuring CO_2 concentrations in the headspace above each soil [17] on day 1, then again on days 8, 15, 22, 29, and 70. Tops of the containers were open during incubation, but sealed for 10 min for CO_2 sampling, at which time 100 mL samples were collected from the 415 mL headspace using a Sensidyne gas detection pump (Sensidyne Inc., Clearwater, FL) connected in series to an RAE gas detection tube (RAE Systems Inc., Sunnyvale, CA).

Effects of organic matter and nitrate on dependent variables CO_2 and redox potential were analyzed by ANOVA for a repeated measures design. Also, separate data analyses for each individual sampling day were conducted by ANOVA for a factorial design [18].

3. Results

Tables 1 and **2** show P values indicating significance of treatment effects. We considered any P value less than 0.05 to be significant. Results from the analysis of variance for repeated measures of the dependent variables CO_2, redox potential, and relative redox potential are presented in **Table 1**. For CO_2, organic matter main effects and the organic matter interaction with time were significant. Nitrate did not significantly affect CO_2 levels. For redox values and relative redox values, interactions between time and organic matter, and between nitrate and time, were very highly significant (P < 0.001). Also for both redox and relative redox values, the main effects of organic matter were very highly significant (P < 0.001) but main effects of nitrate were not significant (P > 0.05). Because of the interactions with time (*i.e.*, sampling day) in a temporally repeated measures design, data were also analyzed for each individual sampling day. **Table 2** indicates results of analysis of variance on redox values and relative redox values performed for individual sampling days.

Microbial respiration as measured by CO_2 evolution (**Figure 1**) increased significantly in response to additions of organic matter as measured on Days 8, 15, 22, and

Table 1. P values for temporally repeated measures of carbon dioxide emissions (CO_2), soil redox potential (redox), and soil redox potential relative to the suboxic-anoxic boundary (relative redox). Variables were nitrate (N), organic matter (O) and time (T).

Source	CO_2 df	CO_2 P value	Redox df	Redox P value	Relative Redox df	Relative Redox P value
N	2	0.281	2	0.143	2	0.094
O	1	<0.001	1	<0.001	1	<0.001
N × O	2	0.374	2	0.384	2	0.355
T	5	0.011	8	<0.001	8	<0.001
N × T	10	0.769	16	<0.001	16	<0.001
O × T	5	0.028	8	<0.001	8	<0.001
N × O × T	10	0.839	16	0.103	16	0.120

Table 2. P values for effects of nitrate (N) and organic matter (O) on soil redox potential (redox) and soil redox potential relative to the suboxic-anoxic boundary (relative redox) as determined by analysis of variance for a factorial design. On no sampling day was the N × O interaction significant.

Sampling Day	Redox N	Redox O	Relative Redox N	Relative Redox O
1	0.940	0.063	0.543	0.046
14	0.098	<0.001	0.016	0.004
28	0.215	<0.001	0.393	<0.001
42	0.042	<0.001	0.010	0.001
56	0.093	<0.001	0.245	0.003
70	0.151	<0.001	0.226	<0.001
84	0.075	<0.001	0.076	<0.001
98	0.137	0.004	0.191	0.006
112	0.274	<0.001	0.216	<0.001

29. Because neither nitrate main effects nor nitrate × organic matter interactions were significant on any sampling day, data from nitrate treatments were pooled such that organic matter effects presented in **Table 1** include averages of all treatments with the organic matter addition vs all treatments without it.

Organic matter additions consistently produced a strong negative effect on soil redox values, with highly significant results observed on all days after Day 1 (**Table 2**; **Figure 2**). Nitrate did not significantly affect soil redox values except on Day 42, when redox values were higher for the 100 ppm nitrate treatment than for the control. No significant interactions between nitrate and organic matter were observed.

4. Discussion

The data strongly support the hypothesis that adding soil organic matter will decrease soil redox potentials. Furth-
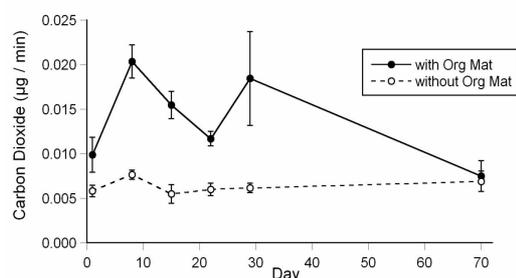
Figure 1. Carbon dioxide emitted from 1.0-kg wet soil microcosms. Values are means of all treatments either with or without a 1% addition of organic matter. Vertical bars indicate standard errors of the means.
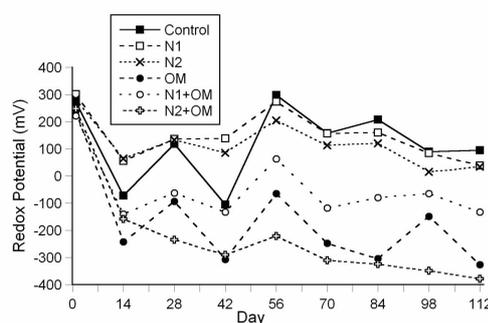
Figure 2. Mean soil redox potentials as affected by organic matter and nitrate. Treatments levels were 100 ppm nitrate (N1), 200 ppm nitrate (N2), and 1% organic matter (OM).

ermore, the control treatment having native levels of organic matter and nitrate resulted in intermediate redox potentials through Day 42, after which it produced the highest redox values of all treatments. This further supports the hypothesis that the negative impact on redox values was caused by the organic matter addition, not by the native organic matter in the soil.

This study uniquely measured redox potential relative to the pH-dependent boundary line separating suboxic and anoxic soils (**Figure 3**). Effects of organic matter were significant on every sampling day, whereas effects of nitrate were significant on Days 14 and 42 only (**Table 2**). Interactions between nitrate and organic matter were not significant on any sampling day. Redox values relative to the suboxic-anoxic boundary can be more telling than absolute redox values because of their relation to actual events or reactions. In suboxic soil sulfate and perhaps other species ranging between O_2 and sulfate in redox susceptibility remain in their oxidized state; whereas in anoxic soil sulfate is absent, having been reduced to sulfide or other forms of reduced sulfur. As one might expect, many data points in **Figure 3** are in close proximity to the boundary line, suggesting that for these soils sulfate may be reducing but not completely reduced. With the disappearance of sulfate, soil redox potentials
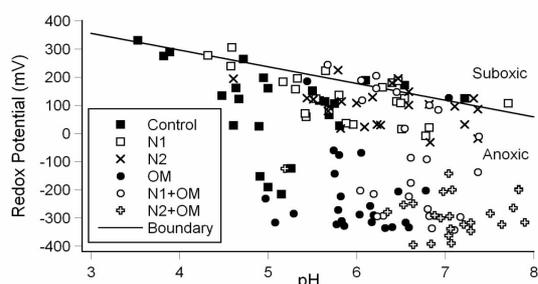
Figure 3. Soil redox potential relative to the boundary separating suboxic and anoxic soils. The boundary is a function of pH. Treatments levels were 100 ppm nitrate (N1), 200 ppm nitrate (N2), and 1% organic matter (OM).

can fall appreciably below the boundary line, as was observed in most measurements here.

The findings raise the question of why redox potentials relative to the suboxic-anoxic boundary were somewhat more strongly influenced by nitrate than were absolute redox values. Denitrification rates have long been known to be inhibited by acidic soil; therefore denitrification may have occurred to a lesser extent where pH values were lower. However, we postulate that a second explanation may also apply, *i.e.*, that denitrification may have produced the mildly alkaline reaction product bicarbonate as suggested Equation (1) [19].

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O. \quad (1)$$
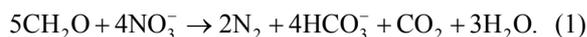
Figure 3 indicates that the combination of N plus organic matter generally tended to increase pH; and illustrates that as pH increases the boundary line slopes downward. With all other factors remaining constant, a treatment causing a slight elevation in pH would increase redox value relative to the boundary line, and would tend to shift equilibria between reduced and oxidized sulfur toward the oxidized state.

Only for Days 14 and 42 did the results support the hypothesis that nitrate would mitigate the impact of organic matter. Because denitrification is one of many possible fates of nitrate, this result was not unexpected. Studies of N attenuation in wetlands reveals a wide range of results, including 100% nitrate and 69% total N attenuation [11], 13% to 73% nitrate attenuation, [9], and 51% total N attenuation [20]. Investigators [10,21] show generally positive correlations between nitrate reduction and soil organic matter. Matheson *et al.* [8] found that in unplanted wetland microcosms 49% of nitrate nitrogen was reduced to ammonium, 29% was denitrified, and 22% was immobilized. Adding to the complexities of quantifying denitrification in wetlands is its tendency to occur simultaneously or sequentially with nitrogen recycling [22]. Whitmire and Hamilton [4] observed nitrate depletion within 5 to 20 hours, leading to the speculation

for the present study that added nitrate might have already been reduced to another form when the first redox reading was taken, perhaps to cycle back as nitrate later in the study, such as on day 42 when nitrate treatment effects were surprisingly significant.

In conclusion, this study of wet soil microcosms described the effects of organic matter and nitrate on respiration, redox potentials, and relative redox potentials (*i.e.*, relative to the suboxic-anoxic boundary). Relative redox potentials are of particular relevance because they provide an indication of the effect of a factor on electron acceptors having lesser susceptibility to reduction than O_2 but greater than sulfate. We found that an organic matter addition of 1% markedly decreased the redox potential of wet soil both in the absolute sense and relative to the suboxic-anoxic boundary. On two sampling days nitrate raised redox potentials relative to the suboxic-anoxic boundary. Our findings suggest that adding organic matter to a wetland may quickly and markedly decrease soil redox potential and increase CO_2 emission. Our findings offer little information from which a response to nitrate could be predicted, but rather serve to corroborate previous descriptions of the complex nature of nitrate in wetlands.

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