Adding organic matter to restore wetland soils may increase methane generation and is not needed for hydric soil development

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Abstract Methane (CH_4) emissions are a potent contributor to global warming and wetlands can be a significant CH_4 source. In a microcosm study we evaluated how the practice of amending soils with organic matter as part of wetland restoration projects may affect CH_4 production potential. Organic amendments including hay, manure, biosolids and compost were evaluated at three different levels. Using 1-liter glass microcosms, we measured the production of biogenic gases over 60 days in two soils, a sandy loam (SL) and a sandy clay loam (SCL). Fresh organic amendments increased CH_4 production, leading to potentially higher global warming potential and wetland C loss, particularly in sandy soils. Organic amendments increased biogenic gas production in two sequential steady state phases: Phase 1 produced some CH_4 but was mostly carbon dioxide (CO_2) followed by Phase 2, two to six weeks later, with much higher total gas and nearly equal amounts of CH_4 and CO_2. The CH_4 from the SCL soil ranged from 0.003 – 0.8 cc/Kg/day in Phase 1 to 0.75 – 28 in Phase 2 and the SL range from 0.03 – 16 cc/Kg/day in Phase 1 to 1.8 – 64 in Phase 2. We had set out to identify an organic amendment that would promote iron (Fe) reduction without excess CH_4, but amendments were not needed to produce Fe and make soils hydric. Adding fresh organic matter (hay) resulted in both excess Fe^{2+} and CH_4 whereas composted amendments had little effect. The potential for excess methanogenesis should be taken into account when considering organic matter amendments in mitigation wetlands.

Keywords Methane emissions, mitigation wetlands, organic amendments

1 Introduction

The ecological benefits of wetlands are well documented, including their role as carbon sinks to stabilize global climate (Mitsch, Bernal, and Hernandez 2015). Driven in part by this ecological contribution, from 1970 to 2015 new (human-made) wetlands have increased 233% (Darrah et al. 2019). Between 2004 and 2009 the United States saw a net gain of 41,200 acres of freshwater wetlands: 891,600 acres of new wetlands to offset 850,400 acres of existing (carbon-sink) wetlands that were destroyed (Dahl 2010). Although human-made wetlands may effectively sequester carbon (C), it may take hundreds of years to offset their radiative forcing due to methane (CH_4) emissions (Neubauer 2014). With such a large number of new human-made wetlands, and their potential to increase global warming, it is vital to consider factors that contribute to CH_4 emissions.

Some researchers have suggested that organic amendments such as straw, wood mulch, manure, and biosolids should be mixed into the soil to accelerate C storage by enhancing the conversion of plant-derived compounds to microbial residues (Richardson et al. 2016). Microbial residues, largely aliphatic-C from cell membrane lipids, can accumulate under anoxic conditions and are not directly accessible by methanogens (Chen et al. 2018). Belowground plant materials are preferentially

35 converted to soil organic matter (Mazzilli et al. 2015). Standing litter in natural wetlands is partially decomposed by fungi (Kuehn et al. 2011), and further decomposed by aerobic bacteria before entering the soil (Yarwood 2018). In saturated soils root residues of wetland plants contain suberin and cutin (Watanabe et al. 2013), which persist reducing biogenic gas production (Mikutta et al. 2006). Organic amendments are more representative of above-ground material, and studies suggest they are less amenable to soil C stabilization compared to natural plant inputs and may produce CH_4 (Scott et al. 2020).

40 Organic amendments may also contribute to CH_4 generated from existing soil organic carbon (SOC) by priming existing SOC (Nottingham et al. 2009).

Iron (Fe) oxides play a dual role in anoxic soils, being both an electron acceptor for organic C metabolism (Straub, Benz, and Schink 2001), and a stabilizing agent for SOC on mineral surfaces (Lehmann and Kleber 2015). As a metabolite, Fe reduction may compete with CH_4 production (Huang, Yu, and Gambrell 2009), couple it (Sivan, Shusta, and Valentine

45 2016), or enhancement it (Zhuang et al. 2015; Prakash, Chauhan, and Ferry 2019). In systems that are near pH neutral, Fe reduction does not have an energetic competitive advantage over CH_4 production (Bethke et al. 2011). Organic matter interacts with metal-oxide mineral surfaces, which can affect both Fe reduction (Poggenburg et al. 2018) and C mineralization and storage (Amendola et al. 2018; Lalonde et al. 2012). Iron reduction is also one of the primary methods for determining if soils are hydric (National Technical Committee for Hydric Soils (NTCHS). 2015), a key indicator of wetland

50 success under mitigation guidelines.

We carried out a lab experiment using organic amendments commonly used in wetland restoration (biosolids (Bloom® - B, manure - M, composted yard waste (LeafGro®) - L, wood chips - W, and hay - H) and measured how they affected CH_4 production and Fe-reduction. A series of 1-liter glass-jar microcosms were incubated with two different soils, a sandy clay loam (SCL), and a sandy loam (SL), both from recently created freshwater wetlands. The microcosms were kept under

55 anaerobic conditions to compare the ability of these substrates to support anaerobic metabolism. We hypothesized that organic amendments would stimulate dissimilatory Fe-reduction in soils (measured as soluble ferrous iron, Fe^{2+}). Further, we hypothesized that amendments promoting Fe reduction would limit methanogenesis. We also tested differences between cured (i.e., aged) and uncured (fresh) organic amendments and hypothesized that uncured amendments would increase Fe reduction due to the presence of more labile, soluble, compounds. In the United States organic amendments are often

60 required in mitigation wetlands. However, there has not been a systematic evaluation of whether or not amendments promote hydric soil conditions (Fe reduction), or may lead to Fe toxicity (excess Fe reduction), or my cause excess CH_4 production.

2 Materials and methods

2.1 Microcosm setup

65   Saturated incubations were established using soil from two recent mitigation wetlands located in Maryland, USA. The first site (76°50'40.35"W, 38°47'5.41"N) was most recently a horse pasture and will be referred to as SCL denoting the texture (sandy clay loam), a mesic anthrudult. The second site (75°47'40.20"W, 39°1'52.42"N) was most recently a corn/soy farm with tile drains and was likely a wetland prior to conversion to farmland. The second site will be referred to as SL for the texture (sandy loam) and is classified as a mesic anthraquult (Supplemental Table S1). Both sites had been recently graded to
70   establish wetland topography, so the upper portion of the soils were mixed endo- and umbr-aquic horizons. All soils were air-dried, sieved (2mm), and homogenized.

Microcosm experiments were conducted in 1000-mL glass straight-sided wide-mouth food canning jars. Each microcosm had a total of 600cc of solid material and was filled with water for a total volume of 660cc. The volumes needed to be precise in order to facilitate headspace and liquid sampling and allow space for soil expansion. When amendments were
75   added, an equal volume of soil needed to be removed so the total volume of solid material was a constant 600cc. At the start of the experiment, the headspace was purged with nitrogen gas. The incubation temperature was 20°C. Jar lids had precision drilled holes fitted with grey butyl rubber stoppers, making it possible to non-destructively remove the overlying liquid (for Fe and pH analyses) using a 3" needle. Since the head-space pressure increased due to biogenic gas production, atmospheric pressure was re-established during gas sampling events by piercing the septa with a 24-gauge needle connected to a 50mL
80   gas-tight syringe. This procedure allowed us to record the total volume of gas produced and collect gas samples (0.01 - 1000 µL) under atmospheric pressure (Supplemental Figure S1). A small coating of silicone applied to stoppers after piercing prevented leaks. All microcosm trials were run with three replicates except where noted.

2.2 Microcosm Experiments

2.2.1 Experiment 1

85   We measured CH_4 and Fe^{2+} production with various organic amendments, including composted yard waste (L), composted wood chips (W), class 1 biosolids (Bloom®) - B, manure - M, and hay - H at three treatment levels: 8.8% (v/v), 26%, and 53%, in two soils, a SL and a SCL. We used horse M for the SCL incubations and cow M for the SL incubations. This matched the wetland mitigation conditions at each field location. The treatment levels reflect the Maryland Department of Environment (MDE) recommendation for wetland restoration (60 cubic yards per acre assuming a 6" mixing depth) = 1x, 3x,
90   and 6x the MDE recommended level. All amendments were sieved to 5mm. Hay was chopped with a Wiley mill, blended, or cut with scissors until it could easily pass a 5mm sieve.

2.2.2 Experiment 2

We measured CH_4 and Fe^{2+} production using cured (aged) and uncured (fresh) organic materials. We used two amendments, B and M. The two cured materials were from the same two sources as the fresh material but were cured for a minimum of 3 months. We added the same amount of amendment to each microcosm based on organic matter (OM) content. Each amendment was evaluated for OM by loss-on-ignition (LOI) (550°C for 2h). Based on the percent OM we adjusted the amount of amendment so the final dose was 20g OM/ 600cc soil. The microcosm setup was the same as Experiment 1 except we used the same volume of soil (600cc) in all microcosms. These microcosms were incubated for 13 days and sampled periodically for Fe^{2+} and biogenic gases.

2.2.3 Experiment 3

We measured a) CH_4 and b) Fe^{2+} production as a function of pH. We used H leachate as a substrate as described in (McMahon et al. 2005). We leached 5.63 g H with 125 cc cold de-ionized water, shaking horizontally at 5°C for 24 hours. The leachate was filtered to 20 μm and immediately placed into jars with 600 cc SL soil and incubated for 22 days. The pH was adjusted to target levels of 5.6, 6.1, and 6.6 using a non-substrate buffer: 2-(N-morpholino) ethanesulfonic acid (MES). To determine the necessary concentration of MES, we titrated SL (pH 5.8) to our maximum desired pH (6.6). We determined that the buffering capacity of the soils corresponded to ~ 2 mN in the 125 cc of liquid (leachate volume), so we prepared microcosms using 125 cc of 20 mN MES buffer.

2.2.4 Experiment 4

We measured Fe^{2+} production using leached H as a substrate (as in Experiment 3) but compared these finding to those with unleached H, and the H residuals.

2.3 Soil, Liquid, and Gas Analyses

Prior to the start of the experiments, we analyzed the SL and SCL for soil texture, percent soil C, and extractable iron. Soil texture was determined by hygrometer method: 50 g soil was added to a 1000 ml cylinder with 0.5% hexametaphosphate. Sand settled after 1 minute and silt after 24 hours. Soil moisture content was determined as weight loss of approximately 5 g of soil dried at 105°C for 48 hours. We determined percent soil C using thermal combustion analysis at 950°C on a LECO CHN-2000 analyzer (LECO Corp., St. Joseph, MI). Iron extractions were performed sequentially with 1 M hydroxylamine hydrochloride in 25% v/v acetic acid; 50 g / 1 sodium dithionite in solution 0.35 M ace-tic acid / 0.2 M sodium citrate buffered to pH 4.8; 0.2 M ammonium oxalate / 0.17 M oxalic acid (pH 3.2) (Poulton and Canfield 2005).

Throughout the experiments we measured Fe^{2+}, pH, and biogenic gases in the headspace. In some cases, Fe^{2+} and pH were measured only at the end of the incubation. Using a 3" needle, we extracted 0.3 - 1 cc (for Fe^{2+}) and 1 cc (for pH) of the supernatant liquid to avoid disturbing soil in the jars. Liquid samples were removed during gas sampling, when atmospheric

pressure was maintained, to avoid loss of biogenic gases and atmospheric contamination. Ferrous iron in supernatant liquid was measured with a HACH DR4000 spectrophotometer. The spectrophotometer was also used to measure Fe in the Fe-oxide extractions. Prior to analysis, extracted Fe-oxides were reduced by adding thioglycolic acid. To confirm the
125 spectrophotometer accuracy, a subset of samples was also analyzed on a PerkinElmer PinAAcle 900T atomic absorption spectrometer. An Orion 9142BN electrode was used to determine pH.

Gas samples were collected in 12 cc N purged exetainer vials and analyzed by injecting 5 cc into a Varian Model 450-GC gas chromatograph. Since sample volume was typically 1 cc or less, 5 cc nitrogen gas was added to the vials immediately prior to analysis for CO_2 and CH_4. For fluorescent spectral scans dissolved organic matter was extracted from organic
130 materials with 1:10 solid (weight) / deionized water (volume) for 24 hours and filtered to 0.45 μm (Fischer et al. 2020). After diluting samples, emission spectra were recorded using an Aqualog fluorometer (Horiba Scientific; Edison, NJ).

2.4 Data analysis

Unless otherwise noted, statistical determinations were done using ANOVA in R or SAS. The Fe^{2+} concentrations were evaluated using contrasts for each of the amendments compared to the control using the multcomp package. The gas curves
135 were modelled as piecewise, bimodal linear functions using the R "Segmented" package (Muggeo 2008). Breakpoints were determined using the total gas curves but, in some cases, Segmented could not identify a breakpoint in the total gas curve, so CH_4 curves were used as noted in Supplemental Figures S2 & S3. Gas curves from H amendments did not fit a piecewise model and were modelled as sigmoidal functions using the SSgompertz function in R. However, Ssgompertz is sensitive to data scatter, particularly at the beginning and end of the curve, so in two cases, the total gas and CO_2 curves for H6x in the
140 SL, we fit the data with a power function in Excel.

3 Results

3.1 Experiment 1a: Effect of organic amendments and soil type on CH_4 gas production

The addition of organic amendments increased CH_4 production (Table 1). The amount of the increase depended on the soil type, when CH_4 samples were collected, amendment, and dose. In general, the SL soil produced 2.4 times as much CH_4 as
145 the SCL (Table 1 and Supplemental Figure S4a). Methane gas production occurred in two distinct steady-state gas production periods identified as Phase 1 and Phase 2. Therefore, we reported Phase 1 & 2 gas production rates, as well as the breakpoint (Table 1). A typical piecewise gas production curve is shown in Supplemental Figure S5 with individual gas curves shown in Supplemental Figures S2 (SCL) and S3 (SL). Supplemental Table S2 shows CH_4 and includes total gas and carbon dioxide (CO_2). Some CH_4 was produced almost immediately upon inundation (Phase 1), but after the breakpoint (40
150 days in both the SL and SCL soils), there is a large increase in CH_4 as well as an average 4.7x ± 1.9 increase in total gas production (Supplemental Table S2). In the SCL soil, CH_4 production in Phase 1 was 0.003 cc/Kg/day and with amendments increased to as much as 0.8 cc/Kg/day (Table 1). In Phase 2 CH_4 was 1.9 cc/Kg/day and with amendments increased to as

much as 28 cc/Kg/day (Table 1). In the SL soil, amendments increased CH_4 from 0.04 to 16 cc/Kg/day in Phase 1 and from 1.8 to 64 in Phase 2.

155 Gas production rates increased with amendment dose. With the exception of L in the SL, all amendments reduced the breakpoint. Biosolids caused the largest shift, decreasing the breakpoint to as little as 5 days. While amendments generally increased CH_4 there were exceptions. Low doses of cured amendments (L and W) had lower CH_4 production rates than unamended soil: L1 in Phase 1 in both soils; L3 in the SL; L3 in the SCL (Phase 2 only); W1 in the SCL (Phase 2). Biosolids (B1) also lowered CH_4 production rates in both soils (Phase 1) (Table 1).

160 Using fresh H, biogenic gas production followed a sinusoidal pattern and we reported maximum CH_4 production rate at the inflection point (Table 1). Hay was prone to floating and at higher doses was mostly present in the water column above the soil surface (not in contact with soil). In the in-stances where this occurred (H3 and H6 in the SCL), there was a decrease in overall gas production rate and very low CH_4 – much lower than unamended soils (Table 1, Supplemental Table S2 and Supplemental Figures S2z & S3z).

165 **3.2 Experiment 1b: Effect of organic amendments and soil type on Fe^{2+}**

The type and dose of organic amendments affected total soluble Fe^{2+} production, compared to the unamended control, in only a limited number of cases (Figure 1, Supplemental Table S3). In the SL soil, L caused a decrease ($p < 0.05$) in supernatant Fe^{2+} concentrations whereas H increased Fe^{2+} in both soils ($p < 0.05$). Soil type affected the amount of soluble Fe^{2+} produced ($p < 0.05$). We did not see a difference in Fe^{2+} in the unamended microcosms even though the SCL had 2.2x

170 the amount of hydrochloramine hydrochloride extractable Fe (Fe_{HHCl}) compared to the SL and had 7.6x more dithionite extractable Fe (Supplemental Table S1). Of the Fe_{HHCl} in soil, 19% or less in the SCL and 61% or less in the SL was reduced to Fe^{2+}. Hay was an exception, where up to 155 % of the Fe_{HHCl} in the SCL and 236 % in the SL was reduced to Fe^{2+} (Supplemental Table S3). During the SL soil incubations, aqueous Fe^{2+} was measured simultaneous to CH_4 production. In the H and M treatments, there was a marked increase in CH_4 production when Fe^{2+} became asymptotic. However, with the

175 other amendments, Fe^{2+} production continued or even increased during periods of high CH_4 production (Supplemental Figure S6).

3.3 Experiment 2a: Effect of cured versus fresh organic amendments on CH_4 production

In Experiment 1a, it appeared that curing may have had an effect on CH_4 production. Fresh H produced the most CH_4. The H1 trials had maximum CH_4 production rates of 18.2 and 27.8 mg/Kg/day in the SCL and SL soils, respectively (Table 1).

180 The H3 and H6 doses would likely have been higher had some portion of the H not floated. The M6 trials produced the most CH_4 at 27.7 and 64.0 mg/Kg/day in the SCL and SL soils, respectively. Of the amendments used, M was cured the least (after fresh H, which was uncured). LeafGro, a commercial composted yard waste, was cured the most and produced very little CH_4, in some cases less than the controls. Since we could not specify how long the organic material had been cured, we conducted a separate experiment with organic materials that had been cured at least 90 days, using B and M. Rather than use

185 the same volumetric quantities, we used the same dose based on OM content. The results confirmed that curing has a strong influence on CH_4 production. Methane production was much higher using fresh material in both cases and cured material resulted in a decrease in CH_4 production (Table 2).

3.4 Experiment 2b: Effect of cured versus fresh organic amendments on Fe^{2+} production

In Experiment 1b, we observed that curing also had an effect on the amount of Fe^{2+} produced. Hay was the only amendment
190 that produced significantly more Fe^{2+} and the use of L saw a significant reduction in Fe^{2+} (Figure 1). In Experiment 2 we used biosolids (B) and manure (M) that had been cured at least 3 months. Whether the material had been cured had a strong influence on Fe^{2+} production and Fe^{2+} was higher using fresh material in both cases (Figure 2).

3.4.1 Spectral analysis of cured and uncured organic matter

We observed differences in CH_4 and Fe reduction rates in cured versus uncured organic material. The fluorescent spectral
195 signatures of the cured materials (B and M) were similar as were the signatures of fresh material (Supplemental Figure S7). The fluorescent signatures varied due to curing, but not due to the source material. The difference in signatures was indicative of higher concentrations of organic (humic) acids and lower nominal oxidation state in the cured materials. We considered other organic matter characterization methods such as the material's carbon to nitrogen ratio, but we did not find another reliable predictor of CH_4 and Fe^{2+} production other than curing.

200 ### 3.5 Experiment 3: Effect of pH on a) CH_4 and b) Fe^{2+} production

The soil pH affected both CH_4 and Fe^{2+} production. In Experiment 1, we observed that on Fe^{2+} varied with pH in the SL soil ($p<0.001$; Supplemental Figure 8a), but there was little variation in the SCL ($p=0.45$; Supplemental Figure 8b). In order to isolate the effect of pH, we performed experiment 3 using a single substrate (H leachate) in the SL soil. Higher pH increased the CH_4 production rate in both Phase 1 and 2 (Table 3) and reduced the production of Fe^{2+} (Figure 3).

205 ### 3.6 Experiment 4: Leached versus unleached H and pH considerations

In Experiment 4 we measured Fe^{2+} produced from H, H leachate, and the H residuals (Figure 4). The H residuals appeared to produce more Fe^{2+} than the leachate. However, as noted on the figure, leaching also resulted in a change in the system pH. Using the results from Experiment 2, we predict that had the pHs been the same, there would have been no difference in Fe^{2+} production between H, H residuals, and leachate (Supplemental Figure S9). Therefore, we re-evaluated the results from
210 Experiment 2b, correcting for pH and confirmed that the organic material age accounts for differences in Fe^{2+} production (Supplemental Figure S10). Similarly, we considered whether pH affected the out-come of Experiment 1 results. However, a MANOVA analysis of the Experiment 1 data (Supplemental Table S4) indicated that pH had a small effect ($p=0.30$) compared to organic matter type and dose ($p<0.0001$).

4 Discussion

215 Net CH$_4$ emissions are a primary factor that determines whether a wetland is a C sink or contributes to long term global warming (Neubauer and Verhoeven 2019). Soil management practices, such as wetland restoration methods, can have a large impact on production of CH$_4$ and total greenhouse gases (Paustian et al. 2016). Our data indicate that organic amendments used in mitigation wetlands can have a large influence on CH$_4$ production. Cured organic amendments (L and W) only slightly increased CH$_4$ emissions, whereas fresh material (M and H) resulted in large increases (Table 1 and

220 Supplemental Table S2). This is consistent with field studies where comparable cured amendments (composted wood and yard waste), did not result in increased CH$_4$ emissions (Winton and Richardson 2015), but straw (Ballantine et al. 2015) and peat bales (Green et al. 2014) increased CH$_4$. Curing produces humic acids and increases the nominal oxidation state (NOSC) of C (Guo, Liu, and Wu 2019). When cured material is then subjected to methanogenic conditions, less CH$_4$ is produced to maintain an electron balance (Yao and Conrad 2000).

225 Following soil inundation, we observed two distinct gas production phases (Phase 1 and 2). This pattern is difficult to distinguish in unamended soils but has been reported previously (Yao and Conrad 1999). The breakpoint was similar: from 5 – 36 days in Yao and Conrad (1999) and 5 – 45 days in our study (Table 1). The Phase 2 CH$_4$ production rates in unamended soils were 0.96 – 3.98 mg/Kg/day in Yao and Conrad (1999) and 1.82 – 1.94 in our study (Table 1). Organic amendments may contribute viable methanogens into the soil matrix. Biosolids, which may contain high populations of

230 inactive methanogens, contributed to the early onset of Phase 2 CH$_4$ production (Table 1). Once established in soil, there is some evidence that methanogens can survive both desiccation and oxic conditions (Mayer and Conrad 1990; Ramakrishnan et al. 2001).

The increased gas production from organic amendments was more pronounced in SL compared to SCL, where there was 2.4x higher CH$_4$ and 2.6x higher gas production (Supplemental Figures 4a & 4b). There are few studies that compare CH$_4$

235 production rates by soil type. (Yagi and Minami 1990) observed respiration rates that were 1.8x higher when using compost (approximate dose the same as our 1x treatment) in a SCL versus a loam soil. (Maietta et al. 2020) observed that respiration rates were higher in a sandy loam soil compared to a silty clay, with and without 3.3% & 23% wetland hay. Our study showed that coarse grain soils are more vulnerable to SOC loss via CH$_4$ production, which may be one reason why they are generally lower in SOC (Scott et al. 2020).

240 We considered the gas production from H microcosms separately because they followed a different pattern than the other amendments (Table 1), but the pattern was similar to other studies using hay (Glissmann and Conrad 2002) and wetland hay (Maietta et al. 2020). Our study adds to these findings by observing that H produced very low CH$_4$ in the water column (after floating) compared to being mixed with soil. If this is generally true, applying organic matter as a mulch, rather than mixed into the soil, could greatly reduce CH$_4$ emissions.

245 Reduction of Fe-oxides occurs in saturated soils in the presence of an organic substrate and is a key biogeochemical process in wetland soils. With sufficient time, hydric soils may develop redoximorphic features from Fe reduction; however, studies

have not shown lasting redoximorphic development due to organic amendments (Gray 2010; Ott et al. 2020). Organizations responsible for constructing mitigation wetlands have an interest in documenting Fe reduction prior to redoximorphic feature development as evidence soils that are hydric. Some mitigation wetland practitioners experience challenges meeting hydric

250 soil testing standards. Although reports in the scientific literature are rare, there are examples of sites meeting vegetation and hydrology wetland indicators, but not hydric soils (Berkowitz, Page, and Noble 2014). Both the soils we tested produced Fe^{2+} and would have passed hydric soils tests without the aid of an amendment.

We observed that fresh organic matter resulted in increased Fe^{2+} compared to cured organic matter (Figure 2), likely due to the presence of carbohydrates, allowing access to more crystalline Fe-oxides (Lentini, Wankel, and Hansel 2012). Fresh

255 material such as hay has been promoted as a soil amendment in wetland construction (Melvin 2003). In some soils fresh organic matter amendments could access crystalline Fe making it more bioavailable. However, excess Fe^{2+} production could lead to Fe toxicity and ferrolysis (Kirk 2004), similar to the way fresh organic matter leads to SOC priming (Blagodatsky et al. 2010). Ferrolysis occurs when immobilized Fe-oxides are reduced to Fe^{2+} and are subject to hydraulic transport. We observed that cured amendments, like L, lowered Fe^{2+} concentrations (Figure 1), likely due to combination of factors

260 including displacement of the Fe-bearing soil by the amendment and the presence of humic acids that are generated during curing (Guo, Liu, and Wu 2019). Humic acids often contain insufficient biogeochemical energy to drive dissimilatory Fe reduc-tion (Keiluweit et al. 2017), bind to Fe^{2+}, removing it from the liquid phase (Catrouillet et al. 2014), and create insoluble precipitates (Shimizu et al. 2013).

Regulating Fe^{2+} production could influence the growth of wetland plants. For example, rice growth may be stimulated under

265 low Fe^{2+} doses of 1 mg/L (Müller et al. 2015), but higher doses can produce detrimental Fe plaque (Pereira et al. 2014). Juncus effusus was stimulated at 25 mg/L Fe^{2+} (Deng, Ye, and Wong 2009). North American native reed Phragmites australis ssp. americanus was stimulated at 11 mg/L Fe^{2+} from ferrous sulfate (Willson et al. 2017), but the invasive Eurasian lineage of Phragmites australis seedling growth was inhibited by Fe^{2+} as low as 1 mg/L (Batty 2003). Soils high in free Fe^{2+} adversely affected P. australis growth by creating an Fe-oxide plaque on roots (Saaltink et al. 2017). Therefore, promoting Fe

270 reduction could have a beneficial effect on native wetland plant growth while limiting invasive species seedling recruitment or growth. In our experiment, fresh organic amendments increase Fe production, but had the detrimental side effect of high CH_4 generation.

Our results show that pH has a significant effect on both the production of Fe^{2+} (Figure 3) and CH_4 (Table 3). Between pH 5.6 and 6.6, the lower pH produced more Fe^{2+} and less CH_4, consistent with thermodynamic predictions (Ye et al. 2012).

275 Hydrogenotrophic methanogens can maximize CH_4 production at pH 5 (Bräuer, Yavitt, and Zinder 2004). In rice paddy soils, CH_4 emissions had a clear peak at pH 7, but almost none below pH 5.5 (Wang et al. 1993). The strong effect of pH underscores the need to take this parameter into account when interpreting data from experiments evaluating Fe-reduction and methanogenesis. Attempting to control the pH of soils could potentially introduce con-founding effects. We used an MES buffer with 10x the quantity we estimated from a soil titration and still saw shifts in the pH after incubation (data not

280 shown). With a high residual soil acidity, the amount of buffer needed to control soil pH may increase the ionic strength to a

level that could influence cellular sorption to mineral and Fe-oxide surfaces (Mills et al. 1994) as well as enzyme activity (Leprince and Quiquampoix 1996).

5 Implications

In our experiment, we saw that organic amendments significantly increased CH_4 production, particularly after extended
285 anaerobic periods. There is mounting concern that CH_4 from mitigation and created wetlands may result in net global warming potential for decades to centuries (Neubauer 2014). Our results suggest that not only do organic amendments increase CH_4 gas production overall, but uncured amendments can also decrease the time it takes before there is a large increase in both total gas production and CH_4. Methane production is not constant and dramatically increases after several weeks; therefore, it may be possible to limit CH_4 by designing systems with shorter flooding or saturation periods,
290 alternating with drier conditions. Our lab study demonstrates the potential for significant CH_4 emissions, but in a real system, methanotrophic activity could attenuate CH_4 some of the emissions (Chowdhury and Dick 2013); however, this would not decrease the overall C loss from soils, it only changes the pathway. Our study suggests using organic amendments in nutrient- and carbon-poor coarse grained (sandy) soils could exacerbate SOC losses and greenhouse gas production. If organic amendments are to be used, cured amendments may be preferable because they are not as prone to high CH_4
295 generation and may attenuate Fe toxicity. Amendments that lower the soil pH would favor Fe reduction and limit methanogenesis (Marquart et al. 2019). When deciding whether or not the use of organic amendments for wetland mitigation is beneficial, or necessary, consideration should be given to whether or not the material has been cured, the material pH, the soil type (coarse or fine grained), and expected hydroperiod.

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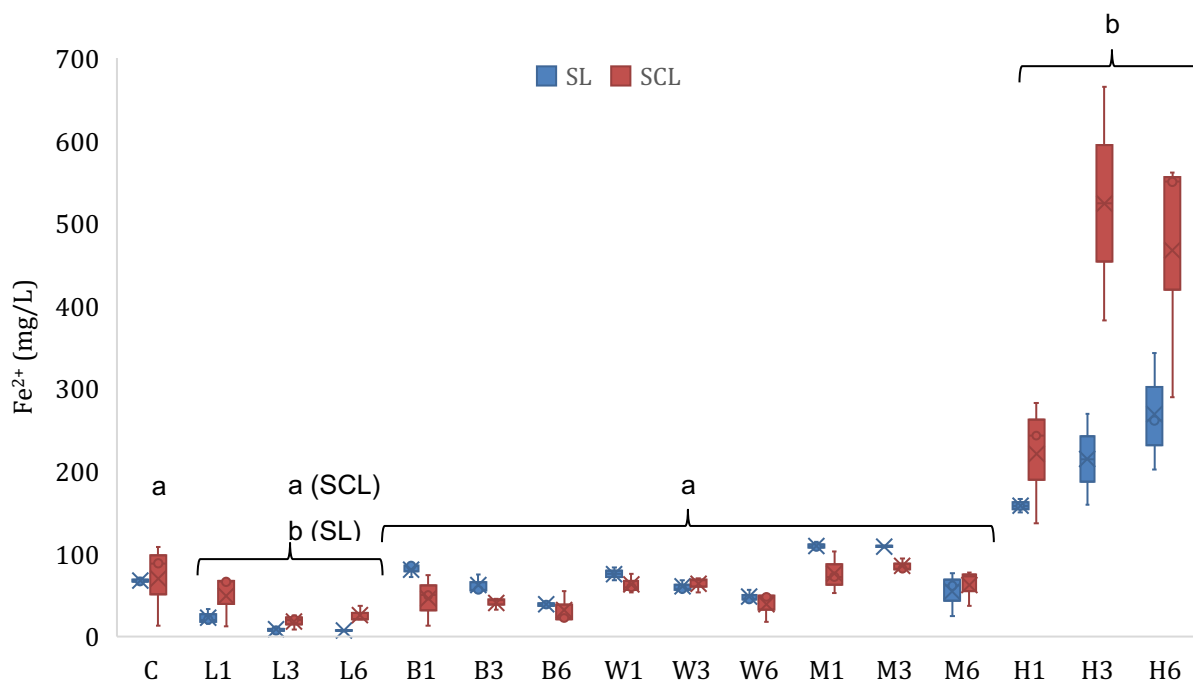
Fig. 1 (Experiment 1b) Ferrous iron (Fe^{2+}) concentration in the liquid phase at the end of the incubation period in microcosms
450 **receiving different organic amendment types and levels in Sandy Clay Loam (SCL) and Sandy Loam (SL) soils. C = no amendment control, L = LeafGro (yard waste), B = biosolids, W = wood chips, M = manure, H = hay. Numbers signify treatment level (1, 3, or 6 times amount of organic matter equivalent to 60 yd3 acre-1 to a depth of 6 inches). Different lower-case letters signify differences (p < 0.05) based on contrasts compared to C and brackets signify all results in the bracketed group were not statistically different. Hay increased total Fe^{2+} production compared to the C in both soils, and L decreased total Fe^{2+} production**
455 **compared to C (SL only)**
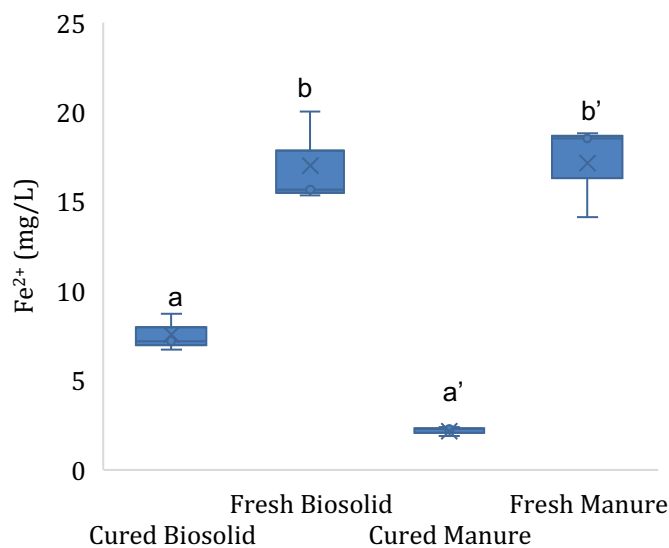
Fig. 2 (Experiment 2b) Ferrous iron (Fe^{2+}) concentration in the liquid phase at the end of the incubation period (13 days). Incubation was carried out with cured and uncured biosolids (B) and manure (M) in SL soil. Letters indicate a difference at p<0.001

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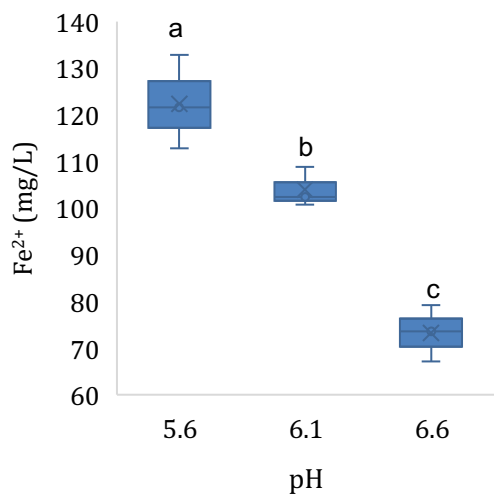
Fig. 3 (Experiment 3) Ferrous iron (Fe^{2+}) concentration in the liquid phase with varied in of microcosms receiving H in Sandy Loam soils. Letters indicate a difference at p<0.05.

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Table 1: (Experiment 1a). CH₄ gas data for incubations of different organic amendment types and levels in silty clay loam (SCL) and sandy loam (SL) soils. Instances where organic amendments did not increase CH₄ production are bolded.

Treatment	r²	Phase 1 CH₄ (cc/Kg/day)	Breakpoint (days)	Phase 2 CH₄ (cc/Kg/day)	r²	Phase 1 CH₄ (cc/Kg/day)	Breakpoint (days)	Phase 2 CH₄ (cc/Kg/day)
		Experiment 1 (SCL)				Experiment 2 (SL)		
Control	0.959	0.003	40.0 ± 4.5	1.94	0.957	0.04	40.0 ± 3.2	1.82
B1	0.987	0.18	29.3 ± 1.9	3.45	0.880	**0.04**	8.6 ± 3.0	8.50
B3	0.974	0.80	20.1 ± 3.4	8.17	0.989	0.79	4.7 ± 1.8	15.28
B6	0.994	0.13	10.3 ± 2.4	23.29	0.992	1.27	9.1 ± 1.2	43.07
M1	0.997	0.04	40.2 ± 2.1	2.22	0.998	0.93	16.7 ± 0.7	21.02
M3	0.997	0.64	20.8 ± 0.8	10.89	0.992	5.85	17.2 ± 1.5	59.10
M6	0.956	0.30	22.1 ± 3.2	27.67	0.974	15.87	29.4 ± 1.4	64.00
L1	0.966	**0.001**	32.2 ± 1.6	**0.090**	0.993	**0.03**	38.3 ± 1.2	2.85
L3	0.983	0.01	32.0 ± 2.2	**0.75**	0.977	**0.03**	40.5 ± 2.0	3.50
L6	0.923	0.09	32.0 ± 3.7	2.24	0.988	0.26	44.8 ± 1.3	7.21
W1	0.986	0.01	34.0 ± 2.9	**0.460**	0.991	0.10	25.6 ± 7.6	2.19
W3	0.989	0.19	24.2 ± 3.1	3.52	0.974	0.36	23.2 ± 2.3	7.51
W6	0.981	0.24	13.0 ± 2.4	5.15	0.991	0.54	23.2 ± 1.1	18.60
H1	N/A	N/A	N/A	18.15	N/A	N/A	N/A	27.81
H3	N/A	N/A	N/A	**0.04**	N/A	N/A	N/A	25.52
H6	N/A	N/A	N/A	**0.27**	N/A	N/A	N/A	41.1

470 **Table 2: (Experiment 2a). Methane gas data for incubations with fresh and cured organic matter in SL (Experiment 1). Control data (*) from Experiment 1a (Table 1) included for reference. Letters indicate a difference at p<0.001.**

Treatment	Phase 1 Methane (cc/Kg/day)	Phase 2 Methane (cc/Kg/day)
Control*[a]	0.04	1.8
Cured Biosolids[a]	0.003	0.37
Fresh Biosolids[b]	3.29	17.48
Cured Manure[a']	0.22	5.4
Fresh Manure[b']	3.85	42.36

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Table 3: (Experiment 3). Methane gas data versus pH of microcosms receiving H in Sandy Loam soils (Experiment 3). Letters indicate a difference at p<0.001.

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pH	Phase 1 CH$_4$ (cc/Kg/day)	Phase 2 CH$_4$ (cc/Kg/day)
5.6[a]	0.44	10.6
6.1[b]	1.0	13.0
6.6[c]	1.8	13.8

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Declarations

Funding

Work was made possible by funding from the Maryland State Highway Administration (SHA/UM/4-53), the Maryland Water Resources Research Center (2017MD340B), and USDA National Institute of Food and Agriculture, Hatch Project

490   Number: MD-ENST-7741.

Conflicts of interest/Competing interests

Authors declare no conflict of interest

Availability of data and material

Significant data detail is available in the supplementary materials. Additional raw data available upon request.

495 **Code availability**

None